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EFFECT OF HIV AND ANTIRETROVIRAL THERAPY ON SEMEN QUALITY

BY

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EFFECT OF HIV AND ANTIRETROVIRAL THERAPY ON SEMEN QUALITY

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CERTIFICATION

This is to certify that, this thesis is the candidate's own account of his research.

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ABSTRACT

Background: Semen is the major vehicle for HIV-1 infection. However, the supposed detrimental effect of HIV on semen quality has been a matter of unsettled debate. Antiretroviral therapy can effectively decrease the levels of viral load in peripheral plasma and seminal fluid of infected men and also relieve the symptoms of the disease, but whether or not it improves semen quality remains unknown currently.

Methods: Semen characteristics of 129 subjects made up of 100 HIV-infected men, (25 HIV-infected men with slight symptoms of opportunistic infections, 25 asymptomatic HIV-infected men, 25 HIV-infected men who have been on ART for less than 1 year, 25 HIV-infected men who have been on ART for 1 or more years) and 29 HIV-negative healthy control men capable of reproducing were evaluated.

Results: Semen quality was negatively affected in the HIV- infected cohorts. Semen parameters that were negatively affected in the HIV-infected groups (G1, G2, G3 and G4), compared with the HIV-negative control (G5) were: reduced semen volume ($P \leq 0.05$), reduced percentages of rapid progressive sperm ($P \leq 0.05$), increased immotile spermatozoa percentages ($P \leq 0.05$), reduced sperm concentration ($P \leq 0.05$), total sperm count ($P \leq 0.05$), increased immature germ cells ($P \leq 0.05$), increased RBC count ($P \leq 0.05$), reduced normal morphology of spermatozoa ($P \leq 0.05$), reduced percentages of sperm vitality ($P \leq 0.05$) and increased pH ($P \leq 0.05$).

Within the HIV-infected groups, G2, G3 and G4 exhibited significantly better semen parameters such as rapid progressive motile sperm, slow progressive motile sperm, sperm concentration, total sperm count, immature germ cell loss, haemospermia, normal morphological sperm and sperm vitality compared with G1 at $P \leq 0.05$. However, only total sperm count, sperm concentration of G5 and sperm concentration of G3 showed significant correlations with absolute $CD4^+$ counts ($P \leq 0.05$)

Conclusions: HIV infection reduced semen quality of infected men compared with HIV-negative men. Within the HIV-infected men, the asymptomatic HIV-infected men showed better semen quality than the symptomatic ART naïve men. Within the HIV-infected men on ART, longer duration of treatment had relative positive effect on semen quality than shorter duration of treatment.

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CHAPTER ONE

1.1 General Introduction

Human Immunodeficiency Virus, (HIV), is a retrovirus which causes AIDS. AIDS is a clinical syndrome characterized by the progressive depletion of $CD4^+$ T-lymphocyte population of the blood, leading to a progressive deterioration of the immune system leaving the infected person vulnerable to a variety of infections(Gurunathan *et al.*, 2009). The clinical syndrome of AIDS was first recognized over 20 years ago and the discovery of HIV-1 (HIV), the causative virus, followed soon after (Gelmann *et al.*, 1983). Since then, more than 25 million people have died of AIDS and more than 40 million are currently infected by HIV(WHO & UNAIDS, 2004). Its transmissibility depends on the type of sexual exposure, frequency of sexual intercourse, level of infectivity of the infected partner, and the susceptibility of the non-infected partner (Politch *et al.*, 1994; Royce *et al.*, 1997).

HIV/AIDS is one of the single most important health issues that threaten the survival of millions in sub-Saharan Africa. The HIV pandemic continues to challenge the development and economy of many countries and Ghana is not an exception (Sauve, 2002). From 1986 to 2006, 121,050 cases of AIDS were reported by the Ghana Ministry of Health, and in 2006, about 297,000 Ghanaians were estimated to be living with HIV (Sauve, 2002). Though the prevalence rate in Ghana has remained below 5%, for the past 16 years, the number of persons living with HIV continues to rise daily (GAIDSC, 2006, 2007).

Mitigation of the effects of HIV/AIDS involves conscientious and systematic efforts by Governments, Ministries, Departments, Agencies, non-governmental organizations and other

stakeholders to develop and implement policies and strategies aimed at decreasing the rippling effects of HIV/AIDS pandemic on the society and our fragile economy.

While genital fluids represent the main vehicle for human immunodeficiency virus transmission worldwide, the origin of the cell free and cell-associated HIV contaminating these body fluids is poorly understood currently (Ho *et al.*, 1996; Zhu *et al.*, 1996; Byrn & Kiessling, 1998; Barroso *et al.*, 2000; Quinn *et al.*, 2000). It is not surprising therefore that lots of investigations have hypothesized local sources within the Male Genital Tract (MGT) which contribute virus particles and infected cells to seminal fluids (Anna Le Tortorec & Roques, 2008).

The relatively high efficiency of male sexual transmission of HIV has made a number of studies focused attention on the histologic findings as well as the functional activity of the testes critically, to unravel undiscovered knowledge in the arena of seminal sexual transmission of HIV. To investigate the origin of HIV in semen, a necessary prerequisite is to determine which semen-producing organs are productively infected by the virus and/ or susceptible to HIV seed virus. Importantly, despite antiretroviral therapy achieving an undetectable blood viral load, virus release persists in semen (Byrn & Kiessling, 1998; Zhang *et al.*, 1998; Anna Le Tortorec & Roques, 2008).

The routine clinical management of HIV seropositive patients in developing nations involves monitoring of absolute $CD4^+$ count. Although monitoring of both the blood and seminal viral loads are the most effective strategies needed to effectively manage and prevent further spreading of the virus into the seronegative population (Porco *et al.*, 2004), this is not factored into routine management strategies in the middle and low income countries probably because of cost. The reproductive compartment serves as a major viral reservoir in infected men and degeneration of major testicular tissue and associated reproductive glands leads to fundamental

deterioration of seminal parameters as the viral burden increases (da Silva *et al.*, 1990; Shevchuk *et al.*, 1999). On the other hand, ART has recently been reported to improve health, reduce blood viral loads and seminal viral loads in most incidences and maintains or reconstitute the immune system by upward adjustment of absolute $CD4^+$ count (Egger *et al.*, 1997; Detels *et al.*, 1998; Vernazza *et al.*, 2000). As the indicator of immune reconstitution, a rise in the absolute $CD4^+$ count might imply a significant reduction in viral load in the testes, a significance increase in testicular tissue regeneration and normal seminal parameters regained in seropositive males after commencement of ART. Seminal indicators should gradually revert to the normal situation (Anderson *et al.*, 1992; Politch *et al.*, 1994; Shevchuk *et al.*, 1999; Ghosn *et al.*, 2008)

1.3 Specific Aims And Objectives

The main objectives of the study are:

1. To investigate the effect of HIV infection on the semen quality.
2. To investigate relationship between $CD4^+$ count and the semen quality.
3. To investigate the effect of ART on semen quality of seropositive men.

1.4 Justification

HIV virus infection is mainly through sexual contact. It is a common knowledge that not all men who are seropositive of the HIV virus infect their partners when they have sexual encounter with them (Fowke *et al.*, 1996; Plummer *et al.*, 1999). These are however, are in the very minority. The vast majority could be infected and the critical determinant of sexual transmission of HIV in the nonresistant populations is the seminal viral load of the seropositive man as well as the cervical and vaginal viral shedding of women (Fowke *et al.*, 1996; Plummer *et al.*, 1999).

There is viral replication in the testes and/ or associated glands (Ghosn *et al.*, 2008). In the light of this, when viral replication in this compartment increases, spermatozoa and the other components of semen in the reproductive glands of infected males would be impaired, hence, the quality of semen produced would be affected negatively. On the order hand, as ART highly reduces the blood viral load, and in some cases the seminal viral load, the quality of the semen would be expected to improve when male patients are treated with ART for at least, three months (Ghosn *et al.*, 2008).

Many studies have established some relationship between blood viral load and the health conditions of the individual. Nonetheless, the effect of ART on semen quality remains to be determined. Since the use of antiretroviral therapies for the treatment of HIV improves the control of opportunistic infections and produces significant improvements in life expectancy and quality of life of HIV seropositive cohorts, it has become justifiable to investigate the effect of the HIV and ART on the semen quality of seropositive men who are on the therapy.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 HIV/AIDS

The virus that causes Acquired Immune Deficiency Syndrome (AIDS) is called Human Immunodeficiency Virus (HIV) and is visible only under an electron microscope (Feldman & Wang, 1998). It was first discovered by Dr. Louis Montagnier of France in 1983 (Feldman & Wang, 1998). The following year, it was called HTLV-III by Dr. Robert Gallo of the United States, and then widely recognized as the cause of AIDS(Feldman & Wang, 1998). AIDS is a severe disease syndrome that represents the late clinical stage of infection with HIV(WHO, 2003). The syndrome was first recognized in 1981, but was thought to probably have existed at a low endemic level in Central Africa during the 1970s , before epidemic HIV spread began to occur in several areas of the world (Feldman & Wang, 1998; WHO, 1999). After the initial infection with HIV, a mononucleosis-like syndrome develops in 40% - 70% of patients at 36 weeks (Feldman & J., 1998; Feldman & Wang, 1998; WHO, 2003). During this acute phase of infection, symptoms may include fever, headache, sore throat, erythematous rash, diarrhoea and generalized lymphadenopathy (Feldman & Wang, 1998; WHO, 1999). There may be a significant depression of the cellular immune system and infected persons at this early stage are considered extremely infectious(WHO, 2003). Subsequently, the immune system rebounds to generally normal levels and the infected person becomes asymptomatic for periods ranging from many months to many years (Feldman & Wang, 1998; WHO, 1999).

HIV infection attacks the cellular immune system (WHO, 2003). Continued damage to the immune system eventually makes HIV-infected individuals susceptible to various opportunistic

infections and cancers (Feldman & Wang, 1998). The first infections described in patients with AIDS were due to ubiquitous organisms that do not usually cause disease in healthy persons; the cancers that developed in AIDS patients were of types that had been diagnosed only rarely in the past (WHO, 2003). Subsequently, it became clear that persons with HIV infection could contract almost any common or uncommon infectious disease, or some malignancies, because of their immune deficiency (WHO, 2003). Based on detailed cohort studies, it is believed that the median period for the development of severe immune deficiency that results in AIDS ranges from 8-10 years (WHO, 2003).There is a consensus however, that the survival period from the development of severe immune deficiency to death is much shorter in most developing countries compared with developed countries, where the advent of Antiretroviral Therapy (ART) has significantly increased survival rates of patients with moderate immune deficiency related to their HIV infection (WHO, 2003).

2.1 Semen

Semen is slightly alkaline with pH range of 7.2-7.6. The average volume of an ejaculate is 2.5-5ml (Chan, 2005). Semen is discharged in a definite sequence. During erection and sexual arousal, pre-ejaculatory fluid from the bulbourethral and urethral glands lubricates the urethra. During ejaculation, the prostate discharges its secretions first. The spermatozoa are then propelled from the epididymis and Vas deferens by strong muscular contractions within the walls of these organs. Finally, the secretions of the seminal vesicles are added to the mass and ejaculation occurs with the attainment of orgasm. Semen is 90% seminal plasma and 10% cells. Each ejaculate volume comprises approximately 10^8 spermatozoa per milliliter of semen and 10^6-10^7 immature germ cells and non-sperm cells (epithelial cells, $CD4^+$ T lymphocytes,

monocytes, polymorphonuclear leukocytes and macrophages) (Chan, 2005). Seminal plasma, the nutritious fluid medium used by spermatozoa in semen, comprises secretions derived from the seminal vesicles (60%), prostate (30%) and the testes, epididymis, urethral glands and bulbourethral glands contribute the remainder (10%); i.e. seminal fluid is mainly derived from structures distal to the vas deferens(Chan, 2005). Testicular and epididymal fluids are dense with spermatozoa and lack protein. Fluid from the seminal vesicles contains protein, fructose and prostaglandins and is slightly alkaline (pH 7.8). Prostatic fluid is proteinaceous and acidic (pH 6.6), due largely to the presence of acid phosphatase and citric acid(Chan, 2005).

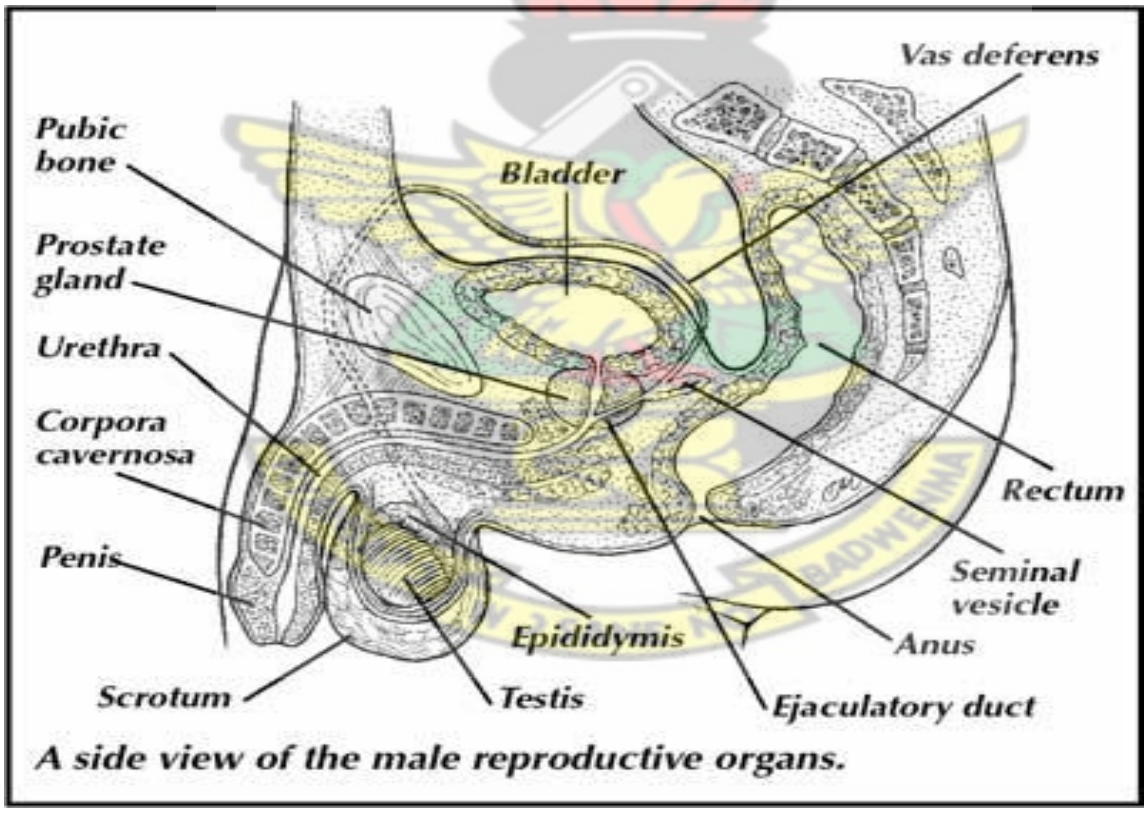

Fig. 1a: A figure showing the male reproductive system indicating the site and storage of spermatogenesis and the assessory glands.

2.2 HIV And Semen Production

There are a number of anatomical reservoirs for HIV-1 in the body and these include the central nervous system, lymphoid tissues and genital tract (Pomerantz, 2002). Histologic findings of testes throughout the early AIDS epidemic reported by a study indicated that large opportunistic infections, tumors, and especially germ cell changes had a direct impact on the quality of semen produced by those patients (da Silva *et al.*, 1990; Shevchuk *et al.*, 1999). In their ground breaking histological investigations, they noticed that all the patients who had systemic opportunistic infections also had testicular involvement (Mobley *et al.*, 1985; Shevchuk *et al.*, 1999). With no exception, the association between systemic infection and testicular involvement remained strong (De Paepe *et al.*, 1989; De Paepe & Waxman, 1989; Shevchuk *et al.*, 1999). Testicular germ cell loss may be associated with testicular infections and may be secondary to inflammatory infiltrates, vascular compromise, necrosis, and granulomatous reactions (De Paepe *et al.*, 1989; De Paepe & Waxman, 1989; Baccetti *et al.*, 1994). Unfortunately, about 75% of the subjects who had very depressed immunity with testicular opportunistic infections had Sertolicell only seminiferous tubules, i.e., total loss of testicular germ cells (Shevchuk *et al.*, 1999).

In another study by Pudney *et al*, (1991; 1999), 50% HIV-1 positive subjects had very high infiltration of lymphocytes and monocytes into the seminiferous tubules and interstitium of the testes, epididymal epithelium and connective tissue of the epididymis and prostate. Shevchuk and colleagues (1999) reported that a prolonged life with viral germ cell infection resulted in exacerbated germ cell death at autopsy which helps explain the trend toward Sertoli cell only histologic features of the seminiferous tubules of patients who died of HIV/AIDS.

Another potential cause of increased germ cell loss apart from opportunistic infections was hypothalamic dysfunction caused by HIV infection of the cerebral compartment (Morley & Melmed, 1979; Aron, 1989; De Paepe *et al.*, 1989; De Paepe & Waxman, 1989). Finally, HIV has been reported to probably have direct cytopathic activity, causing germ cell death, as evidenced by the pervasive germ cell degeneration(Nuovo *et al.*, 1994b).

While HIV-1 and simian immunodeficiency virus (SIV) were found in a number of male genital organs from AIDS deceased men and macaques respectively, very little is known about HIV/SIV infection of the male reproductive tract during the asymptomatic phase of the infection (Miller *et al.*, 1994; Nuovo *et al.*, 1994a; Benjamin *et al.*, 2001). In adults, HIV-1 infection is transmitted predominantly by sexual routes (Gupta *et al.*, 1997). Other reports have indicated the presence of HIV-1 in semen shortly after primary infection and at all stages of the disease (Learmont *et al.*, 1992; Tindall *et al.*, 1992; Gupta *et al.*, 1997; Pilcher *et al.*, 2001). Some model estimates suggest HIV transmission rates to be highest during the acute stage of the infection, when semen is the most infectious and lowest during the chronic phase (Pilcher *et al.*, 2004; Anna Le Tortorec & Roques, 2008).

Though high blood plasma HIV viral load correlates with the risk of HIV sexual transmission (Quinn *et al.*, 2000),the most important compartment that is very crucial in the sexual transmission of the virus is the genital compartment viral loads (Chan, 2005). Male genital tract is a distinct compartment from blood in which HIV-1 can evolve separately in terms of viral phenotype, genotype, infectivity, quasi-species, drug resistant mutations and quantity of RNA (Chan, 2005). This compartment is absolutely crucial because antiretroviral drugs generally penetrate endothelial membranes poorly and therefore do not readily reach therapeutic levels to control viral replication within this compartment (Ho, 1995; Ball *et al.*, 1999; Kashuba *et al.*,

1999; Pomerantz, 2002; Taylor *et al.*, 2003). Semen is, in fact, considered the major vehicle for sexual transmission of the virus, as it contains free and/or cell-associated virions, mostly lymphocytes and macrophages (Borzy *et al.*, 1988; Mermin *et al.*, 1991; Van Voorhis *et al.*, 1991; Vernazza *et al.*, 1994; Hrishikesh *et al.*, 2001; Paranjpe *et al.*, 2002). It is however possible this correlation is mediated through shedding of HIV in genital secretions (Vettore *et al.*, 2006). The first critical step in HIV sexual transmission is the shedding of infectious virus in the genital secretions of an infected person (Delwart *et al.*, 1998). The most robust predictor of HIV sexual transmission within HIV serodiscordant couples is the level of plasma HIV RNA viremia in the infected partner (Quinn *et al.*, 2000). Even though it probably does not reflect direct contact with blood during sex, the amount of HIV RNA and/or DNA in genital secretions correlates broadly with the plasma viral load as shown by some studies (Mostad, 1998; Speck *et al.*, 1999; Hrishikesh *et al.*, 2001). Several studies have demonstrated that the presence of HIV-RNA in the semen secretions were associated with higher blood HIV viral load (Kaul *et al.*, 2008). It therefore means that the testes are a potential target for HIV-1, as AIDS and asymptomatic seropositive patients are known to suffer from serious testicular infections and other abnormalities relating to the testicular functions such as orchitis, oligo- or azoospermia and some germ cell tumors (Sellmeyer & Grunfeld, 1996; Dejucq & Jegou, 2001; Anna Le Tortorec & Roques, 2008); (Zagury *et al.*, 1984; Alexander, 1990).

It is indubitable viral shedding in the male genital tract is a well known phenomenon as different viruses such as HIV-1 and Hepatitis B viruses have been observed in the semen of infected subjects (Tindall *et al.*, 1992; Barroso *et al.*, 2000; Dejucq & Jegou, 2001; Pilcher *et al.*, 2004; Anna Le Tortorec & Roques, 2008).

However, whether HIV infection decreases fertility in males has become an unresolved dispute (Alexander, 1990; Nuovo *et al.*, 1994a). As has been stated earlier, autopsy samples of the male reproductive tract and seminal fluid of HIV-seropositive men with advanced disease revealed marked abnormalities of the seminiferous epithelium, destruction of germinal cells, inflammation, opportunistic infections, and abnormalities of the accessory reproductive organs (da Silva *et al.*, 1990; Pudney & Anderson, 1991; Anderson *et al.*, 1992). In addition, other histological studies have reported loss of testicular germ cells and maturation arrest of spermatozoa during spermatogenesis (Shevchuk *et al.*, 1999)

2.3 HIV infection and Semen Parameters

Although a number of studies have assessed the effects of HIV infection on semen parameters with inconsistent results (Gupta *et al.*, 1997; Tachet *et al.*, 1999), Coombs and colleagues (1998) reported no difference in any parameter. However, Crittenden and colleagues (1992) reported a decrease in the percentage of motile sperm, reduced motility and increased frequency of round cells in infected men. Other studies have also reported decreases in motility, sperm concentration and total sperm count (Dulioust *et al.*, 1998; Muller *et al.*, 1998; Nicopoullos *et al.*, 2004), and ejaculate volume decreases (Muller *et al.*, 1998). Reduced percentages of morphologically normal spermatozoa have also been reported by other investigators (Nicopoullos *et al.*, 2004; Gilling-Smith *et al.*, 2006). Others however, have reported a more significant effect of HIV on semen parameters when compared to controls (Tachet *et al.*, 1999; Bujan *et al.*, 2007c). Finally, some studies have reported fundamental defects resulting from the effect of HIV on semen quality. These reported that the presence of HIV in sperm jeopardizes the fundamental structure and viability of sperm, and hence, semen quality (Dussaix *et al.*, 1993; Baccetti *et al.*, 1994;

Barboza *et al.*, 2004). Studies have shown that, all the semen obtained from HIV positive male patients had very low sperm concentrations and higher fraction of the sperms had abnormal morphologies and marked degenerated nuclear materials as given by their Terminal Uridinedeoxynucleotidyl transferease phosphate Nuclear End Labeling (TUNEL) values: a technique which allowed for immunohistochemical detection of DNA strand breaks at the level of the individual spermatozoa, which was 35.4% for HIV positive semen samples compared with HIV seronegative control of 2.5% (Gandini *et al.*, 2000b; Muciaccia *et al.*, 2007). All together, HIV-1 seropositive subjects produce semen that contains few motile sperm cells, increased immotile and abnormal sperm and numerous non sperm cells (NSC) (Quayle *et al.*, 1997; Muller *et al.*, 1998; Pudney *et al.*, 1999; Debono *et al.*, 2000; Bujan *et al.*, 2007a; Bujan *et al.*, 2007b).

2.4 Effect of Antiretroviral Therapy On Viral Shedding In Semen: Implication On Semen Quality

Potent antiretroviral therapy against HIV infection results in a marked suppression of HIV-RNA concentration in the blood of infected individuals (Vernazza *et al.*, 2000).The effect of the therapy has also been associated with quite significant increases in absolute $CD4^+$count, $CD4^+$ cell function and a reduction in mortality (Egger *et al.*, 1997). Currently, the standard care for the treatment of HIV-1 infection involves combination of antiretroviral drugs which includes the use of Nucleoside Reverse Transcriptase Inhibitors (NRTIs) with either Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) or Protease Inhibitors (PIs) (Carpenter *et al.*, 1998).The drugs which are combined for the treatment of HIV infection are: Zidovudine (ZDV), Lamivudine (3TC), Stavudine (D4T), Efavirenz (EFV), Nevirapine (NVF), Combivir (CBV), and Abacavir (ABC) (WHO, 2008). Antiretroviral therapy has dramatically improved the survival of HIV

infected individuals and is critically needed to save millions of lives (GAIDSC, 2006, 2007).In a substantial fraction of patients receiving triple drug combination, HIV-RNA can no longer be detected in blood, even with the use of highly sensitive Polymerase Chain Reaction (PCR) technology (Persico *et al.*, 2006; Savasi *et al.*, 2007).

In 2008, World Health Organization, (WHO), estimated that 2,015,000 people living with HIV/AIDS were receiving treatment in Low- and Middle-Income Countries (LMIC), representing 28% (24%-34%) of the estimated 7.1 million people in need (WHO, 2008).Antiretroviral therapy (ART), if prescribed and taken appropriately, is associated with dramatic reductions in blood HIV RNA load, often to undetectable levels (Pettengell *et al.*, 2006; Kaul *et al.*, 2008). However, whether potent antiretroviral therapy is associated with a reduction of the infectiousness of treated individuals is not really certain currently (Porco *et al.*, 2004). Some studies have reported a significant reduction of the HIV-RNA concentration in the seminal plasma of patients treated with antiretroviral therapy (Egger *et al.*, 1997; Vernazza *et al.*, 1997b; Shevchuk *et al.*, 1999; Vernazza *et al.*, 2000). However, the number of patients used for these studies were too small to merit any generalization (Byrn *et al.*, 1997; Delwart *et al.*, 1998). In fact, there is actually a paucity of longitudinal study data on semen quality before and after ART. However, according to one of the few studies, semen parameters were normal according to WHO criteria and remained stable after ART mono-therapy in 5 HIV-1 infected men(van Leeuwen *et al.*, 2008). Another study also reported improved semen quality in 20 men after 4 to 12 weeks of ART (Barroso *et al.*, 2000; Ghosn *et al.*, 2008).

There is very strong evidence supporting the compartmentalization of body cavities with respect to HIV-1 shedding between semen and blood (Vernazza *et al.*, 1997a; Byrn & Kiessling, 1998; Coombs *et al.*, 1998; Ghosn *et al.*, 2004; Lowe *et al.*, 2004).

Buttressing these findings, Pettengell and colleagues (2006) reported significant genital HIV RNA shedding in over 20% of patients who were on ART despite undetectable HIV RNA load in blood. On the other hand, another study reported substantial reduction of HIV seminal viral load when ART was initiated (Barroso *et al.*, 2000). Another study reported a higher proportion of patients (74%) who had undetectable HIV RNA in semen and normal semen quality compared with 25% who showed detectable HIV RNA levels in their semen after ART, with higher number of spermatozoa with reduced motility which might suggest that, the load of HIV virus in the reproductive compartment influences the quality of the semen produced (Carpenter *et al.*, 1998). In addition, other studies have reported a statistically significant reduction in seminal volume, which is a parameter of semen quality, in patients with higher HIV viral load when they received a double nucleoside regimen (Carpenter *et al.*, 1998; Barroso *et al.*, 2000).

2.5 Absolute $CD4^+$ Count Dynamics: Implication on Semen Quality

The absolute numbers of $CD4^+$ lymphocytes and their percentage values within the total lymphocyte population ($CD4^+$T%/ lymphocytes) are the two tests which have retained clinical significance in HIV infection management in clinical diagnostic settings for the assessment of the degree of immune deterioration and speed of progression towards AIDS (Farzadegan *et al.*, 1989; Mandell *et al.*, 1995; Dorrucci *et al.*, 2007). To enter a target cell, HIV-1 requires a $CD4^+$ receptor (Maddon *et al.*, 1986). $CD4^+$, a cluster of differentiation 4, is a glycoprotein that is found primarily on the surface of helper T cells. $CD4^+$ is one of the major receptors needed by HIV-1 to infect humans in addition to CXCR4, CCR3 and CCR5 co-receptors and therefore, cells bearing these receptors make up the host within the human host for the virus (Quayle *et al.*, 1997; Berger *et al.*, 1999; Murakami *et al.*, 1999; Pudney *et al.*, 1999).

Some studies have correlated clinical parameters of CD4 T-cell count with testicular histologic findings to predict germ cell loss after death of the patients (Shevchuk *et al.*, 1999). The absolute $CD4^+$ T-cell count has been the most clinically useful measure of cellular immunocompetence in HIV/AIDS patients and has therefore been used in staging of HIV/AIDS disease probably exclusively in developing nations (Turner *et al.*, 1994). Absolute$CD4^+$counts are important in developing country setting to monitor disease progression of HIV / AIDS. Currently, it serves as an indicator for when prophylactic treatment of ART should be commenced (Nowakowski *et al.*, 1992; Blower *et al.*, 1995; Kaplan & Heimer, 1995). There is also global evidence that the overall incidence of opportunistic diseases increase with the degree of immunosuppression resulting from HIV disease progression which is given by the absolute $CD4^+$ count of the patient (Yazdanpanah *et al.*, 2001; Salami *et al.*, 2006). Hence, it is logical to evaluate the relationship between germ cell loss by semen quality analysis and absolute $CD4^+$ T-cell population dynamics over time. This is because, the degree of germ cell loss may be related to absolute CD4 T-cell count and/ or patient age (Shevchuk *et al.*, 1999).These clinical parameters may be refined to aid clinicians to accurately gauge testicular histology of HIV positive male patients, and by extension, intra-testicular viral burden and potential sexual infectivity (Shevchuk *et al.*, 1999).

Correlations between blood absolute $CD4^+$ counts or viral loads and sperm motilities or sperm counts have been reported in some studies (Gupta *et al.*, 1997; Pilcher *et al.*, 2004; Bujan *et al.*, 2007c). The dynamics of absolute $CD4^+$ counts have been reported to have a relation with semen volume, sperm concentration, motility and morphology of infected men (Crittenden *et al.*, 1992; Politch *et al.*, 1994; Dulioust *et al.*, 1998). If clinicians could predict the degree of germ cell preservation i.e., testicular histologic findings, this information might help to possibly predict the

HIV testicular load, ejaculate load, and infective potential somehow when the actual relationship between semen quality and seminal viral loads have been well established (da Silva *et al.*, 1990).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Site

The data on the HIV-infected men were taken at the VCT-HIV/ AIDS Clinic of the Kumasi South Regional Hospital, Ashanti region of Ghana: this hospital is about sixty minutes drive from Kwame Nkrumah University and Science and Technology. This hospital provides services to 56 communities which contain roughly 400,000 people. They currently run clinical (pharmacy, surgical, general, maternity, x-ray, regional reference laboratory, ultra-sound, dental and HIV Unit) as well as non-clinical (general paramedics) services. The hospital operates 24 hours a day and its staff work on a 3 shift schedule. The HIV Unit is housed in a six roomed facility with at least three resident specialists who attend to patients each day. It carries out counseling as well as treatment of HIV-infected persons in Ashanti and Brong-Ahafo Region of Ghana to reduce the pressure on Komfo-Anokye Teaching Hospital at Kumasi, Ashanti Region, Ghana.

The controls were recruited at the Komfo-Anokye Teaching Hospital. The selection of the study participants was done as follows: a convenient number of seropositive men who reported at the HIV-Clinic each day at the time the study was going on was screened for presence or absence of sexual transmitted infection aside HIV and the level of AIDS symptoms present in the individual. They were also screened for other factors which were possible confounders of the study such as whether or not one has been vasectomized, whether or not they smoke, drink as well as permitting psychological state.

A hundred of the seropositive men attending the hospital out-patient VCT-HIV clinic were recruited for the convenient study. The participants filled Consent forms that were approved by the Kwame Nkrumah University of Science and Technology Ethics Committee. Four groups of twenty-five were the total for the seropositive men. This sample size was chosen because of the nature of the study. It is highly difficult to obtain higher numbers for such a study in Ghana. After recruitment, each participant was registered with a unique identification code that corresponded with the code on the containers for both semen and blood samples. A hundred of the HIV seropositive male participants/ clients took part in the study in addition to 29 seronegative male subjects in the Kumasi Metropolis, recruited to serve as the control for the study.

3.2 Categories of HIV-Infected Participants

The HIV-infected male clients selected were either slightly symptomatic (HIV-positive men who showed at most two symptoms of AIDS) or asymptomatic HIV-infected. It was observed that most of the seropositive men with their absolute $CD4^+$ less than 250 cells/ ml exhibited more opportunistic infection which actually was going to be difficult for them to produce the semen by masturbation (semen obtained by masturbation is complete and avoids the loss of first sperm rich ejaculate that is normally lost when other method such as coitus interruptus is used). Even though, most of the seropositive men with absolute $CD4^+$ count equal to or above 250 cells/ µl of blood also exhibited numerous opportunistic infections, the HIV-infected men selected were in a better health conditions that permitted them to be enrolled in the study. The seropositive men selected were grouped into two categories on the basis of whether the client had already started Antiretroviral therapy (ART) or not. Furthermore, clients who were not on ART were also

divided into two groups: those who were asymptomatic and those who were partially symptomatic. In addition, seropositive participants who were on ART were also subdivided into two broad categories: clients who had been on ART for at least one (1) year and those who had been on ART for less than one (1) year.

Summary of the criteria for selection and the number of clients and controls that were recruited for the study was as follows:

1. Group 1: Made up of 25 HIV-infected men who had not started ART but exhibited some degree of opportunistic infection (symptomatic) but strong enough to partake in the study.
2. Group 2: Made up of 25 HIV-infected men confirmed by the Kumasi South Regional Hospital HIV Clinic, but had not started ART and were asymptomatic of AIDS.
3. Group 3: Made up of 25 HIV-infected men who have been on ART for less than one (1) year.
4. Group 4: Made up of 25 HIV-infected men who have been on ART for at least one (1) year
5. Group 5: Made up of 29 HIV seronegative men which constituted the control.

All male patients who had any sexual transmitted co-infection (STCI) with HIV or had been administered with any drug apart from ART that might confound the results were excluded from the study. This was ensured because infection of the reproductive organ causes serious lymphocyte infiltration into the reproductive organs and their accessory organs. This would definitely affect the semen quality as a result of localized inflammation which directly affects spermatogenesis and other processes involved in semen production. Finally, all male clients who

had undergone vasectomy were excluded from the study. This was because those patients could not produce complete semen (Muller *et al.*, 1998).

3.3 Instruction to the Participants And Semen Sample Collection Procedure

The subjects were given clear, written and oral instructions concerning the collection and where possible, transportation of the semen sample to the VCT-HIV Clinic at the Kumasi South Regional Hospital.

The samples were collected after a minimum of 72 hours of sexual abstinence to make enough room for a complete cycle of spermatogenesis to take place to reduce the variability of the results (WHO, 1999). The number of days of sexual abstinence was maintained constant whenever possible.

Patients were also informed of the effects of smoking, alcohol, aphrodisiacs and other stimulants such as coffee could have on the spermogramme or semen quality (Zhang *et al.*, 2000; Martini *et al.*, 2004). The clients' identification codes were $G_1$01-$G_1$25, for the group with slight symptoms of HIV-infection, G_2 01-G_2 25, for the group with asymptomatic HIV-infected men, G_3 01-G_3 25, for the HIV-infected men who have been on ART for less than 1 year, and G_4 01-G_4 25, for the HIV-infected men who have been on ART for at least a year and G_5 01-G_5 29, which made up of the control. Also, 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,. Two samples were collected from each participant for evaluation. The interval between the two collections was kept at exactly 7 days (WHO, 1999). It was emphasized to the participants that the semen samples needed to be complete (WHO, 1999).

The samples were obtained by masturbation. Participants ejaculated into clean, wide-mouthed sterile plastic containers obtained from the Microbiology Laboratory of Komfo-Anokye Teaching Hospital, Kumasi, Ghana.

The samples were either produced in a private room at the hospital or at home and brought to the laboratory within an hour. The samples that were produced at home were received in a private room at the HIV Clinic and sent to the laboratory in the same facility within 5 minutes.

The samples that were produced at home and transported to the lab were protected from extremes of temperature during transportation process by keeping the samples close to the body during transportation (WHO, 1999).

All clients/ subjects 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.

All the samples presented were registered with the unique label with the sample form as well as the interview guides. When a sample was collected, the time for ejaculation was marked on the container, as well as written in the protocol. The samples were placed without delay on a moving tray in an orbital mixer (37^{o}C) to keep the samples at the normal body temperature and to also thoroughly mix the semen sample.

The samples were analyzed within 60 minutes after semen liquefaction. For each subject, ejaculate volume, sperm concentration, total sperm count, motility and morphology were evaluated according to WHO guidelines on semen examination (WHO, 1999).

3.4 Collection of Peripheral Blood Sample

The blood samples were taken at the same time the participant presented their semen samples for the study. New sterile 5ml syringes with needles were used to take 3ml peripheral whole blood sample from each participant into well labeled sterile test tubes (vacutainers) with an anticoagulant (Heparin), obtained from the Public Health Laboratory at the Kumasi South Regional Hospital. The blood was mixed thoroughly and analyzed within five hours of collection.

3.5 Semen Examination

The reagents and solutions used for the semen analysis were saturated NH_4Cl solution (250 g/l), Na_2EDTA, 50 g/l in phosphate buffer, Ortho-toluidine (0.25 mg/ml), 30% v/V Hydrogen peroxide (H_2O_2), Distilled water, 100% v/V Eosin Stock solution, Phosphate buffer, 100% v/V Ethanol, Crystal violet-0.5% w/V of solution prepared, Potassium iodide, Iodine crystal, Acetone (decolourizer), Safranin (counter stain), 5% $NaHCO_3$ (serves as diluents for semen).

3.5.1 Macroscopic Examination of Semen

3.5.1.1 Liquefaction

Each sample was examined for its duration of liquefaction, i.e., liquefaction time. Normal semen sample would liquefy within 60 minutes at room temperature, although usually this occurs within fifteen minutes (WHO, 1999). The liquefaction time was determined by placing the sample on a moving tray in an orbital mixer (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 shaker and swirled for 20 seconds to ensure that the samples were well mixed. All the samples produced outside the laboratory were warmed up to a temperature of 37^{o}C in the incubator for 10 minutes before examination. Continuous mixing of the samples wasensured to reduce the errors that could have been created when determining the sperm concentration (WHO, 1999).

3.5.1.2 Appearance

The seminal appearances were determined immediately after liquefaction. This was done by first inspection of the colour of the samples 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 observation of the semen samples were grouped under two main categories: Normal and Abnormal. The abnormally coloured samples were also subcategorized into Blood-stained and Non-blood stained (WHO, 1999).

3.5.1.3 Volume of Semen

Seminal volume was determined for each sample by pouring the liquefied specimen into a 10.0 ml graduated glass measuring cylinder (Pyrex). The volumes were then observed and recorded.

3.5.1.4 Viscosity of Semen

The viscosity of each of the specimen was determined as follows:

A clean dried glass rod was introduced into each of the samples in their original labeled specimen-container after liquefaction and withdrawn from the specimens. 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 which showed threads that were less than 2 cm were classified under low viscous semen (WHO, 1999).

All samples with very high viscosity were noted on their respective sample record form. High viscosity interferes with determination of sperm motility and concentration (WHO, 1999). 1 to 2 ml of phosphate-buffered saline (PBS) 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.

3.5.1.6 Seminal pH

pH paper strips that were obtained from AccuBioTech Company Limited, China, were used for the determination of the pH of the semen. The seminal pH was determined for each of the specimen as followed:

The pH was determined within an hour of ejaculation, by pipetting a 5µl of each of the semen after liquefaction onto a pH paper. The colour of the impregnated zone became uniform after 30 seconds and then it was matched with the calibration strip and the pH determined for each seminal sample.

3.6 Sperm Motility And Vitality

Preparation of a wet mount for sperm motility analysis was done by pipetting 15 µl of a well mixed liquefied semen using a positive displacement pipette onto a clean dry microscope slide. The same volume of eosin solution was measured and added to the semen on the slide and mixed thoroughly using a microstirrer. Finally, the slide was then covered with a clean cover-slip (22 mm x 22 mm). This gave a preparation depth of approximately 20µm. Examination of the wet preparation began as soon as the 'flow' in the preparation ceased.

3.6.1 Sperm Motility

Sperm motility was the first microscopic examinations that was carried out on the semen samples to prevent other unfavourable conditions that might affect sperm motility as a result of sperm metabolism be created (WHO, 1999). This assessment began immediately after the flow ceased to avoid temperature drop or dehydration of the preparation (WHO, 1999). 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, and then vitality (WHO, 1999; Franken & Kruger, 2006). The total sperms observed were scored as percentages, ie, the sperm motility as well as sperm vitality 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, C, D according to whether it showed;

A. Rapid progressive motility (that is, greater or equal to 25µm/s at 37 ^{o}C and greater or

equal to 25µm/s at 20^{o}C. 25µm is approximately equal to 5 heads lengths and half a tail length)

B. Slow or sluggish progressive motility (ie, from 5 µm/s to 25 µm/s at 37 ^{o}C).

C. Non progressive motility(less than 5 µm/s)

D. Immotility

All spermatozoa with grade A and B motility were estimated first to prevent them from losing their energy and/or ability with time. Subsequently, spermatozoa with non-progressive motility (grade C) and immotile spermatozoa (grade D) were counted in the same area. The counting was done by estimating the number of each of the category per field and recording in percentages such as A%, B%, C% and D% (Aitken *et al.*, 1982; WHO, 1999; Franken & Kruger, 2006).

3.6.2 Estimation of Sperm Vitality by Dye Exclusion

Sperm vitality is reflected in the proportion of spermatozoa that are alive. This was determined by the Dye-exclusion method based on the principle that dead cells with damaged plasma membrane take up certain stains. This procedure was performed simultaneously with the sperm motility assessment on all the samples investigated. After the sperm motility assessment, viable sperm, which remained unstained were recorded in percentage. Also, non-viable sperms which became stained red were also noted and recorded (WHO, 1999).

3.6.3 Sperm Concentration

The concentration of the sperm was determined using the haemocytometer method on two separate preparations of the semen samples, one for each side of the counting chamber. Each

semen sample was diluted based on the preliminary observation done when estimating the sperm motility. A dilution factor was selected for a semen sample based on the conjectured estimate of the sperm concentration of the undiluted semen. In view of that, the dilution factor used was not constant but depended on the initial sperm concentration (WHO, 1999). If the wet preparation observed for the determination of the motility gave estimation of N number of spermatozoa per field or part of a field under ×400 magnification, the field of view varied in diameter, usually in the range between 250 and 400µm corresponding to 1 to 2.5 nl with a 20 µm depth of sample. With this field of view estimated by scanning the slide and estimating the number of spermatozoa per field equivalent to the diameter of field, a field equivalent to 1 nl gives an approximate sperm concentration in 10^6 cell/ ml (WHO, 1999). This estimation was then used to decide the dilution for determining the sperm concentration. Generally, the following dilutions were used: <15 spermatozoa, dilution 1:5; 15-40 spermatozoa, dilution 1: 10; 40-200 spermatozoa, dilution 1:20, > 200 spermatozoa, dilution 1: 50 (WHO, 1999). The diluent used was the sodium bicarbonate-formalin solution. A counting chamber of the improved Neubauer heamocytometer was made set by wetting either side of the wells by using drop of distilled water on the finger at the sides of the counting chamber. A cover slip was then secured gently on the chamber by pressing firmly onto the chambers so that iridescence (Newton's rings) was observed between the two glass surfaces. A displacement micropipette was used to transfer 10 µl of the thoroughly mixed diluted specimen from each duplicated dilution to each of the counting chambers of the haemocytometer. This was done by carefully touching the edge of the cover glass with the pipette tip and allowing each chamber to be filled by capillary action. The haemocytometer was allowed to stand for about five minutes in a humid chamber to prevent drying out. The cells sedimented after that period and counting was done afterwards.

The cells were counted as follows:

The central square of the grid in an improved Neubauer haemocytometer contains 25 large squares, each contained 16 smaller squares. Spermatozoa which lay on the line dividing two adjacent squares were counted only if they were on the upper or the left side of the square being assessed. For samples which contained more than 40 spermatozoa per large square, spermatozoa in five large squares were assessed. For samples which contained 10 to 40 spermatozoa per large square, ten large squares were assessed. For each sample, two fresh duplicate dilutions of the semen were prepared, sperm counted and recorded. The number of spermatozoa in 1 ml of fluid was calculated by dividing the average sperm count by the appropriate conversion factor on the table below. For example, if the average count was 100 on a 1:20 dilution, and ten large squares were counted, then the concentration would be 100 divided by 2, which is 50 ($\times 10^6$) sperms/ ml (WHO, 1999).

Spermatozoa per 400× field	Dilution (Semen + dilution)	Conversion Factors Number of large squares counted		
		25	**10**	5
<15	1: 5 (1 + 4)	20	8	4
15- 40	1: 10 (1+ 9)	10	4	2
40- 200	1: 20 (1 + 19)	5	**2**	1
> 200	1 : 50 (1+ 50)	2	0.8	0.4

3.6.4 Sperm Morphology Determination

3.6.4.1 Preparation of smear

Two smears were prepared for duplicate assessment of each sample. The slides were thoroughly cleaned, washed in 70% ethanol and air-dried. 5µl of semen was applied to each slide. Another slide faced down, was placed on top so that the semen spreads between them. The two slides were gently pulled apart to make two smears simultaneously. These slides were fixed with 95% v/V ethanol for 10 minutes after they were air-dried (WHO, 1999). The smear was washed with sodium bicarbonate-formalin solution to remove any mucus which may be present and afterwards rinsed several times with changes of water. The smears were then flooded with crystal violet solution and allowed to stain for 2 minutes and then the stain was washed off with water. Lugol's solution (mordant) was added to the smear for 1 minute and washed with distilled water. The smear was then counterstained with safranin (0.1%) solution for 2 minutes and washed with distilled water, drained and air-dried.

3.6.5 Performing Sperm Morphology Count

The ×40 objective was used to observe the slide and oil-immersion bright-field objective was used to corroborate the morphology of the spermatozoa and the other cellular elements in the smear. The slides were then examined systematically from one microscopic field to another and 100 spermatozoa were assessed and the percentages of normal and abnormal spermatozoa were recorded. The following abnormalities were all grouped under abnormal sperms

Head

- Greatly increased or decreased in size.
- Abnormal shape and tapering head (pyriform)
- Acrosomal cap absent or abnormally large.
- Two heads.

Middle piece

- Absent or markedly increased in size.
- Appears divided (bifurcated).
- Angled where it meets tail.

Tail

- Absent or markedly reduced in length.
- Double tail.
- Bent or coiled tail.
- Defective morphology included irregular shaped heads, neck, mid-piece and tail (WHO, 1999).

3.6.6 Estimation of Cellular Elements Other than Spermatozoa

The ejaculates invariably contain cells other than spermatozoa collectively called round cells (WHO, 1999). These include epithelial cells from the genitourinary tract, prostate cells, spermatogenic cells and leukocytes. A solution was prepared by combining 1ml of saturated

NH_4Cl solution, 1ml of 5% of Na_2EDTA solution, 9ml of orthotoluidine solution and 1 drop of H_2O_2. This solution was mixed thoroughly before used (WHO, 1999). The procedure for the determination of the leukocytes concentration consisted of mixing 0.1ml of semen with 0.9ml of the working solution to achieve a total volume of 1 ml. This mixture was shaken for 2 minutes. It was then allowed to stand for 30 minutes at room temperature and mixed again by shaking before transferring onto a haemocytometer chamber for leukocytes and the immature sperm cells to be estimated. Leukocytes are peroxidase-positive cells and therefore stained brown while the immature sperm cells remained unstained. Both the leukocytes and the Peroxidase-negative immature sperm cells were counted in the haemocytometer chamber in the same way the sperm were counted and recorded in percentages in relation to other cellular elements (WHO, 1999).

3.7 Blood Absolute $CD4^+$ Count

3.7.1 Background

Management of HIV patients in Low and Middle Income Nations has widely relied on the absolute $CD4^+$ T lymphocyte count in the assessment of disease progression and prognosis (CDC, 1987; Blatt *et al.*, 1993; Turner *et al.*, 1994). Absolute $CD4^+$ count was equally employed in this study as a yardstick for measuring the disease progression of the HIV-infected men and all the semen parameters were correlated with their respective absolute $CD4^+$ count. Due to the strict criteria employed in the selection process of the HIV-infected men as participants, no seropositive male client whose $CD4^+$ count was below 250cells/µl was included for the reason that those men showed much symptomatology of AIDS. Some seropositive men who fell within the criteria but showed much AIDS symptoms were also excluded from the study.

3.7.2 Materials and Reagents

Material necessary for collection of peripheral blood, 150 pieces of vacutainers (12 × 75 mm), an automatic positive displacement pipettes with disposable tips, BD FACSCount controls [Isotype control antibody (mouse IgG1 PE)], FACSCount Coring Station, FACSCount Workstation, 5ml syringes and needles, a piece of tourniquet for stopping blood flow, 75% (v/V) alcohol for disinfection, CD4 PE [mouse monoclonal antibody against human $CD4^+$ antigen (clone MEM-241) labeled with R-Phycoerythrin (PE)].

3.7.3 Blood Absolute $CD4^+$ Count Determination

The FACSCount Counter was first calibrated before used for the determination of the absolute $CD4^+$ cell count of the blood samples as followed:

The machine was first switched on and allowed 15 minutes to warm up. It was then made to absorb distilled water to humidify its system for about thirty minutes followed by the introduction of the FAS Clean and then FAS Rinse. The Counter at that stage was ready for calibration. Three already formulated controls known as BD FACSCount® controls with already pre-determined absolute $CD4^+$ counts were used in the calibration of the counter. The three controls were known as Low, Medium and High tubes, with absolute $CD4^+$ as 22 cells/µl, 239 cells/ µl and 944 cells/ µl of blood respectively.

First, the Low control cork was opened with the aid of the FACSCount Coring Station. It was then put on the specimen holder of the counter and the calibration button pressed for the counter to aspirate the reagent of the Low control. The corresponding absolute $CD4^+$ cells/µL was then keyed in and the counter allowed to initialize and store the information given to it into its memory. This stage took about fifteen minutes. The result was then printed out. The same

procedure was used for the Medium and High controls without any intermediate cleaning and rinsing. After the calibration, a complete cleaning and rinsing was then performed with the FAS Clean and FAS Rinse respectively.

After calibrating the FACScount counter, CD4 PE [mouse monoclonal antibody against human $CD4^+$ antigen (clone MEM-241), labeled with R-Phycoerythrin (PE)] in their small plastic bottles which were tightly corked were first vortexed in inverted state for 5 seconds and upright also for 5 seconds. The CD4 PE was then opened with the Coring Station. The blood sample from a client was also vortexed to ensure uniform distribution of the blood cells and the plasma. 50µL of the blood was measured with the aid of an electronic positive displacement pipette into the CD4 PE reagent. The CD4 PE plus the blood was then capped and vortexed upright for 5 seconds. The solutions was then incubated at room temperature in the dark for 60 minutes by putting the corked tube containing the solution into a FACSCount Workstation® which is one of the BD instruments which works in conjunction with the FACSCount counter. The FACSCount Workstation keeps the temperature of the solution at 25^{o}C and prevents the effect of light on the reacting solution. After 1 hour, the solution was vortexed upright and 50µl of FACSCount System Fixative solution was then added to the solution to form the final solution. The final solution was then vortexed and fed into the FACSCount counter. After 15 minutes, the counter printed the result of the absolute $CD4^+$ count of the sample of blood that was used.

3.8 Statistical Analysis

Non-parametric methods were used for all comparisons of the semen characteristics. Distributions in the matched HIV-infected groups and control men were compared by t-test and the mean values were compared by the Fisher's least significant difference (LSD). Correlation was estimated by the Durbin-Watson (DW) statistic test. Absolute $CD4^+$ cell count and each semen parameter were compared and the association between this indicator and semen characteristics were verified. The chosen level of significance was $P \leq 0.05$. Data were analyzed using Portable Stat Graphics Centurion 15.2.11.0.

CHAPTER FOUR

4.0 RESULTS

The semen samples were taken from 129 participants that comprised of 100 HIV infected men and 29 uninfected men. 23.26% (30/129) of the men were employed in the formal sector whiles 68.22% (88/129) of the men were employed in the informal sector of the economy. 8.52% (11/129) were unemployed (Fig.1). 61.45% of the infected participants were married whiles 10.45% of the men were single. However, about 28% of the participants were divorced, widowed or separated from their spouse(s) (Fig. 2). 66.67% of the HIV-infected participants had basic education, 14.49% had secondary, 7.25% had tertiary and 11.59% had none (Fig. 3). 57.14% of the infected participants also had infected partner(s), 14.29% had non-infected partner(s) and 28.57% didn't know the status of partner(s) (Fig. 4). 63.24% of the infected participants had a high knowledge about HIV transmission and prevention, 23.53% had moderate knowledge, 10.29% had low knowledge and 2.94 had very low knowledge about HIV transmission and prevention (Fig. 5). 43.48% used herbal medication post HIV-infection and 56.52% did not use herbal medication (Fig. 6). 61.9% of the infected participants are sexually active and 38.1% were not (Fig. 7). 70.83% of the sexually active infected men use condom and 28.1% do not (Fig. 8). 67.16% of the infected men desired to have offspring(s) but 32.84% didn't (Fig. 9). 40% of the HIV-infected men were diagnosed less than a year before their recruitment for the study and the remaining 60% of the seropositive men were diagnosed a year or more before partaking in the study (Fig. 10). For those patients who were on ART, 41%, 18%, 22% and 13% were taking d4T/3TC/NVP, d4T/3TC/EFV, AZT/3TC/NVP and AZT/3TC/EFV respectively (Fig. 11). 88.24% of the men on ART were convinced about ART's therapeutic ability but 1.96% were not convinced. 9.8 could not ascertain the therapeutic power of ART (Fig. 12). 40.58% of the HIV-

infected men showed reduction in the volume of their testes whiles 59.42% did not show any reduction in testicular volume (Fig. 13). 24.56% of the HIV-infected participants who were on ART responded yes to a question about reduction in testicular volume after initiation of ART whiles 71.93 responded no to testicular volume whiles 3.51 responded that they don't know (Fig. 14). Semen parameters in the whole population of HIV-infected men and control men matched by their absolute $CD4^+$ count and duration of ART are presented in Table 1.

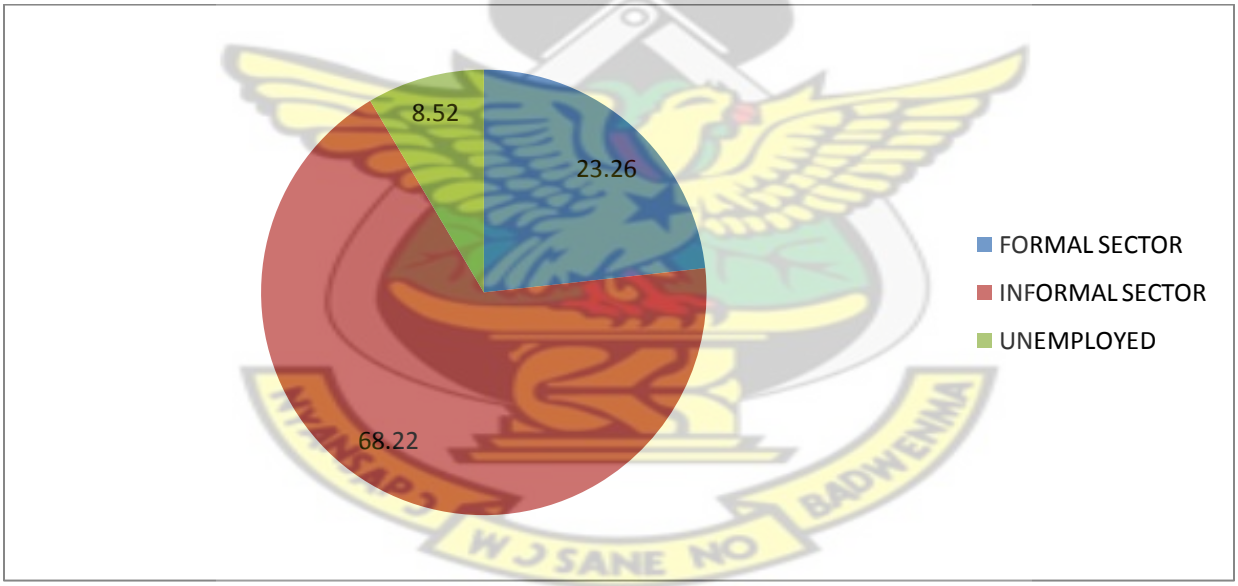

Fig. 1: A chart showing the distribution of the employment status of the HIV-infected participants.

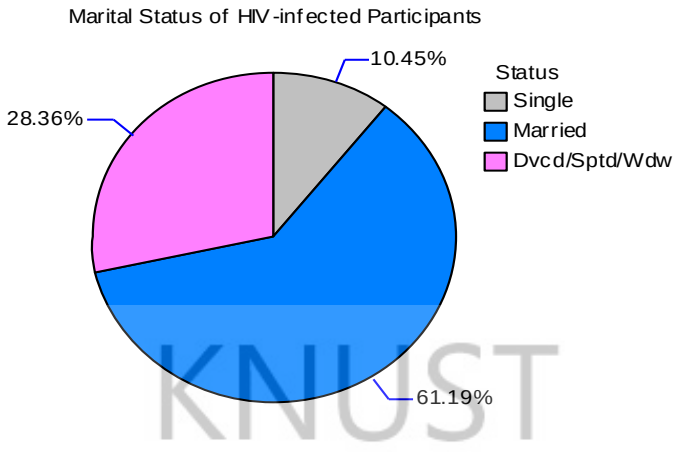

Fig.2: A chart showing the distribution of the marital status of the HIV-infected participants.

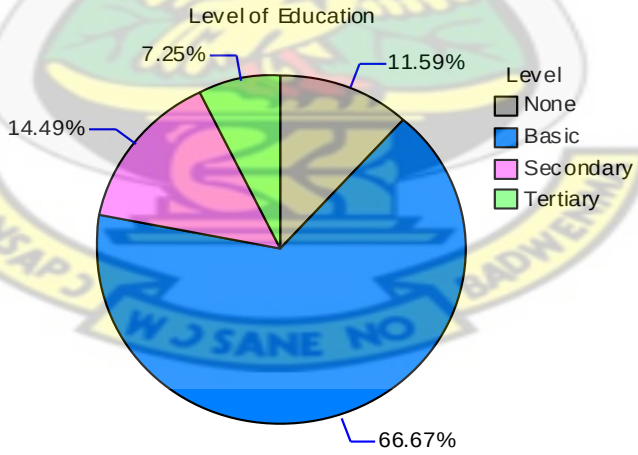

Fig. 3: A chart showing the distribution of the level of education of the HIV-infected participants.

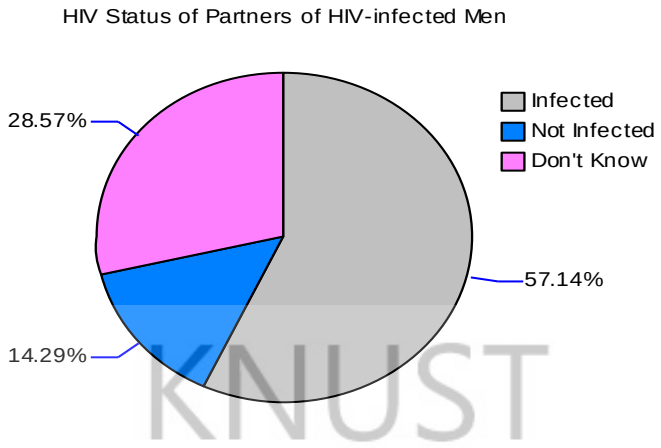

Fig. 4: A chart showing the distribution of HIV status of the partner(s) of the HIV-infected participants.

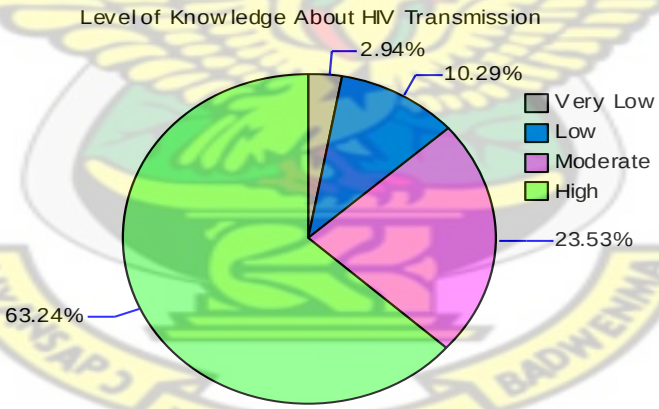

Fig. 5: A chart showing the distribution of the level of knowledge about HIV Transmission of the HIV-infected participants.

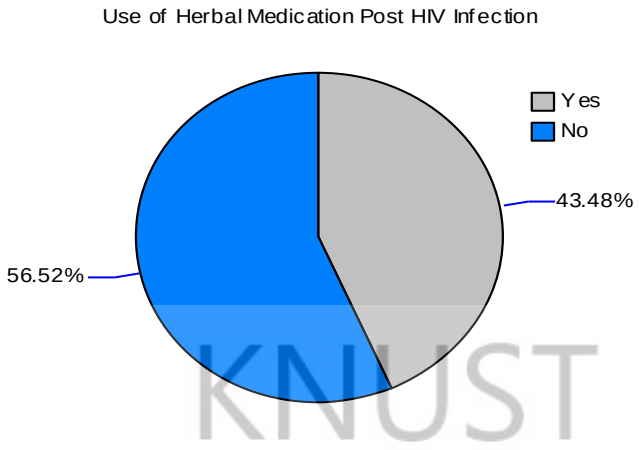

Fig. 6: A chart showing the distribution of the use of herbal medication post HIV infection by the HIV-infected participants.

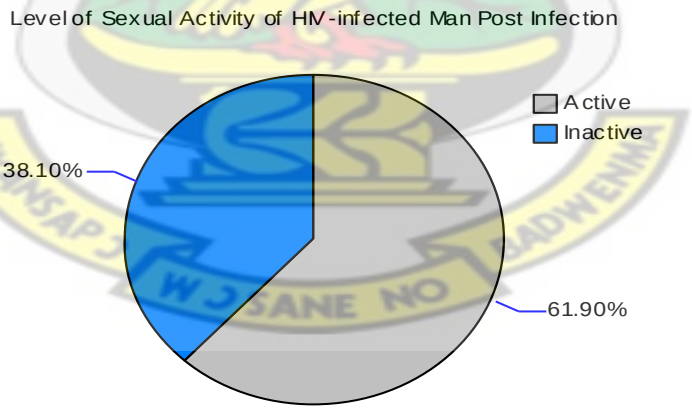

Fig.7: A chart showing the distribution of the level sexual activity by the HIV-infected participants post infection.

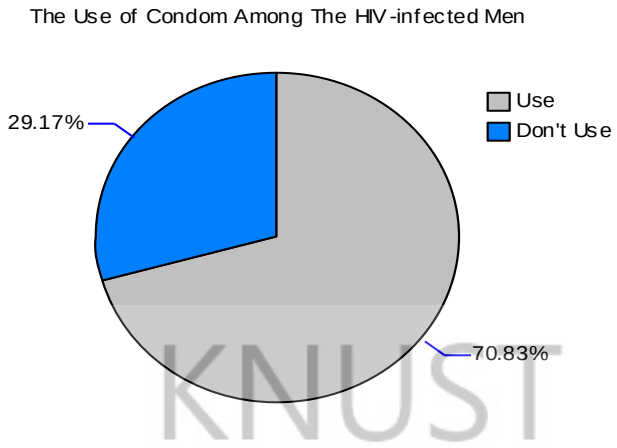

Fig. 8: A chart showing the distribution of the use of condom by the HIV-infected participants post infection.

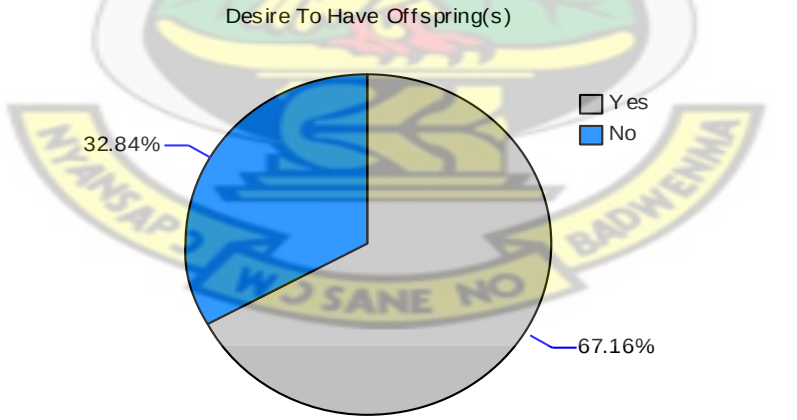

Fig. 9: A chart showing the distribution of the desire for offspring(s) by the HIV-infected participants.

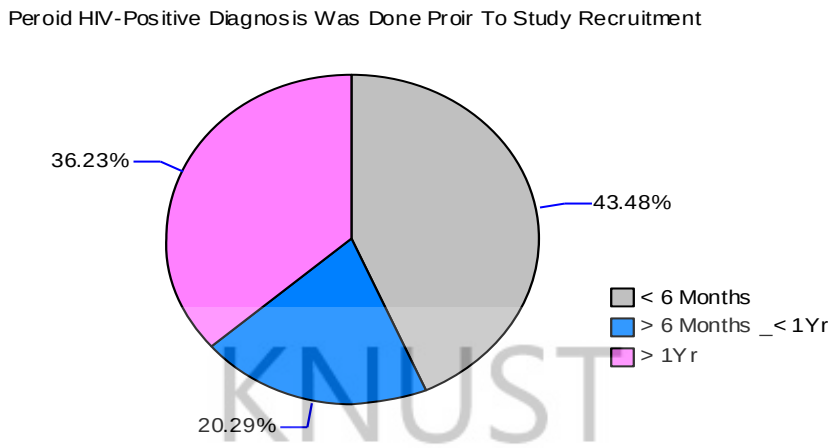

Fig. 10: A chart showing the distribution of the level of the period of HIV diagnosis before recruitment for the study.

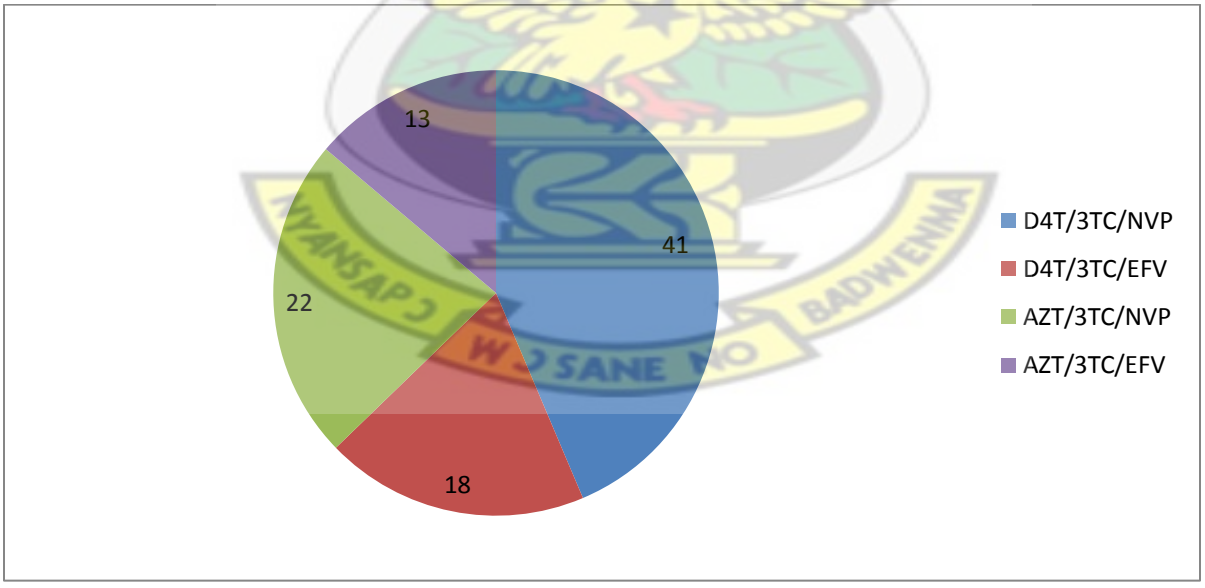

Fig. 11: A chart showing the distribution of the various ART combinations that were being administered to the HIV-infected participants who were on ART.

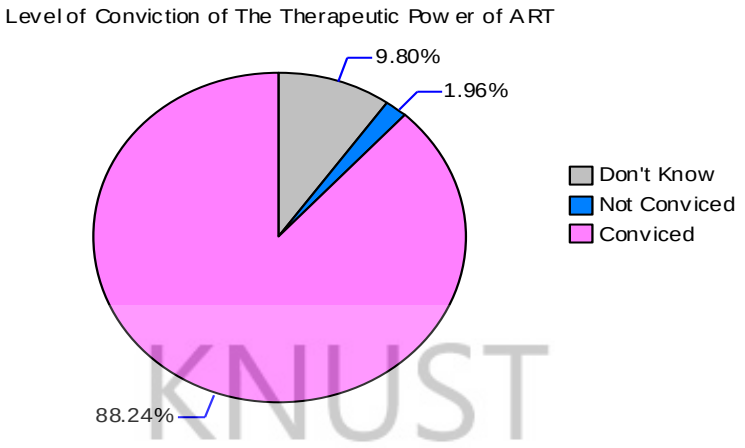

Fig.12: A chart showing the distribution of the level of conviction of the therapeutic activity of ART shown by the HIV-infected participants.

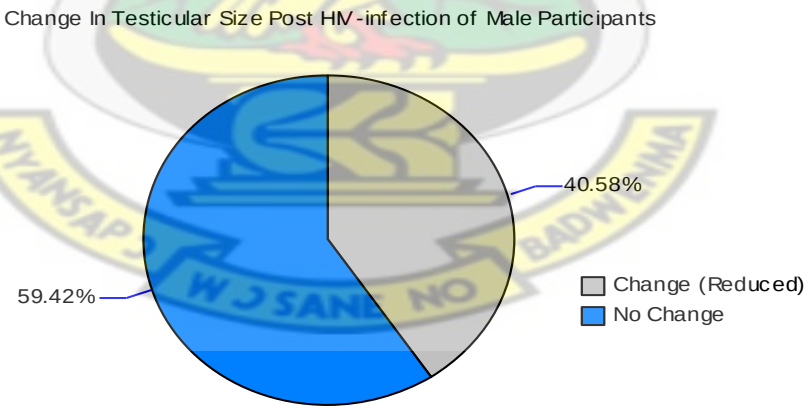

Fig. 13: A chart showing the distribution of the effect of HIV-infection on testicular volume of the HIV-infected participants.

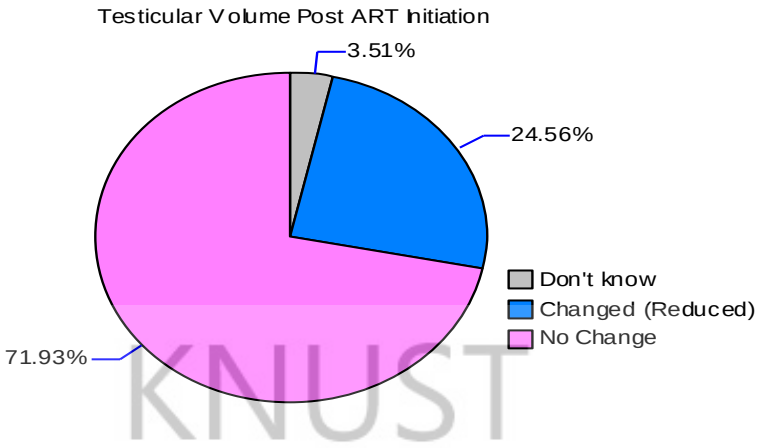

Fig. 14: A chart showing the distribution of the effect of ART on testicular volume of the HIV-infected participants.

Tab. 1: Semen Parameters in HIV-Infected and Control Groups

	Groups					*p*-value
Parameter	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5(Control)**	
Age (Years)	41.0±1.4	37.8±1.4	41.5±1.4	42.1±1.4	38.3±1.3	>0.05
Abstinence Period (Days)	26.9±1.7^{c}	12.6±1.7^{b}	9.4±1.7^{a,b}	12.6±1.7^{b}	7.9±1.6^{a}	<0.05
Volume (ml)	1.6±0.2^{b}	1.6±0.2^{b}	1.7±0.2^{b}	1.7±0.2^{b}	2.8±0.2^{a}	<0.05
pH	8.2±0.1^{b}	8.1±0.1^{b}	8.1±0.1^{b}	8.1±0.1^{b}	7.1±0.1^{a}	<0.05
Rapid Progressive Motile (%)	3.6 ±3.6^{a}	10.7±3.6^{b}	7.2±3.6^{b}	14.6±3.6^{b}	42.4±3.3^{c}	<0.05
Slow Progressive Motile (%)	11.0±3.4	16.8±3.4	16.8±3.4	21.8±3.4	17.0±3.1	>0.05
Non-Progressive Motile (%)	19.4±3.3	28.3±3.3	30.6±3.3	29.7±3.3	27.4±3.0	>0.05
Immotile (%)	47.1±5.4^{c}	40.6±5.4^{bc}	42.9±5.4^{bc}	31.4±5.4^{b}	13.2±5.0^{a}	<0.05
Sperm concentration ($\times 10^{6}$)	16.0±6.3^{b}	33.3±6.3^{c}	29.1±6.3^{c}	35.7±6.3^{c}	68.1±5.8^{a}	<0.05
Total sperm count ($\times 10^{6}$)	21.4±21.8^{c}	56.2±21.8^{b}	54.7±21.8^{b}	63.2±21.8^{b}	207.4±20.2^{a}	<0.05
Immature Germ Cell (%)	41.8±2.2^{c}	20.4±2.2^{b}	24.6±2.2^{b}	18.2±2.2^{b}	1.6±2.1^{a}	<0.05
White Blood Cells (%)	12.8±1.1^{b}	5.9±1.1^{a}	10.7±1.1^{b}	3.9±1.1^{a}	4.1±1.1^{a}	<0.05
Red Blood cells (%)	13.9±2.0^{c}	6.5±2.0^{b}	5.4±2.1^{b}	5.6±2.8^{b}	0	<0.05
Morphology (Normal) (%)	48.4±3.7^{a}	61.4±3.7^{b}	55.2±3.7^{a,b}	60.1±3.7^{b}	82.1±3.4^{c}	<0.05
Morphology (Abnormal) (%)	31.6±3.2^{b}	34.6±3.2^{b}	44.7±3.2^{c}	39.8±3.2^{b,c}	17.6±2.9^{a}	<0.05

Vitality (%)	58.1 ± 3.5^{d}	84.3 ± 3.4^{b}	$76.6\pm3.4^{b,c}$	70.7 ± 3.4^{c}	92.5 ± 3.2^{a}	<0.05
Absolute $CD4^{+}$ count (cells/µl)	311.2 ± 49.2^{b}	564.1 ± 49.3^{c}	371.7 ± 49.2^{b}	338.3 ± 49.2^{b}	991.5 ± 47.4^{a}	<0.05

*They were run by Fischer Least Significant Difference test. The values on the table are therefore the mean ranks.

Different superscripts on means in the same row designate means which are significantly different from one another at $P\leq 0.05$.

Tab. 2: Correlation Between Absolute $CD4^+$ count and Semen Parameters

	Semen Parameter													
	Immotile	**Normal**	**Abnormal (Morphology)**	**Rapid-**	**Slow-**	**Non- (progressive motility)**	**Vitality**	**Volume**	**pH**	**IGC**	**Sperm concen**	**WBC**	**RBC**	**Total sperm count**
Group 1	0.0	-0.3	0.1	-0.4	0.0	0.3	0.1	0.1	0.2	-0.2	-0.1	-0.2	0.3	-0.1
Group 2	-0.3	-0.1	-0.4	-0.0	0.2	-0.2	0.2	0.2	-0.6	-0.0	0.2	-0.2	0.1	0.4
Group 3	0.0	0.2	-0.2	0.1	-0.1	-0.1	0.0	0.0	-0.1	0.1	0.5	-0.2	-0.2	0.4
Group 4	0.3	-0.4	0.4	-0.3	0.2	-0.2	-0.1	0.1	0.1	0.0	0.4	0.4	-0.2	0.2
Group 5	-0.1	-0.1	0.1	0.2	-0.2	-0.2	0.1	0.0	-0.1	-0.1	0.7	-0.2	-	0.5

* Correlation of equal to or greater than 0.5 was significant at $P \leq 0.05$. The meanings of the abbreviations used are:

IGC- Immature germ cell

RBC- Red blood cell

WBC- White blood cell

Concen- Concentration

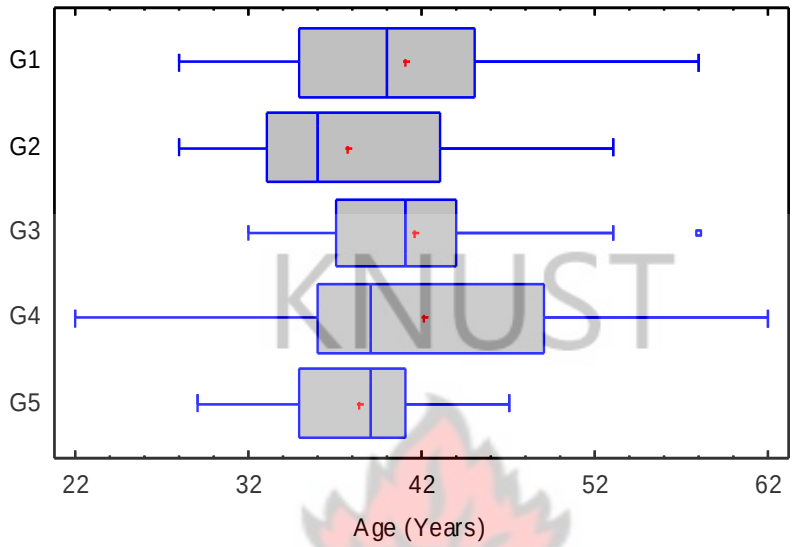

Fig. 15: Age variation between the groups.

The mean ages for G1, G2, G3, G4 and G5 respectively were 37 ± 7.19, 38 ± 6.38, 42 ± 6.75, 42 ± 9.89 and 38 ± 5.09. Even though, there were little variations between the ages of the five groups, they were not significantly different at 5% level.

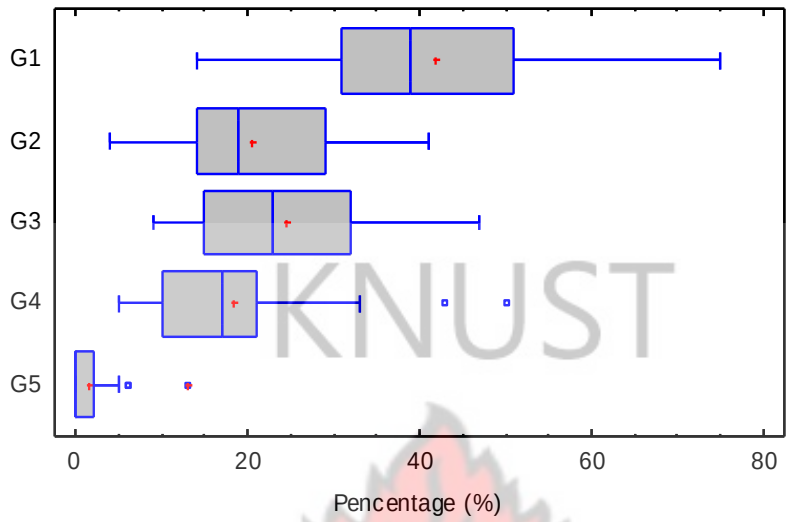

Fig. 15: Estimated variation of number of immature germ cells per 100 seminal cells between HIV infected groups and the seronegative control.

The mean IGC percentage of the control group was significantly ($P \leq 0.05$) lower than that of the HIV-infected populations. Within the HIV infected population, the mean IGCs of G2, G3, and G4 were significantly lower than that of G1 at 5% confidence interval (Table 1; Fig. 2). None of the groups showed any significant correlation with their corresponding absolute $CD4^+$ count (Tab. 2).

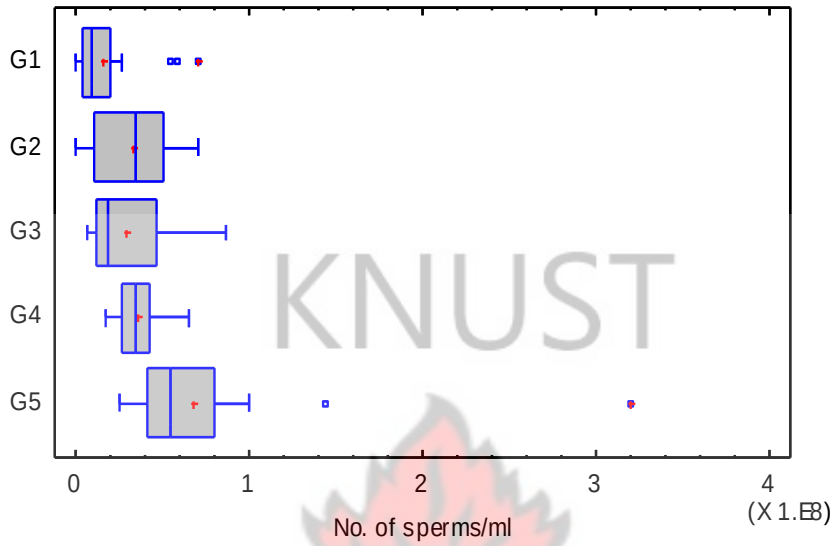

Fig. 17: The effects of HIV and ART on sperm concentrations compared with HIV-negative control.

The mean sperm concentration of the control group was significantly ($P \leq 0.05$) higher than that of the HIV-infected populations. Within the HIV infected populations, the mean sperm concentration of the group G1 was significantly ($P \leq 0.05$) lower than the others. The mean concentrations for groups G2, G3 and G4 were not significantly ($P \leq 0.05$) different (Table 1; Fig. 3). The control and G3 groups however showed significant positive correlation between their sperm concentration and absolute $CD4^+$ counts (Tab. 2)

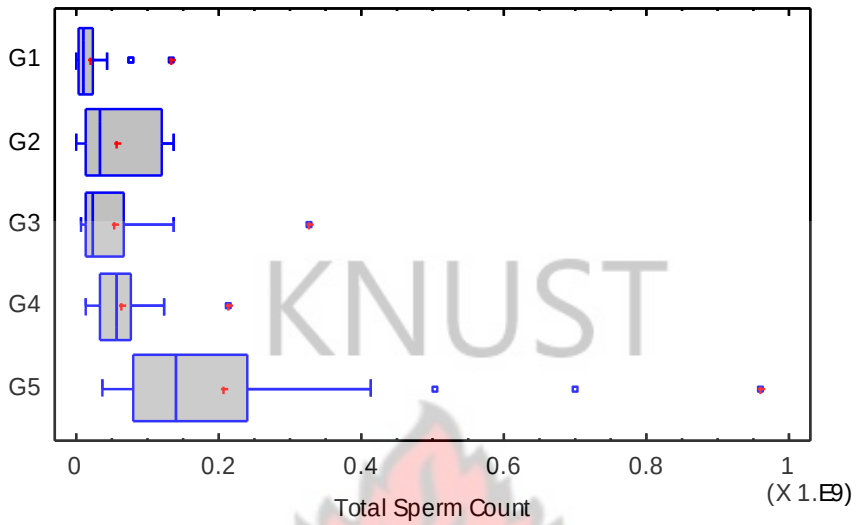

Fig. 18: The effects of HIV and/ or ART on the total sperm count compared with seronegative control group.

The mean total sperm count of the control group was significantly ($P \leq 0.05$) higher than that of the HIV-infected populations. Within the HIV infected populations, the mean total sperm count of the group G1 was significantly ($P \leq 0.05$) lower than the others. The mean concentrations for groups G2, G3 and G4 were not significantly ($P \leq 0.05$) different (Table 1; Fig. 4). The control group only, however showed significant positive correlation between its total sperm count and absolute $CD4^+$ counts (Tab. 2)

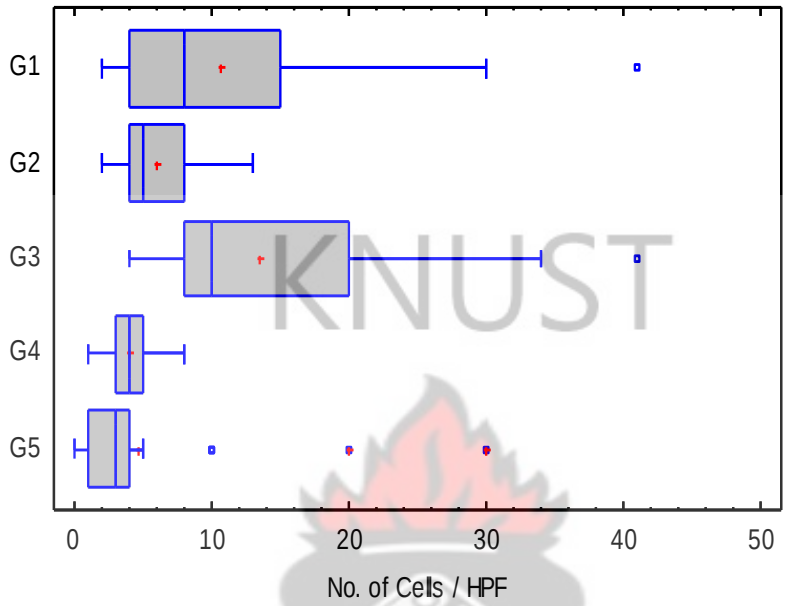

Fig. 19: The effects of HIV and ART on number of white blood cells per high power microscopic field compared with seronegative group.

The mean WBC percentages of control, G2 and G4 were not significantly different from each other, but their means were significantly lower than that of G1 and G3. The mean WBC percentages of these two groups were not significantly different (Table 1; Fig. 5). There was no significant correlation between white blood cells count and absolute $CD4^+$ count in all the groups.

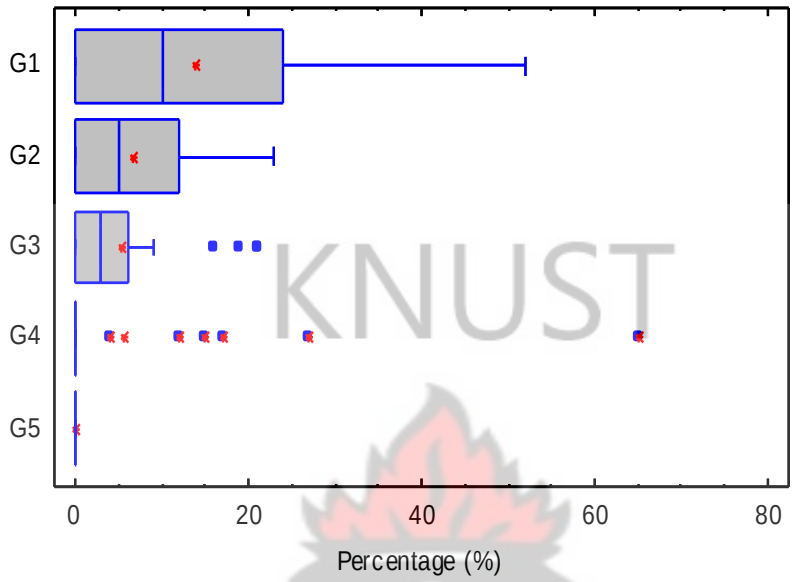

Fig. 20: The effects of HIV and ART on Number of Red Blood Cells in semen.

There was no red blood cells (RBCs) shown by the HIV-negative group. However, within the HIV-infected populations, G1 exhibited a significantly higher semen RBC percentage than the others at $P \leq 0.05$. There were no significant differences between the means RBC percentages of G2, G3 and G4, (Tab. 1 and Fig. 6). There was no significant correlation between RBC and absolute $CD4^+$ count in any of the groups (Tab. 2).

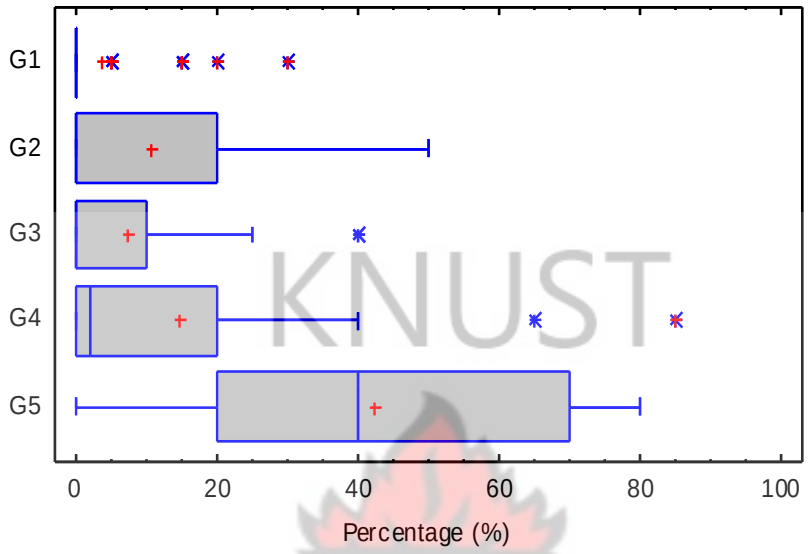

Fig. 21a: The effects of HIV and ART on percentage of rapidly progressive motile sperm in semen compared with seronegative control.

The mean percentage of rapidly progressive motile sperm (RPM) of the control was significantly higher than that of the HIV infected populations. Within the infected population, the mean percentages of rapidly progressive motile sperms of G2, G3 and G4 were not significantly different but were significantly different from that of G1 at $P \leq 0.05$ (Tab. 1; Fig. 7a). There was no significant correlation between RPM and absolute $CD4^+$ count in any of the groups (Tab. 2)

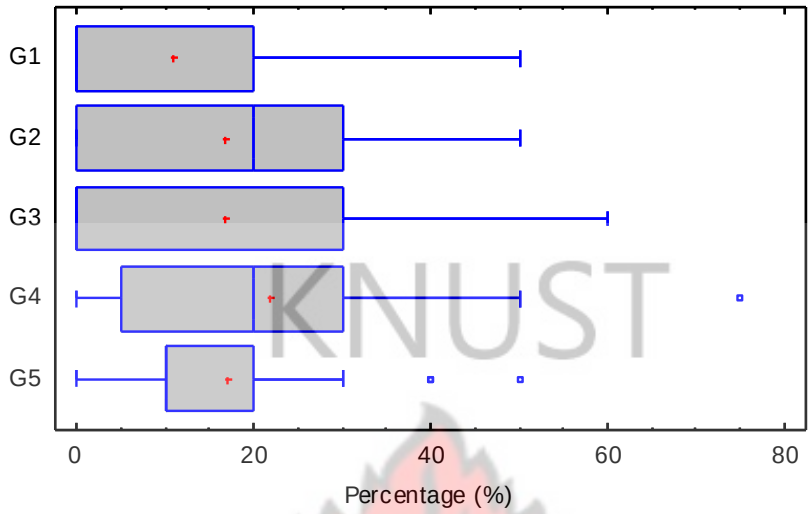

Fig. 21b: The effects of HIV and ART on percentage of slow progressive motile sperm in semen of HIV-infected men compared seronegative control.

There was generally no significant ($P \geq 0.05$) difference between the means of slow progressive motile sperm (SPM) of the control and HIV infected groups. However, between G4 and G1, there was significant difference between their means at $P \leq 0.05$ (Tab. 1; Fig. 7b). In addition, there was no significant correlation between SPM and absolute CD^+ count in any of the groups (Tab. 2).

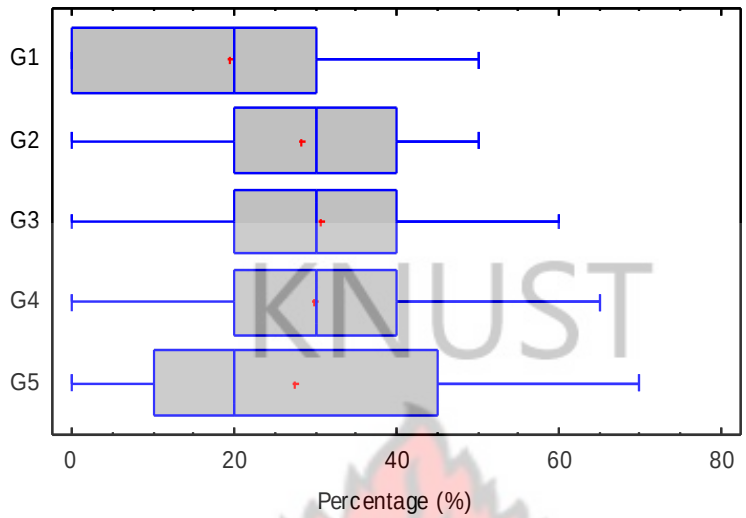

Fig. 21c: The effects of HIV and ART on percentage of non-progressive motile sperms in semen compared with seronegative control.

The percentage of non-progressive motile sperm (NPM) for the G1 was significantly lower than that of the control and the other HIV infected populations. That for the control was not significantly different from that of the other HIV-infected populations at $P \leq 0.05$ (Tab. 1; Fig. 7c). There was no significant correlation between NPM fraction and absolute $CD4^+$ count (Tab.2).

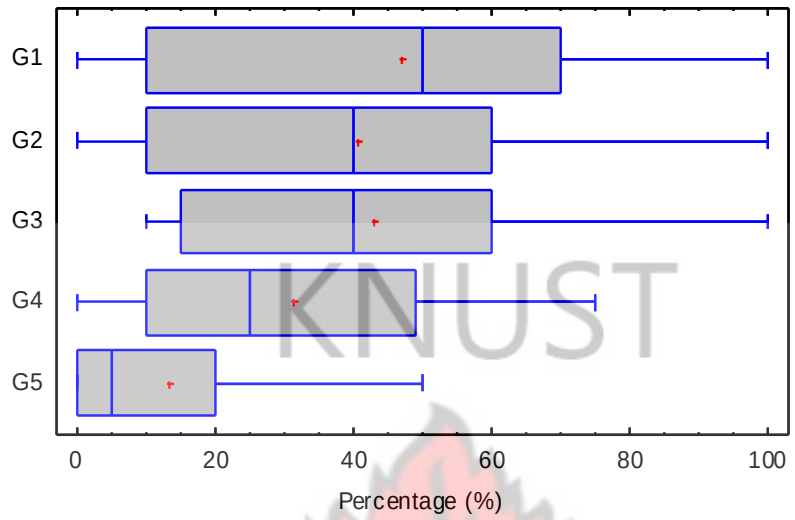

Fig. 21d: The effects of HIV and ART on percentage of immotile sperms in semen among the between HIV-infected groups compared with a seronegative group.

The percentage immotile sperm fraction of the G5 was significantly lower than that of the HIV infected groups ($P \leq 0.05$). Within the HIV-infected population, G4 had a significantly lower mean than G1.There was however, no significant differences between the percentage immotile fractions of the G2, G3 and G4 of HIV infected groups (Tab. 1; Fig. 7d). In addition, between G1, G2 and G3, there was no significant difference at $P \leq 0.05$. There was no significant correlation between immotile sperm fraction and absolute $CD4^+$ count in any of the groups (Tab. 2).

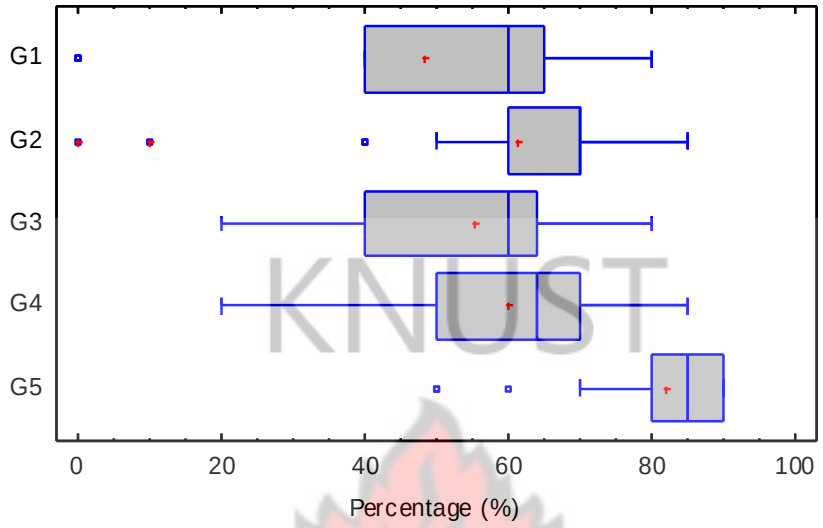

Fig. 22a: The effects of HIV and ART on percentage of morphologically normal sperms in semen between the seropositive groups and seronegative control.

The control group had a significantly higher percentage of normal morphological sperm compared to the HIV infected population. Within the HIV-infected population, the normal sperm morphology for G2 and G4 were significantly higher than that for G1 but not significantly different from that of G3. The percentages of normal sperm for G1 and G3 were not significantly different at $P \leq 0.05$ (Tab. 1; Fig. 8a). There was no significant correlation between morphologically normal sperm percentage and absolute CD4 count in any of the groups (Tab. 2)

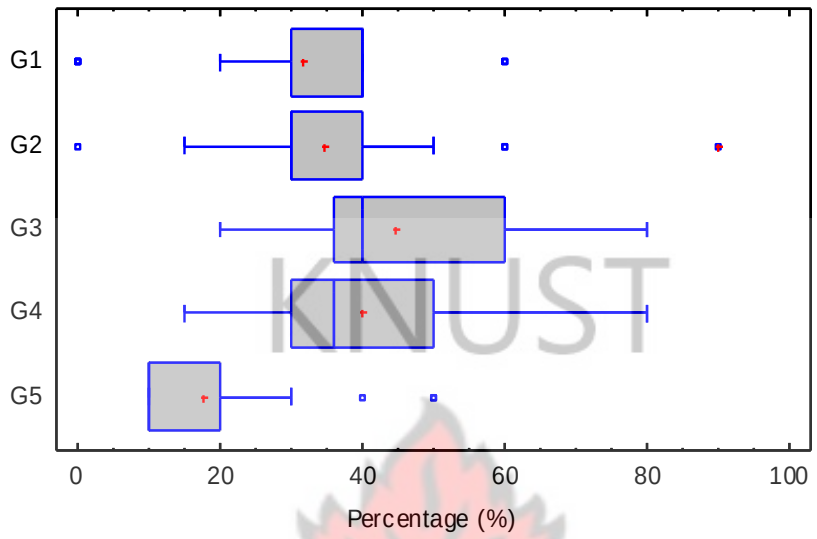

Fig. 22b: The effects of HIV and ART on percentage of morphologically abnormal sperms in semen compared with a seronegative control group.

The control group had a significantly lower percentage of abnormal morphological sperm compared to the HIV-infected populations. Within the HIV-infected groups, G1 and G2 had significantly lower percentage abnormal morphological sperm compared to that of G3 but not significantly different from G4. There was no significant difference between the ARTs (G3 and G4) (Tab. 1; Fig. 8b). There was no significant correlation between morphologically abnormal sperm proportions and absolute CD4 count in any of the groups (Tab. 2).

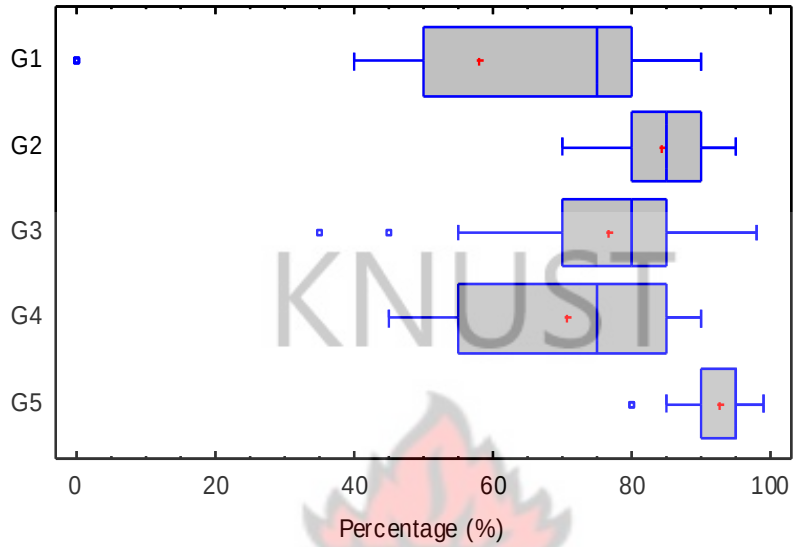

Fig. 23: The effect of HIV and/ or ART on sperm vitality between seropositive groups and seronegative control.

The mean sperm vitality was significantly higher in the control group than HIV-infected groups at $P \leq 0.05$. Within the infected groups, G2 mean was significantly higher than G1 and G4 but not significantly different from that of G3 ($P \leq 0.05$). There was however no significant difference between G3 and G4, though significantly higher than from G1 at $P \leq 0.05$ (Tab. 1; Fig. 9). There was no significant correlation between sperm vitality and absolute $CD4^+$ count in any of the groups (Tab. 2).

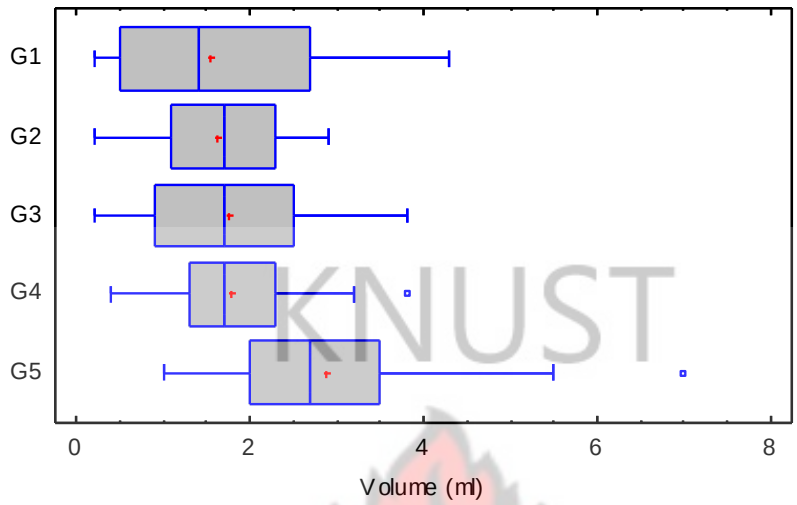

Fig. 24: The effects of HIV and ART on ejaculate volume of seropositive men compared with the seronegative control group.

The control group had a significantly higher mean volume compared to the HIV-infected groups. The mean volumes of the HIV-infected groups were not significantly different at $P \leq 0.05$ (Tab. 1; Fig. 10). Correlation analyses between ejaculate volume and absolute $CD4^+$ did not show any significant correlation between any of the groups (Tab. 2).

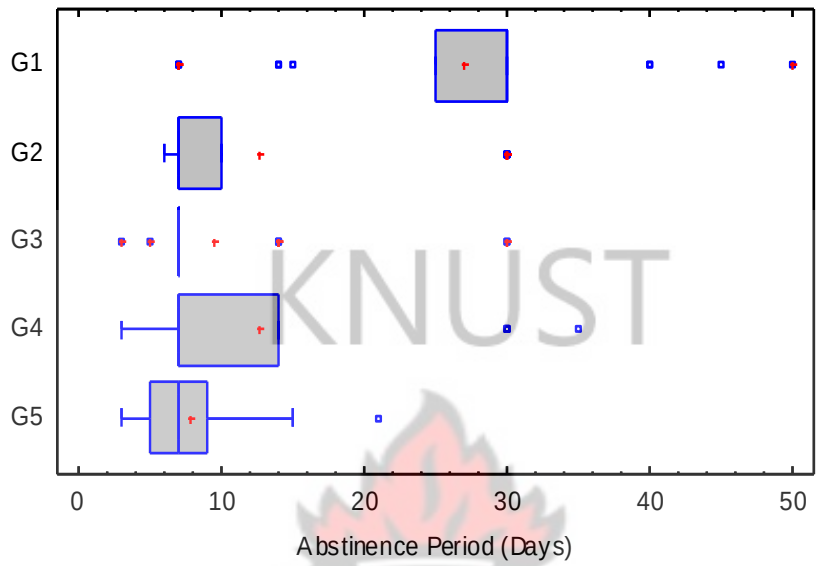

Fig. 25: Variations of the abstinence period observed by the participants before the semen samples were taken.

With the exception of the G3, the mean period of abstinence for the control groups was significantly shorter than the rest of the HIV-infected groups. Within the HIV infected groups, G1 had significantly higher period of abstinence than the others. There was however, no significant difference between the G2, G3 and G4 groups (Tab. 1; Fig. 11).

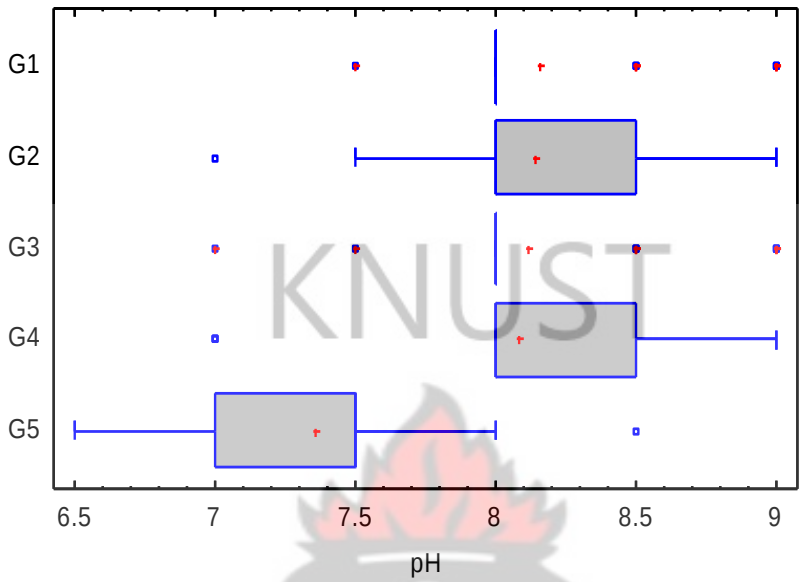

Fig. 26: The effect of HIV and ART on pH of ejaculates from seropositive men compared with that of from seronegative men.

The mean pH of the control groups was significantly lower than that of the HIV-infected groups, but there were no significant differences between the mean pH within the HIV-infected groups at $P \leq 0.05$ (Tab. 1; Fig. 12). There was a significant negative correlation between seminal pH and absolute $CD4^+$ count within G2. However, there was no significant correlation between pH and absolute $CD4^+$ count within the other groups (Tab. 2).

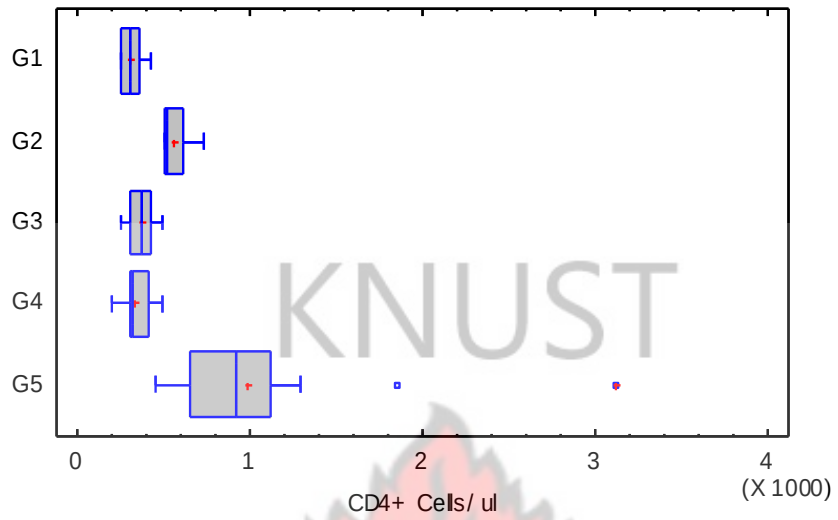

Fig. 27: The differences between the Absolute CD4$^+$ count/µl of blood between the seropositive groups and the seronegative group.

The mean absolute CD4$^+$ count of the control group was significantly higher than that of the HIV-infected groups. Within the HIV-infected groups, the mean CD4$^+$ count of G2 was significantly higher than the others. Absolute CD4$^+$ count for the G1, G3 and G4 were not significantly different at $P \leq 0.05$ (Tab. 1; Fig. 13).

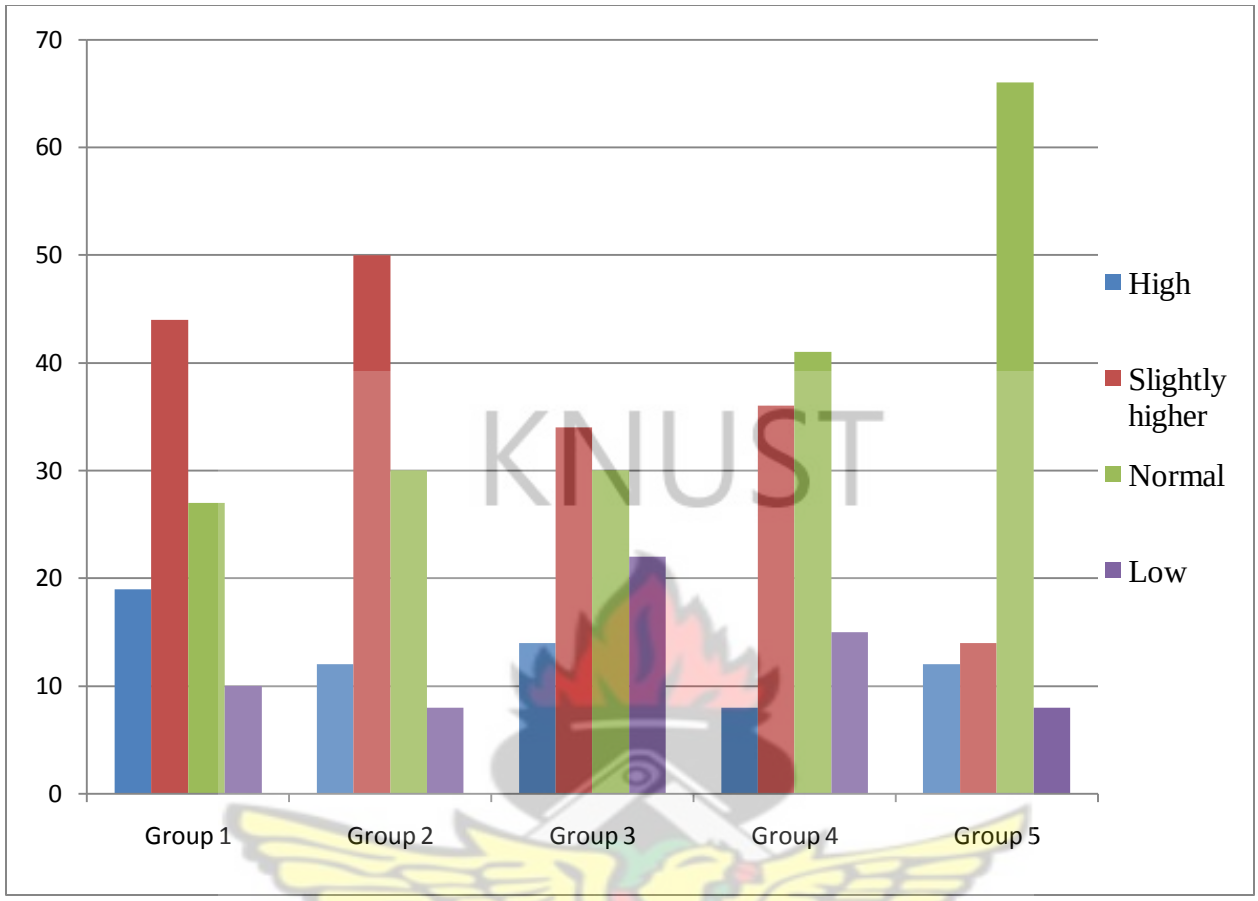

Fig. 28: The variation of viscosity of semen samples obtained from the participants.

The viscosity of the samples obtained from the HIV-infected groups and that from the Control groups were categorized under high, slightly higher, normal and low. The control had 66% of its samples being normal compared with 27%, 30%, 30%, and 41% for G1, G2, G3 and G4 respectively. However, there was much difference in the ejaculates which had slightly higher viscosity in all the HIV-infected groups (G1, G2, G3 and G4) with respective percentages of 34%, 50%, 34% and 36% compared with the control, with corresponding value of 14%. Ejaculates with low viscosity was observed in all the groups with G3 exhibiting the highest percentage of 22%. Also, groups G4, G2, G1 and Control had respective percentages of 15%, 8%, 10% and 8%.

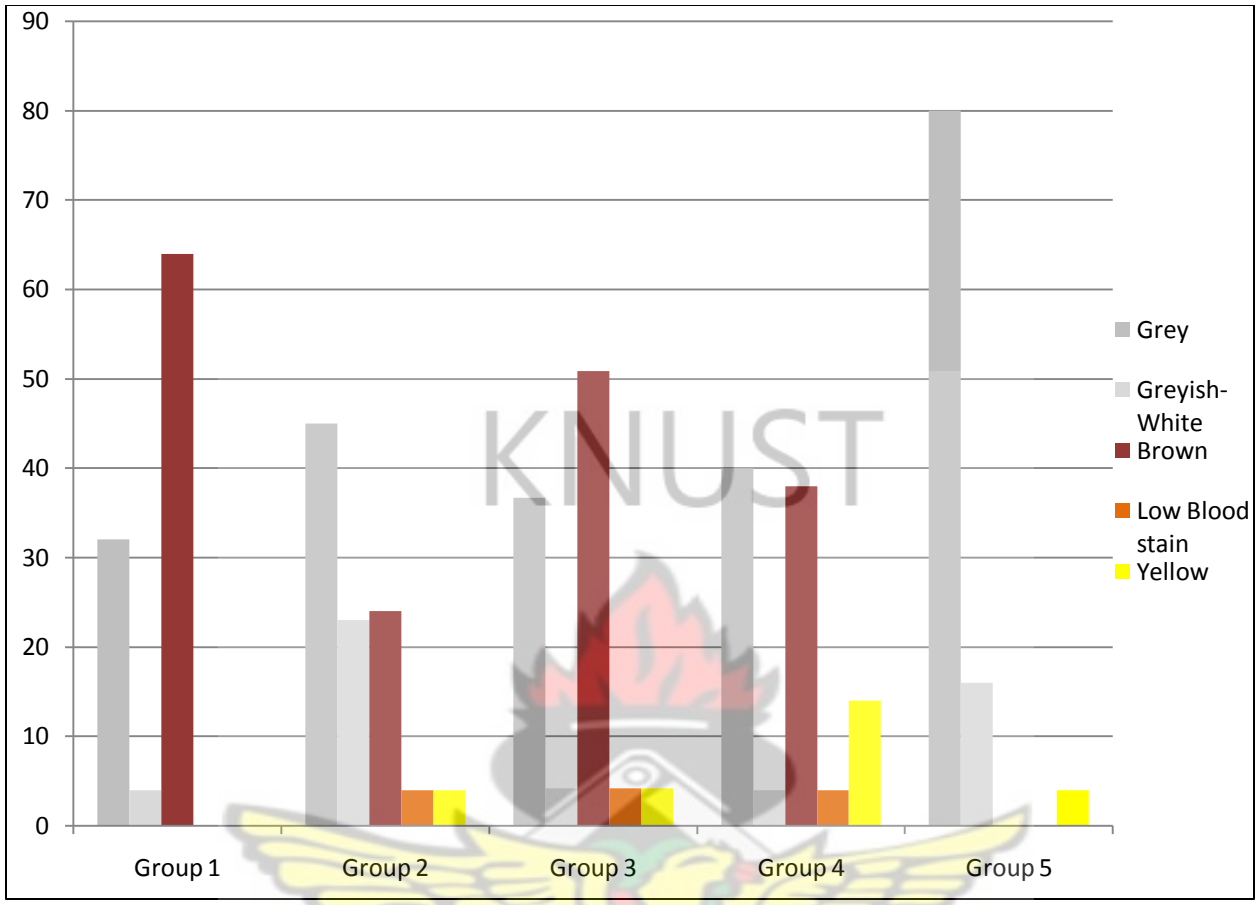

Fig. 29: Colour variations of ejaculates obtained from seropositive groups and seronegative groups.

The seronegative control had 80% of its samples having the 'normal' semen colour of grey. Nevertheless, G1, G2, G3 and G4 respectively gave 16%, 45%, 36.66 and 40% for the normal semen colour. Most of the seropositive samples were brown/ reddish. The seronegative group showed no haemospermia. G1 had the highest number of semen samples with red blood cells with corresponding percentage of 44. Groups G3, G4 and G2 on the other hand respectively had 30.83%, 28% and 24% of the haemospermic semen samples.

CHAPTER FIVE

5.1 Discussion

Currently, HIV/AIDS is the fourth leading cause of death globally and it's been known to be the leading cause in Africa (UNAIDS, 2009). Sexual transmission of HIV has been admitted by UNAIDS to be the most complex mode of transmission of the virus and it has remained the most difficult mode to control (UNAIDS, 2009). From this study, it was realized that a number of factors might have also contributed to the spread of HIV in Ghana. People without basic education may find it difficult to understand HIV transmission dynamics. Basic education is, at least, what one might need to understand the dynamics of HIV transmission and prevention, and from this study, 88% of the HIV-infected participants, at least, had basic education and only about 12% had no education. It thus therefore appears that education level has not increased the level of awareness or consciousness about HIV transmission and prevention since only about 12% of the infected participants did not have any formal education. With regard to knowledge about HIV and its transmission modes, at least, 87% of the infected participants had good knowledge about HIV transmission modes and prevention and only 13% had generally low knowledge. This might suggest that lack of knowledge about HIV and its mode of transmission and prevention have not solely been the key factor responsible for the spreading of the infection. About 67% of the infected men desired to have offspring(s) and others want to satisfy their sexual desires and because of that about 62% of the infected men were sexually active. About 38% of the infected men were not sexually active. The probable reason might be as a result of separation from their partners, death of their spouses or divorced, as about 28% of the infected men had their partner(s) either dead, separated from them or divorced. Even though, about 71%

of the sexually active infected participants used condom to prevent super-infection and also avoid infecting other seronegative partners, about 28% responded they do not use condom during sexual relations with their partner(s) post-infection. Also, about 43% of the infected men responded that they used some form of herbal medication (traditional medicine) to mitigate the general weakness and illness they were experiencing post-infection. However, about 57% responded that they never used any form of herbal medication post-infection.

The exact effect of HIV infection on semen quality has been largely a debate and currently, the effect of the virus and its mechanism of action on the male reproductive system and hence, semen quality have remained inconclusive. This study provides a description of the main semen parameters of a cohort of HIV-infected men; both asymptomatic and symptomatic HIV clients, who were seeking medical attention at the Kumasi South Regional Hospital, Ghana. The study was made up of male participants (seronegative control and HIV-infected male clients) of age ranging from 22.0 to 62.0 years with the mean age across the entire population being 40.12 ± 7.28 years. Even though, a higher proportion of the participants' ages was skewed to the left, the age between the participants in the subgroups were not significantly different at 5% level. Therefore, age could not be a confounder of the observed differences in the semen quality exhibited between the seronegative and the seropositive groups.

It has been well demonstrated that the duration of sexual abstinence can influence semen quality and some prior studies have reported that semen volume, sperm concentration, percentage motile sperm and total sperm count per ejaculate positively and significantly correlate sexual abstinence period (Levitas *et al.*, 2005; Gao *et al.*, 2007). In this study, significant longer abstinence durations were observed by the HIV-infected men and could not conform to the stipulated period of sexual abstinence as compared with the control group. Most of these men because of the

infection had decided to abstain from sex and by the time they were recruited for the study, some had already abstained for some period and therefore the study saw the need to factor those days into its computation of sexual abstinence period to eliminate confounding the upshot. Even though most of the HIV-infected men had longer abstinence periods, this did not translate into good semen quality. There was no significant positive relationship with any of the critical semen parameters, such as sperm concentration, motility, and the duration of abstinence.

With respect to blood absolute $CD4^+$ count, the control group (G5) exhibited the highest mean absolute $CD4^+$ count of 991.5 ± 47.4 cells/µl of blood (Tab. 1) due the fact that there was no underlying medical condition responsible for diminishing their absolute $CD4^+$ cells. Group G2 which had slightly similar semen quality compared with the control in a number of semen indices however had a lower mean absolute $CD4^+$ count of 564.1 ± 49.3cells/µl of blood at 5% level of significance. Within the HIV-positive groups, apart from G2, there was no significant difference between the means of G1, G3 and G4.There was however a clear difference between G1 and G2 seminal indices which may solely be accounted for by differences in absolute $CD4^+$ count between the groups as ART was not a confounder within these groups. A critical examination between G1 and G4 shows a vast improvement in the semen quality in G4 than G1 even though they showed statistically similar absolute $CD4^+$ counts. This might suggest the therapeutic capability of ART in the improvement of fundamental physiological processes, even though there was not significant upward adjustment of absolute $CD4^+$ count in the ART treated seropositive cohorts comparing G4 with G3 (Tab. 1).

Many critical semen parameters such as: semen viscosity, sperm motility and morphology did not show any significant correlation with their respective absolute $CD4^+$ count; however sperm concentration, total sperm count of the control group and pH of G2 did (Tab. 2). This finding

partly corroborates results of other studies (Politch *et al.*, 1994; Dondero *et al.*, 1996; Lasheeb *et al.*, 1997; Nicopoullos *et al.*, 2004). This observation may imply that, apart from the fact that HIV itself might have a direct effect on the male reproductive system, the immune strength, indicated by the absolute $CD4^+$ count, might generally be a fair indicator in the prediction of the dynamics of semen quality in the absence of ART administration.

The observed semen pH showed much distinct difference between the control and HIV-infected population. On the average, there was a relatively higher pH exhibited by most of the HIV-infected ejaculates irrespective of whether the ejaculate was obtained from a client on ART or not. This observation is actually in consonance with other investigators' reports (Anderson *et al.*, 1992; Crittenden *et al.*, 1992; Politch *et al.*, 1994). Increased seminal pH might probably be due to a partial or total dysfunctional prostate glands or loss of its ability to concentrate citric acid and which is responsible for reduction in pH of semen in the prostatic fluid (Knobil & Neill D, 1993). It is possible the prostate glands might have been compromised due to a direct or indirect action of HIV such that even ART administration was not able to ameliorate the condition to normalcy as evinced by the consistency of higher pHs in groups G3 and G4 as well.

There were palpable differences in the seminal viscosity between the control and the HIV-infected groups from this study. The control had about 66% of the specimens having normal consistency or viscosity whiles 34% showed some abnormalities. On the other hand, G1 showed 27% normal and 73% abnormal, (19-very high, 44- slightly high, 10-low), which exhibited much deviation from the control. Nevertheless, the higher viscous semen samples observed in the seropositive ejaculates was an observation that is in consonance with other studies (Crittenden *et al.*, 1992; Dondero *et al.*, 1996; Muller *et al.*, 1998). In addition, group G2 showed similar findings: 30% normal and 70% abnormal (12% very high, 50% slightly high, 8% low). Groups

G3 and G4 respectively did not show much difference from that of G1 and G2, however, the striking observation was the consistently high percentages of abnormal seminal consistency exhibited by HIV-infected ejaculates. This could possibly be due to colonization and interference with the associated glands of the male reproductive system: seminal vesicles and prostate gland, by HIV. The seminal vesicles are known to secrete a fibrinogen-like precursor which is basically responsible for the viscosity exhibited by human semen. The prostate is one of the leading organs in the male prone to infection and inflammatory processes, producing both acute and chronic prostatitis (Knobil & Neill D, 1993; Anna Le Tortorec & Roques, 2008). Although the influx of lymphocytes and inflammatory cells into the prostate was not exhibited in the form of severe leukocytospermia in the HIV-infected groups compared with the seronegative control, the inability of most of the seropositive semen to liquefy readily suggested more pronounced dysfunctional prostate glands. The prostate glands have the primary function of secreting proteolytic enzymes (fibrinolysin) for hydrolyzing the protein responsible for the viscosity in semen into amino acids to effect liquefaction (Smith *et al.*, 2004).

In addition to the general aberrations observed in pH and viscosity inherent to semen obtained from HIV-infected men, the semen volume obtained from the seropositive participants also showed a marked significant reduction compared to that of the seronegative control at 5% confidence level. Seminal volume was the first observed difference when the ejaculates were obtained from the seropositive participants. There were no differences between the volumes obtained for the HIV infected groups which might be as a result of the reason stated above. Leruez-Ville *et al* (2002) reported similar findings. As it is known that the prostate and seminal vesicles are responsible for about 90–95% of the ejaculate volume, dysfunctional glands due to past or silent inflammation as a result of the HIV colonization might be responsible for the

observed reduced ejaculate volume(Dobs *et al.*, 1988; Johnston *et al.*, 1995; Taylor *et al.*, 2001; White *et al.*, 2001).

There is a general speculation of a direct disruption of spermatogenesis and spermiophagy induction by the viral activity in the testicular tissue (Dondero *et al.*, 1996; Muciaccia *et al.*, 2007). The viral activity has also been thought to be responsible for the defoliation of immature germ cells in HIV-infected men (Muciaccia *et al.*, 2007). The results evince the palpable difference between control and HIV-infected population with respect to the mean immature germ cells counts. The higher mean percentage of immature germ cell observed in the group G1 shows the apparent continuous heightened defoliation of immature germ cells in the absence of ART. This might be as a result of a heightened activity of uninhibited viral activity in the testicular tissues over an extended period. Within the HIV-infected groups, there were varied degree of defoliation of immature germ cells with G2, G3 and G4 not showing any significant differences between their means. Immature germ cells defoliation reduction in G3 and G4 could be due to the effect of ART, which reduced viral replication and activity in the body. Group 2 however showed reduced defoliation basically because of the relatively stronger immune strength, as indicated by its mean absolute $CD4^+$ count, which might have prevented the uninterrupted immature germ cell degeneration as was apparent in G1. Other studies have also reported germ cell depletion phenomenon which was attributed to the viral activity in the male reproductive tract (Rogers & Curran, 1988; De Paepe *et al.*, 1989; De Paepe & Waxman, 1989). Since then, other authors have described the arrest of spermatogenesis at various points, numerous foci of degenerating germ cells, and epididymal block in some histological investigations (Shevchuk *et al.*, 1999; Muciaccia *et al.*, 2007). Interestingly, a study of 140 testicular autopsy specimens from AIDS patients with and without antiviral treatment reported that treatment and prolongation of

survival in AIDS patients is associated with a shift in the histology of the testicular tissues toward a more pronounced loss of germ cells (Shevchuk *et al.*, 1999).

Seminal colour was also substantially varied as the number of haemospermic ejaculates from the HIV-infected cohorts was high, especially in G1. Indeed, comparison of the RBC counts that were observed in ejaculates among the five groups showed significant difference at 5% level. Nevertheless, none of the control ejaculate was haemoospermic, even though all the HIV-infected groups showed varying degrees of blood in their semen with G1 group exhibiting the highest estimated average (Tab. 1; Fig. 5). Due to the presence of red cells in 43 pairs out of 129 pairs of the total ejaculates, there was a substantial percentage of ejaculates whose colour, as a result, was masked by the presence of blood (Fig. 14). This observation was never witnessed in any of the control ejaculates. 64% of group G1ejaculates were haemospermic as against 28%, 45%, 32% and 0% for groups G2, G3, G4 and control respectively. The control group on the other hand had a profoundly higher percentage (80%) of the 'normal' semen colour of opaque and translucent-grey compared with the HIV-infected population (Fig. 14). Within the HIV-infected groups, G1 exhibited the highest abnormal seminal colours, probably because of heightened and unchecked viral activity within the reproductive compartment. Group 2 on the order hand, showed the highest of the normal seminal colour within the HIV-infected groups. G3 and G4 even though showed slightly higher normal semen colour compared with G1, ART administration seems not to have been able to halt the inflammation which might be as a result of viral activity within the accessory glands, hence, the persistence of blood stained semen. The occurrence of these seminal colours could partly be explained by silent inflammatory responses in the associated reproductive glands and/ or tract which administration of ART did not completely ameliorate as was evinced in G3 and G4 (Tab. 1) (Knobil & Neill, 1993).

In totality, there was a slight aberration in the number of seminal leukocytes observed per high power microscopic field (HPF) between the groups. Groups 2, 4 and 5 exhibited lower number of leukocytes per HPF compared with the observed means in G1 and G3. These latter groups shared similar means with the abnormal morphological spermatozoa recorded. This might suggests a sort of relation between high leukocytes number and abnormal morphological spermatozoa. Leukocytes are cells which fight infections and are also attracted to any site of inflammation in the body and this might suggest that, G1 and G3 exhibited more inflammatory responses in their testicular tissues and associated organs than the other HIV-infected groups and the control as well. The probable reason why G1 and G3 seem to be the only ones with higher mean leukocytes number per HPF may be an unhalted viral activity in the reproductive compartment in G1 which tend to attract high leukocytes into this site of viral activity. Most G3 clients might have not been on ART for a substantially long enough period to reduce the infiltration of leukocytes into the reproductive compartment. Since the viral activity might not be fully repressed as in G3, this reason might therefore account for the relatively high mean leukocytes per HPF recorded with the group (Quayle *et al.*, 1997; Wolff, 1998; Ball *et al.*, 1999). The seemly association between seminal leukocytes number and abnormal morphological sperms has been attributed to the production of reactive oxygen species (ROS) capable of destroying cellular membranes and nuclear materials. The higher the number of leukocytes, the more ROS produced and the more damages done to sperm and the higher the abnormal morphological sperm produced (Wolff, 1998).

The mean sperm concentrations of the HIV-infected populations were significantly lower than that of seronegative control at $P \leq 0.05$. These apparent differences between the two major groups could be due to a number of very critical reasons. First of all, some studies have reported

that several endocrine and testicular dysfunctions in men infected with HIV are caused by the HIV infection, depending, in part, on the stage of the disease (Merenich *et al.*, 1990; Christeff *et al.*, 1992). This was evinced in some other studies in which very low testosterone profile were observed in HIV-infected men such that there was significant negative correlation with stage of the disease (Dobs *et al.*, 1988; Villette *et al.*, 1990; Raffi *et al.*, 1991; Schurmeyer *et al.*, 1997). Fundamentally, testosterone is an androgen produced by special endocrine cells in the testes known as interstitial endocrinocytes, to sustain the process of spermatogenesis once it has been initiated by the priming of Sertoli cells by Follicle Stimulating Hormone. It therefore stands to extrapolate that, as has been observed by a number of studies including this that there might be testicular tissue dysgenesis which might definitely have affected the interstitial endocrinocytes also. Hence, a possibility of reduced production of testosterone as reported by other studies might contribute to the decreased sperm concentration observed in the HIV-infected population in this study (Chabon *et al.*, 1987). Secondly, a significant number of studies have also attributed the reduced sperm concentration to germ cell depletion in the testes as it was evinced by higher defoliation of immature germ cells in the HIV-infected groups compared with the seronegative control. The germ cell reserves which are the precursors of the spermatozoa in the testes are directly or indirectly depleted by the HIV activity in the testes (Leruez-Ville *et al.*, 2002; Nicopoullos *et al.*, 2004). If germ cells are depleted, it directly decreases the final cell type that results from them, spermatozoa. Within the HIV-infected groups, striking differences were also observed. Although, Group 2 exhibited a relatively higher absolute $CD4^+$ count, there was also a heightened immature germ defoliation compared with the other infected groups. This could be the reason for the reduced sperm concentration similar to G3 and G4 with strikingly similar mean immature germ cell loss. Group 1 which gave the lowest sperm concentration was very

different from the other HIV-infected groups statistically at 5% level and showed much heightened immature germ cell loss; approximately double of that exhibited by G3.

Multiple factors including anatomical and physiological states of the sperms as well as some physicochemical properties within the seminal fluid are the major determinants of sperm motility. The results obtained showed varied differences in the sperm motility of the seronegative control compared with the HIV-infected populations as well as within the HIV-positive cohorts. There was much significantly higher mean percentage of aggressively motile sperm (a-type) compared with the means observed within the infected cohorts. Between the control and the infected cohorts, what might have accounted for the reduction in rapidly motile sperm could be attributed to a number of factors; high viscosity, a possible reduction in mitochondrial number in the neck of spermatozoa produced by infected cohorts, or a probable reduction in the number of channels which allow cat ion (possibly, Ca^{2+}) (Dejucq-Rainsford & Jegou, 2004), into the tail to effect maximum aggressive motility. The high semen viscosity observed in the HIV-infected groups could account for the reduced aggressive forward thrust of spermatozoa seen (Crittenden *et al.*, 1992; Dondero *et al.*, 1996; Muller *et al.*, 1998; Leruez-Ville *et al.*, 2002; Nicopoullos *et al.*, 2004). Secondly, calcium ions are highly needed by sperm for effective motility and other key physiological processes in living organisms. In both vertebrate and invertebrates, sperm functions could be optimized when sperm has the normal capacity to mobilize Ca^{2+} ions which is highly necessary for hyperactivation of sperm (rapid progressive motility) (Conner *et al.*, 2007; Publicover *et al.*, 2007).Therefore, development abnormality associated with Ca^{2+} channels or Ca^{2+} sequestration might be the possible cause of the abnormally reduced sperm motility that characterized sperms obtained from HIV-infected men (Dejucq-Rainsford & Jegou, 2004; Conner *et al.*, 2007; Publicover *et al.*, 2007). Thirdly, mitochondria are well known energy

producing organelles found in most living cells, except RBCs. All mitochondria found within cells have a number of DNA that regulates all the functions of these organelles (St John *et al.*, 2001). Some mitochondrial studies have reported abnormal functioning of these organelles when multiple DNA deletion occurs as a result of interference with the either mitotic or meiotic processes of new cell formation (cell division) (Muciaccia *et al.*, 1998; St John *et al.*, 2001). Sperm cells enormously utilize mitochondria for production of energy for motility. Sperm with abnormal mitochondria as a result of mitochondrial DNA deletions exhibit abnormal functioning and low motility (St John *et al.*, 2001; Yang *et al.*, 2005; Kao *et al.*, 2008).

And also, the relatively high a-type motile sperm in G3 and G4 might have occurred as a result of the therapeutic effect by ART administration which could have ameliorated those possible effects of HIV's activity to some degree and hence, a fairly improved condition than that observed within G1. Group 2, on the other hand, showed mean a-type motility similar to that of G3 and G4 basically because of probably moderate viral activity as compared with the degree of viral activity in G1, hence, the moderately reduced condition observed within G2 with regards to the mean a-type motile fraction sperm. Generally, the mean slow progressive motile sperms observed within the five groups were not significantly different. However, within the infected cohorts, G4 exhibited much higher mean than G1, though not significant at $P \leq 0.05$. G1 basically showed about 5 semen samples out of 25 of the samples as azoospermic, which might have contributed to the overall reduced means of sperm assessed for motility within this group. With respect to the non-progressive motile sperm, similar pattern as observed in the slow-progressive was evinced and therefore there was no significant difference between the groups generally. However, G2, G3, G4 and G5 exhibited higher mean non-progressive motile sperm of compared with G1 though not significant at $P \leq 0.05$. It would be plausible to predict that

increased viral activity should lead to very high degree of immobilization of sperms from the above discussion. This was apparent, however, because of the 5 out of 25 semen samples were azoospermic in G1, their overall mean sperm count and motility determination were highly reduced such that, the means from G1 looked like that in G2 and G3, though slightly higher. Within the infected groups, G4 exhibited the least mean of immotile sperms; though more than double that of the control. This result apparently shows the ameliorating effect of ART on sperm motility as could be ascertained between G3, G4 and G1 with very similar immune strength depicted by their similar absolute $CD4^+$ counts. However, the results on motility, especially, with respect to the infected on ART and samples from those not on ART was contradictory to assertion by other studies that ART administration retards sperm motility in infected cohorts (Johnston *et al.*, 1995; Lewis & Dalakas, 1995; Brinkman *et al.*, 1998). From a generalized view, almost all the living sperms observed in HIV-infected population had reduced motility compared with the control, which actually corroborated reports by other studies (Folgero *et al.*, 1993; Ruiz-Pesini *et al.*, 1998; Donnelly *et al.*, 2000; May-Panloup *et al.*, 2003). The significant increase in sperm motility in both G3 and G4compared with G1 suggests that even though immune reconstitution by ART does little to actually improve absolute $CD4^+$ count, sperm motility as an index of semen quality was improved compared to G1, though significantly lower than that of the HIV-negative control. It has even been reported by other studies that several ARTs have mitochondrial toxicity and that the observed changes in motility could be due partly to the ART itself (Johnston *et al.*, 1995; Lewis & Dalakas, 1995; Brinkman *et al.*, 1998). Even though, some studies have attributed the reduced percentage of rapid progressive motile spermatozoa in HIV-patients receiving ART to the mitochondrial toxicity and multiple DNA deletions in the sperm caused by ART, the observed changes in motility from this study could not be attributed only to

ART (Johnston *et al.*, 1995; Lewis & Dalakas, 1995; Brinkman *et al.*, 1998). The results on sperm motility observed between the infected groups rather suggest there might be some direct or indirect pathologic action by the virus on the spermatogenetic process itself which may be responsible for the general retardation of sperms observed in the HIV-infected male cohorts rather than the exclusive consequence of ART toxicity.

The higher percentages of the immotile sperm fraction observed in the HIV-infected population were found not to be dead by the sperm vitality assessment employed for distinguishing between dead and living cells. G2 exhibited 84.3 ± 6.3 mean sperm vitality which was close to the control which showed 92.5 ± 5.3 as the highest mean vitality, even though significantly different at $P \leq 0.05$. There was however, progressively lower sperm vitality observed in the other groups within the HIV-infected population with group G1 showing the least mean vital sperm. Sperm vitality was performed to further give a clearer estimate of the percentages of the spermatozoa that were actually alive, because much of the sperms observed from the HIV-infected clients were immotile and therefore it was necessary to distinguish immotile sperms from dead ones to ascertain whether HIV's pathological effect on sperm is immobilizing or lethal. Within the infected population, G2 exhibited significantly higher mean sperm vitality than G1, G3 and G4. Group 1 showed the lowest mean vital sperm compared with G3 and G4 which did not exhibit much difference between them. The reason for the reduced percentage of vitality in the HIV-infected groups may be rather vague currently, but some studies have attributed it to the entry of the virus via CCR5 and CCR3 co-receptors into the spermatozoa and subsequent genomic disruption by HIV virions; as these receptors are present on sperms from healthy subjects (Muciaccia *et al.*, 2005a; Muciaccia *et al.*, 2005b; Muciaccia *et al.*, 2007). Evidence adduced by TUNEL-positive staining of spermatozoa from HIV-positive males with much abnormal genetic

materials have also implied or attributed the teratozoospermia observed in HIV-infected men to impaired spermatogenesis which might have been caused by entry and disruption of nuclear materials. This may be the cause of the much abnormal morphology and dead spermatozoa produced by the seropositive men (Muciaccia *et al.*, 2005a; Muciaccia *et al.*, 2005b; Muciaccia *et al.*, 2007). Within the infected population, the difference in vitality could basically be attributed to heightened activity of the virus in certain groups, especially, G1, which also showed 5/25 of semen samples as azoospermic which might have reduced the group's mean as a whole. Group 2 exhibited relatively higher mean percentage vitality compared with G3 and G4 basically because of probably low viral activity in their system in the absence of excessive leukocytes in sperm which are believed to produce ROS with the capability of destroying cell membranes and nuclear material thereby rendering most sperm cells dead. In addition, the probable adverse effects of ART administration was absent in G2 which together might have accounted for its higher vitality. Aside reduced sperm motility, increased mean percentages of sperm with abnormal morphology in the infected cohorts was a key feature this study observed. This finding was highly supported by a number of other studies (Dejucq-Rainsford & Jegou, 2004; Muciaccia *et al.*, 2005a; Muciaccia *et al.*, 2005b; Muciaccia *et al.*, 2007).There was significantly higher mean abnormal morphological sperm in the HIV-infected ejaculates compared with the control (Tab. 1; Fig. 7a; Fig. 7b). Conversely, the control exhibited a significantly higher mean normal morphological sperm compared with the infected population. The mean normal morphological sperm in the control was approximately twice that observed in G1. However, within the infected cohorts, G1 showed the least mean normal morphological sperm compared with the means of the other infected groups at 5% significant level. Within the infected cohorts, G3 and G4 exhibited the highest mean of the abnormal morphological sperm compared with G1, G2. The cause of the

differences within the infected groups, basically, might be as a result of slightly impaired immunity and the effect of ART on spermatogenesis or on the sperm storage sites, vas deferens and epididymis. Within G3 and G4, which did not have significantly different means, the elevated abnormal morphological spermatozoa could be attributed to viral activity as well as a probable side-effect of ART. G1 strikingly showed lower mean abnormal morphological sperm compared with G2, G3 and G4 basically because, 5 out of 25 of its semen samples were technically azoospermic, hence, the reduced overall means from that particular group with respect to mean sperm morphology (normal and abnormal), sperm concentration and total sperm count.

In this study, a low sperm concentration was often associated with high mean abnormal morphological sperm which suggests spermatogenesis might have been compromised in those subjects. This finding has been partly explained by a study in which higher TUNEL-positive stained sperms were observed in ejaculates produced by seropositive men compared with seronegative control men (Muciaccia *et al.*, 2007). In addition, other studies have reported the presence of HIV-1 DNA in most abnormal spermatozoa of HIV-1 infected subjects whose semen contained a high percentage of spermatozoa with abnormal morphologies (range 58–80%) and the percentage of spermatozoa with fragmented DNA (range 9.5–35.4%) which greatly exceeded normal values (range 0.9–4.4%) (Antonelli *et al.*, 2000; Gandini *et al.*, 2000a; Gandini *et al.*, 2000b; Muciaccia *et al.*, 2007). In a similar arena, Nuovo*et al*, (1994b) reported that HIV-1 DNA-positive sperm cells clearly showed abnormal morphologies and also unraveled by PCR technology that there was localization of HIV-1 DNA in the sperm head. From their observation, they suggested that the virus could either be 'trapped' in the sperm nucleus or integrated into the

sperm genome which is believed might be responsible for the teratozoospermic conditions which were very apparent in the seropositive ejaculates (Tab. 1; Fig. 7a; Fig. 7b).

These multiple abnormal seminal indices that characterized semen samples obtained from group G1, especially, raises lots of questions about the mode of action of HIV in the testis. On the one hand, the virus was first thought to have an indirect effect due to the chronic debilitating illness and cachexia of the patients. In this school of thought, it has been suggested that opportunistic infections might be involved (De Paepe *et al.*, 1990). On the other hand, another study hasindicated that only 32% of patients with opportunistic infections had Cytomegalovirus (CMV), *Mycobacterium avium-intracellulare*, or *Toxoplasma gondii*in the testes in which the reduced sperm quality was attributed to opportunistic infection by Cytomegalovirus, as this virus has been found to cause primary hypogonadism in CMV-infected men; but 68% of the patients did not show CMV infection (Eggert-Kruse *et al.*, 2009). This suggests that these opportunistic infections are probably not the key factor causing the observed reduced semen quality in the HIV-infected men and therefore, HIV might have a direct effect as a cause of low seminal indices (De Paepe *et al.*, 1989; De Paepe *et al.*, 1990). Few reports have asserted HIV acts directly by disrupting the well coordinated physiological activity in the hypothalamic-pituitary-testis axis. Those studies reported that men with AIDS often showed low testosteronemia and a dysfunction in hypothalamic gonadotropin releasing hormone secretion which may account for this phenomenon (Dobs *et al.*, 1988). The low serum testosterone levels in men with AIDS are in some cases associated with high serum LH and FSH levels, implying that there is primary testicular failure (Croxson *et al.*, 1989). A possible reason for the testosterone secretary defect in these men was explained by reports that cytokines released by the activated phagocytic cells of

the immune system inhibit the steroidogenic response to human chorionic gonadotropin in vitro and presumably also the response to LH in vivo (Calkins *et al.*, 1988).

HIV infection of the testis has now been described in some recent studies, indicating a direct local action, which might be responsible for the observed changes within the gonads (da Silva *et al.*, 1990).

5.2 Conclusion And Recommendations

The study suggests that HIV infection has detrimental effect on testicular tissues, hence the low semen quality produced by HIV-infected male. The study has indeed shown that the semen quality of seropositive males majorly declines with declining blood absolute $CD4^+$count of the individual seropositive ART naïve males. ART significantly improved a number of critical semen parameters such as progressive motile sperm, reduced immotile sperm, sperm concentration and total sperm count, loss of immature germ cells, reduced leukocyte number (HPF), reduced red cells (HPF) and improved sperm morphology compared with ART naïve group with equivalent absolute $CD4^+$ count at $P \leq 0.05$. However, the ameliorating effect of ART was dependent on the duration of treatment.

I recommend further studies be carried out on the following to make the findings conclusive:

effect of HIV/ ART on functions of the associated reproductive glands; effect of very long term ART on semen quality of seropositive men; effect of HIV-infection and/ or ART on Chromosomal competence and effect of HIV-infection on plasma testosterone levels.

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APPENDIX

Age distribution and comparison

Table 1a: Summary Statistics

Groups	*Count*	*Average*	*Standard deviation*	*Coeff. of variation*	*Minimum*	*Maximum*	*Range*	*Stnd. skewness*	*Stnd. kurtosis*
Age $_{G5}$	29	38.3793	5.0879	13.2569%	29.0	47.0	18.0	-0.453	-0.738
Age $_{G3}$	25	41.56	6.75204	16.2465%	32.0	58.0	26.0	1.611	0.075
Age $_{G4}$	25	42.16	9.88551	23.4476%	22.0	62.0	40.0	0.847	-0.017
Age $_{G1}$	25	41.04	7.19073	17.5213%	28.0	58.0	30.0	1.179	-0.056
Age $_{G2}$	25	37.76	6.3854	16.9105%	28.0	53.0	25.0	0.851	-0.322

Table 1b: ANOVA Table

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	404.148	4	101.037	1.96	0.10
Within groups	6383.87	124	51.4828		
Total (Corr.)	6788.02	128			

Since the P-value of the F-test is greater than 0.05, there is no statistically significant difference between the means of the 5 variables at the 95.0% confidence level.

Table 1c: Table of Means with 95.0 percent LSD intervals

Groups	*Count*	*Mean*	*Stnd. error (pooled)*
Age $_{G5}$	29	38.38	1.3324
Age $_{G3}$	25	41.56	1.4350
Age $_{G4}$	25	42.16	1.4350
Age $_{G1}$	25	41.04	1.4350
Age $_{G4}$	25	37.76	1.4350

This is a table shows the mean for each column of data. It also shows the standard error of each mean, which is a measure of its sampling variability. There are no differences between the five groups statistically.

Immature Germ Cell (IGC)proportions and comparison between groups

Table 2a: Summary Statistics

Groups	*Count*	*Average*	*Standard deviation*	*Coeff. of variation*	*Minimum*	*Maximum*	*Range*	*Stnd. skewness*	*Stnd. kurtosis*
IGC $_{G3}$	25	24.64	12.1891	49.4686%	9.0	47.0	38.0	1.15304	-1.054
IGC $_{G4}$	25	18.28	11.4656	62.7221%	5.0	50.0	45.0	2.55668	1.555
IGC $_{G1}$	25	41.84	15.4776	36.9924%	14.0	75.0	61.0	0.78889	-0.290
IGC$_{G2}$	25	20.48	10.6931	52.2126%	4.0	41.0	37.0	0.865094	-0.799
IGC $_{G5l}$	29	1.655	2.93106	177.085%	0.0	13.0	13.0	5.38542	7.920

Table 2b: ANOVA Table

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	22226.1	4	5556.52	44.58	0.00
Within groups	15455.0	124	124.637		
Total (Corr.)	37681.0	128			

Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the means of the 5 variables at the 95.0% confidence level. Multiple Range Test (Fischer Least Significant Difference) was thus used to determine which means are significantly different from which others.

Table 2c: Table of Means with 95.0 percent LSD (least significant difference) intervals

Groups	*Count*	*Mean*	*Stnd. error*
IGC $_{G3}$	25	24.64^{b}	2.2328
IGC $_{G4}$	25	18.28^{b}	2.2328
IGC $_{G1}$	25	41.84^{c}	2.2328
IGC $_{G2}$	25	20.48^{b}	2.2328
IGC$_{G5}$	29	1.655^{a}	2.0731

This is a table shows the mean for each column of data. It also shows the standard error of each mean, which is a measure of its sampling variability.Means with the different superscript are significantly different at the 95% confidence Interval.

Sperm Concentration and comparison between groups

Table 3a: Summary Statistics

Groups	*Count*	*Average*	*Standard deviation*	*Coeff. of variation*	*Minimum*	*Maximum*	*Range*	*Stnd. skewness*	*Stnd. kurtosis*
Sperm $Conc_{G5}$	29	6.81172E7	5.5112E7	80.9075%	2.5E7	3.2E8	2.95E8	8.0715	17.8143
Sperm $Conc_{G3}$	25	2.9108E7	2.27764E7	78.2479%	6.E6	8.6E7	8.E7	2.1091	0.0019
Sperm $Conc_{G4}$	25	3.5784E7	1.36264E7	38.0797%	1.68E7	6.5E7	4.82E7	1.2846	-0.3375
Sperm $Conc_{G1}$	25	1.6088E7	1.90752E7	118.568%	0.0	7.E7	7.E7	3.5179	2.4504
Sperm $Conc_{G2}$	25	3.3348E7	2.31817E7	69.5147%	0.0	7.E7	7.E7	0.0921	-1.4113

Table 3b: ANOVA Table

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	4.0912E16	4	1.0228E16	10.26	0.00
Within groups	1.23582E17	124	9.96629E14		
Total (Corr.)	1.64494E17	128			

Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the means of the 5 variables at the 95.0% confidence level. Multiple Range Test (Fischer LSD) was thus used to determine which means are significantly different from which others.

Table 3c: Table of Means with 95.0 percent LSD intervals

Groups	*Count*	*Mean*	*Stnd. error (pooleds)*
Sperm $Conc_{G5}$	29	6.81172E7[a]	5.8623E6
Sperm $Conc_{G3}$	25	2.9108E7[b,c]	6.3138E6
Sperm $Conc_{G4}$	25	3.5784E7[b,c]	6.3138E6
Sperm $Conc_{G1}$	25	1.6088E7[b]	6.3138E6
Sperm $Conc_{G2}$	25	3.3348E7[b,c]	6.3138E6

This is a table shows the mean for each column of data. It also shows the standard error of each mean, which is a measure of its sampling variability. Means with the different superscript are significantly different at the 95% confidence Interval.

Total Sperm Count Between The Groups

Summary Statistics

Groups	*Count*	*Average*	*Standard deviation*	*Coeff. of variation*	*Minimum*	*Maximum*	*Range*	*Stnd. skewness*	*Stnd. kurtosis*
TSC_{G1}	25	2.14856E7	3.14401E7	146.331%	0.0	1.33E8	1.33E8	4.88509	6.2966
TSC_{G2}	25	5.62888E7	5.20624E7	92.4917%	0.0	1.38E8	1.38E8	0.940488	-1.5880
TSC_{G3}	25	5.4738E7	7.03501E7	128.521%	5.40E6	3.27E8	3.21E8	5.51633	9.0868
TSC_{G4}	25	6.32888E7	4.25513E7	67.2335%	1.40E7	2.13E8	1.99E8	4.01843	5.5402
TSC_{G5}	29	2.07396E8	2.09514E8	101.021%	3.51E7	9.60E8	9.25E8	5.04506	6.2824

ANOVA Table

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	5.90557E17	4	1.47639E17	12.37	0.00
Within groups	1.48009E18	124	1.19362E16		
Total (Corr.)	2.07065E18	128			

Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the means of the 5 variables at the 95.0% confidence level. Multiple Range Test (Fischer LSD) was thus used to determine which means are significantly different from which others.

Table of Means with 95.0 percent LSD intervals

Groups	*Count*	*Mean*	*Stnd. error (pooled)*
TSC_{G1}	25	2.14856E7[a]	2.18506E7
TSC_{G2}	25	5.62888E7[a]	2.18506E7
TSC_{G3}	25	5.4738E7[a]	2.18506E7
TSC_{G4}	25	6.32888E7[a]	2.18506E7
TSC_{G5}	29	2.07396E8[b]	2.02878E7

This is a table shows the mean for each column of data. It also shows the standard error of each mean, which is a measure of its sampling variability. Means with the different superscript are significantly different at the 95% confidence Interval.

White Blood Cells (WBC) proportions and comparison between the groups

Table 4a: Summary Statistics

Groups	*Count*	*Average*	*Standard deviation*	*Coeff. of variation*	*Minimum*	*Maximum*	*Range*	*Stnd. skewness*	*Stnd. kurtosis*
WBC $_{G1}$	25	12.80	7.5498	58.9831%	4.0	34.0	30.0	2.2028	1.0735
WBC $_{G2}$	25	5.96	3.0343	50.9102%	2.0	13.0	11.0	1.6729	-0.1523
WBC $_{G3}$	25	10.72	9.0669	84.5800%	2.0	41.0	39.0	4.0512	4.6963
WBC $_{G4}$	25	3.92	1.4697	37.4922%	1.0	8.0	7.0	0.8286	1.5623
WBC $_{G5}$	29	4.00	4.6522	116.305%	0.0	20.0	20.0	4.2616	4.5217

Table 4b: ANOVA Table

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	1694.3	4	423.575	12.45	0.00
Within groups	4219.84	124	34.031		
Total (Corr.)	5914.14	128			

Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the means of the 5 variables at the 95.0% confidence level. Multiple Range Test (Fischer LSD) was thus used to determine which means are significantly different from which others.

Table 4c: Means with 95.0 percent LSD intervals

Groups	*Count*	*Mean*	*Stnd. error (pooled)*
WBC $_{G1}$	25	12.80^{b}	1.1667
WBC $_{G2}$	25	5.96^{a}	1.1667
WBC $_{G3}$	25	10.72^{b}	1.1667
WBC $_{G4}$	25	3.92^{a}	1.1667
WBC $_{G5}$	29	4.00^{a}	1.0832

This is a table shows the mean for each column of data. It also shows the standard error of each mean, which is a measure of its sampling variability.Means with the different superscript are significantly different at the 95% confidence Interval.

Red Blood Cells proportions and comparison between the grups

Table 5a: Summary Statistics

Groups	*Count*	*Average*	*Standard deviation*	*Coeff. of variation*	*Minimum*	*Maximum*	*Range*	*Stnd. skewness*	*Stnd. kurtosis*
RBC_{G2}	25	6.56	6.8195	103.957%	0.0	23.0	23.0	1.7630	-0.1828
RBC_{G3}	25	5.44	6.8012	125.023%	0.0	21.0	21.0	2.8602	0.9069
RBC_{G4}	25	5.6	14.2068	253.693%	0.0	65.0	65.0	7.0484	13.5508
RBC_{G1}	25	13.96	16.2031	116.068%	0.0	52.0	52.0	1.8592	-0.2651
RBC_{G5}	29	0.0	0.0	-	0.0	0.0	0.0	-	-

Table 5b: ANOVA Table

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	2645.98	4	661.494	6.13	0.00
Within groups	13371.3	124	107.833		
Total (Corr.)	16017.3	128			

Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the means of the 5 variables at the 95.0% confidence level. Multiple Range Test (Fischer LSD) was thus used to determine which means are significantly different from which others.

Table 5c: Means with 95.0 percent LSD intervals

Groups	*Count*	*Mean*	*Stnd. error (pooled)*
RBC_{G2}	25	$6.56^{b,a}$	2.07685
RBC_{G3}	25	5.44^{a}	2.07685
RBC_{G4}	25	5.60^{a}	2.07685
RBC_{G1}	25	13.96^{c}	2.07685
RBC_{G5}	29	0.0^{a}	1.92831

This is a table shows the mean for each column of data. It also shows the standard error of each mean, which is a measure of its sampling variability.Means with the different superscript are significantly different at the 95% confidence Interval.

Rapid Progressive Motile Sperm proportions and comparison between the groups

Table 6a: Summary Statistics

Groups	*Count*	*Average*	*Standard deviation*	*Coeff. of variation*	*Minimum*	*Maximum*	*Range*	*Stnd. skewness*	*Stnd. kurtosis*
RPM_{G1}	25	3.60	7.84219	217.839%	0.0	30.0	30.0	4.7715	5.1093
RPM_{G2}	25	10.72	15.5952	145.478%	0.0	50.0	50.0	2.6332	0.5309
RPM_{G3}	25	7.28	12.1261	166.568%	0.0	40.0	40.0	3.7479	2.7394
RPM_{G4}	25	14.60	22.0454	150.996%	0.0	85.0	85.0	4.0096	3.9499
RPM_{G5}	29	42.41	25.8644	60.981%	0.0	80.0	80.0	-0.1318	-1.3416

Table 6b: ANOVA Table

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	26684.8	4	6671.2	20.06	0.00
Within groups	41237.1	124	332.557		
Total (Corr.)	67921.9	128			

Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the means of the 5 variables at the 95.0% confidence level. Multiple Range Test (Fischer LSD) was thus used to determine which means are significantly different from which others.

Table 6c: Means with 95.0 percent LSD intervals

Groups	*Count*	*Mean*	*Stnd. error (pooled)*
RPM_{G1}	25	3.60^{a}	3.6472
RPM_{G2}	25	10.72^{b}	3.6472
RPM_{G3}	25	7.280^{b}	3.6472
RPM_{G4}	25	14.60^{b}	3.6472
RPM_{G5}	29	42.41^{c}	3.3864

This is a table shows the mean for each column of data. It also shows the standard error of each mean, which is a measure of its sampling variability. Means with the different superscript are significantly different at the 95% confidence Interval.

Slow Progressive Motile Sperm proportions and comparison between the groups

Table 7a: Summary Statistics

Groups	*Count*	*Average*	*Standard deviation*	*Coeff. of variation*	*Minimum*	*Maximum*	*Range*	*Stnd. skewness*	*Stnd. kurtosis*
SPM_{G1}	25	11.04	15.3283	138.843%	0.0	50.0	50.0	2.25285	0.149
SPM_{G2}	25	16.88	16.6591	98.6915%	0.0	50.0	50.0	1.07199	-0.868
SPM_{G3}	25	16.8	22.0737	131.391%	0.0	60.0	60.0	2.09584	-0.422
SPM_{G4}	25	21.84	18.9905	86.9529%	0.0	75.0	75.0	1.86925	1.060
SPM_{G5}	29	17.069	11.3009	66.207%	0.0	50.0	50.0	3.15825	2.008

Table 7b: ANOVA Table

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	1466.22	4	366.554	1.25	0.29
Within groups	36224.8	124	292.136		
Total (Corr.)	37691.0	128			

*Since the P-value of the F-test is greater than 0.05, there is no statistically significant difference between the means of the 5 variables at the 95.0% confidence level.

Table 7c: Means with 95.0 percent LSD intervals

Groups	*Count*	*Mean*	*Stnd. error (pooled)*
SPM_{G1}	25	11.04^{a}	3.4184
SPM_{G2}	25	16.88^{ab}	3.4184
SPM_{G3}	25	16.80^{ab}	3.4184
SPM_{G4}	25	21.84^{b}	3.4184
SPM_{G5}	29	17.07^{ab}	3.1739

The whole groups pooled together did not show significance at 5% level. However, some groups were differences statistically. Groups with different means statistically have different superscripts.

Non-Progressive Motile Sperm proportions and comparison between the groups

Table 8a: Summary Statistics

Groups	*Count*	*Average*	*Standard deviation*	*Coeff. of variation*	*Minimum*	*Maximum*	*Range*	*Stnd. skewness*	*Stnd. kurtosis*
NPM_{G1}	25	19.40	17.4595	89.9973%	0.0	50.0	50.0	0.02691	-1.6344
NPM_{G2}	25	28.36	13.8048	48.6771%	0.0	50.0	50.0	-0.9030	-0.3254
NPM_{G3}	25	30.60	14.7422	48.1772%	0.0	60.0	60.0	-0.3901	0.61082
NPM_{G4}	25	29.76	16.6490	55.9443%	0.0	65.0	65.0	0.4816	-0.5852
NPM_{G5}	29	27.41	19.5311	71.2457%	0.0	70.0	70.0	1.4488	-0.9458

Table 8b: ANOVA Table

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	2007.9	4	501.975	1.81	0.13
Within groups	34439.4	124	277.737		
Total (Corr.)	36447.3	128			

*Since the P-value of the F-test is greater than 0.05, there is no statistically significant difference between the means of the 5 variables at the 95.0% confidence level.

Table 8c: Means with 95.0 percent LSD intervals

Groups	*Count*	*Mean*	*Stnd. error (pooled)*
NPM_{G1}	25	19.40^{c}	3.3331
NPM_{G2}	25	28.36^{a}	3.3331
NPM_{G3}	25	30.60^{ab}	3.3331
NPM_{G4}	25	29.76^{ab}	3.3331
NPM_{G5}	29	27.41^{a}	3.0947

This is a table shows the mean for each column of data. It also shows the standard error of each mean, which is a measure of its sampling variability. There were differences between the five groups statistically. Groups with different means statistically have different superscripts.

Immotile Sperm proportions and comparison between the groups

Table 9a: Summary Statistics

Groups	*Count*	*Average*	*Standard deviation*	*Coeff. of variation*	*Minimum*	*Maximum*	*Range*	*Stnd. skewness*	*Stnd. kurtosis*
$Immotile_{G1}$	25	47.16	36.7136	77.85%	0.0	100.0	100.0	0.150	-1.319
$Immotile_{G2}$	25	40.68	28.0234	68.89%	0.0	100.0	100.0	0.564	-0.770
$Immotile_{G3}$	25	42.92	28.669	66.80%	10.0	100.0	90.0	0.723	-1.142
$Immotile_{G4}$	25	31.40	23.4236	74.60%	0.0	75.0	75.0	1.015	-0.974
$Immotile_{G5}$	29	13.28	17.5904	132.50%	0.0	50.0	50.0	2.548	-0.097

Table 9b: ANOVA Table

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	20036.8	4	5009.2	6.70	0.00
Within groups	92754.4	124	748.02		
Total (Corr.)	112791.	128			

Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the means of the 5 variables at the 95.0% confidence level. Multiple Range Test (Fischer LSD) was thus used to determine which means are significantly different from which others.

Table 9c: Means with 95.0 percent LSD intervals

Groups	*Count*	*Mean*	*Stnd. error(pooled)*
$Immotile_{G1}$	25	47.16^{c}	5.4699
$Immotile_{G2}$	25	40.68^{bc}	5.4699
$Immotile_{G3}$	25	42.92^{bc}	5.4699
$Immotile_{G4}$	25	31.40^{b}	5.4699
$Immotile_{G5}$	29	13.28^{a}	5.0787

This is a table shows the mean for each column of data. It also shows the standard error of each mean, which is a measure of its sampling variability. Means with the different superscript are significantly different at the 95% confidence Interval.

Normal Morphology Sperm proportions and comparison between the groups

Table 10a: Summary Statistics

Groups	*Count*	*Average*	*Standard deviation*	*Coeff. of variation*	*Minimum*	*Maximum*	*Range*	*Stnd. skewness*	*Stnd. kurtosis*
Morphgy Normal $_{G3}$	25	55.24	15.7567	28.5241%	20.0	80.0	60.0	-1.10429	-0.3632
Morphgy Normal $_{G4}$	25	60.12	16.7166	27.8053%	20.0	85.0	65.0	-2.22962	0.9347
Morphgy Normal $_{G1}$	25	48.4	26.3676	54.4785%	0.0	80.0	80.0	-2.38054	-0.1341
Morphgy Normal $_{G2}$	25	61.4	20.4898	33.3711%	0.0	85.0	85.0	-3.69436	3.4777
Morphgy Normal $_{G5}$	29	82.069	11.4578	13.9612%	50.0	90.0	40.0	-4.02679	3.1825

Table 10b: ANOVA Table

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	17543.0	4	4385.75	12.62	0.00
Within groups	43103.1	124	347.605		
Total (Corr.)	60646.1	128			

Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the means of the 5 variables at the 95.0% confidence level. Multiple Range Test (Fischer LSD) was thus used to determine which means are significantly different from which others.

Table 10c: Means with 95.0 percent LSD intervals

Groups	*Count*	*Mean*	*Stnd. error (pooled)*
Morphology Normal $_{G3}$	25	$55.24^{b,a}$	3.7288
Morphology Normal $_{G4}$	25	60.12^{b}	3.7288
Morphology Normal $_{G1}$	25	48.40^{a}	3.7288
Morphology Normal $_{G2}$	25	61.40^{b}	3.7288
Morphology Normal $_{G5}$	29	82.07^{c}	3.4621

This is a table shows the mean for each column of data. It also shows the standard error of each mean, which is a measure of its sampling variability. Means with the different superscript are significantly different at the 95% confidence Interval.

Abnormal Morphology Sperm proportions and comparison between the groups

Table 11a: Summary Statistics

Groups	*Count*	*Average*	*Standard deviation*	*Coeff. of variation*	*Minimum*	*Maximum*	*Range*	*Stnd. skewness*	*Stnd. kurtosis*
Morphgy Abnormal $_{G3}$	25	44.72	15.6964	35.0993%	20.0	80.0	60.0	1.0969	-0.3508
Morphgy Abnormal$_{G2}$	25	34.6	17.5547	50.7361%	0.0	90.0	90.0	2.4801	3.4635
Morphgy Abnormal $_{G5}$	29	17.5862	11.5434	65.6392%	10.0	50.0	40.0	4.1216	3.2686
Morphgy Abnormal $_{G4}$	25	39.88	16.7166	41.9171%	15.0	80.0	65.0	2.2296	0.9349
Morphgy Abnormal$_{G1}$	25	31.6	18.5809	58.8003%	0.0	60.0	60.0	-1.2195	-0.2749

Table 11b: ANOVA Table

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	11616.2	4	2904.05	11.24	0.00
Within groups	32032.7	124	258.328		
Total (Corr.)	43648.9	128			

Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the means of the 5 variables at the 95.0% confidence level. Multiple Range Test (Fischer LSD) was thus used to determine which means are significantly different from which others.

Table 11c: Means with 95.0 percent LSD intervals

Groups	*Count*	*Mean*	*Stnd. error (pooled)*
Morphology Abnormal $_{G3}$	25	44.72^{c}	3.2145
Morphology Abnormal $_{G2}$	25	34.60^{b}	3.2145
Morphology Abnormal $_{G5}$	29	17.59^{a}	2.9846
Morphology Abnormal $_{G4}$	25	39.88^{b,c}	3.2145
Morphology Abnormal $_{G1}$	25	31.60^{b}	3.2145

This is a table shows the mean for each column of data. It also shows the standard error of each mean, which is a measure of its sampling variability. Means with the different superscript are significantly different at the 95% confidence Interval.

Sperm Vitality proportions and comparison between the groups

Table 12a: Summary Statistics

Groups	*Count*	*Average*	*Standard deviation*	*Coeff. of variation*	*Minimum*	*Maximum*	*Range*	*Stnd. skewness*	*Stnd. kurtosis*
Vitality $_{G5}$	29	92.52	5.5396	5.98764%	80.0	99.0	19.0	-1.7989	-0.1004
Vitality $_{G3}$	25	76.68	14.859	19.3788%	35.0	98.0	63.0	-2.6195	1.7895
Vitality $_{G4}$	25	70.72	15.257	21.5746%	45.0	90.0	45.0	-0.8650	-1.3055
Vitality $_{G1}$	25	58.12	32.049	55.1443%	0.0	90.0	90.0	-2.2979	-0.3126
Vitality $_{G2}$	25	84.32	6.3424	7.52188%	70.0	95.0	25.0	-1.6486	0.3839

Table 12b: ANOVA Table

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	18222.1	4	4555.52	15.12	0.00
Within groups	37363.8	124	301.321		
Total (Corr.)	55585.9	128			

Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the means of the 5 variables at the 95.0% confidence level. Multiple Range Test (Fischer LSD) was thus used to determine which means are significantly different from which others.

Table 12c: Means with 95.0 percent LSD intervals

Groups	*Count*	*Mean*	*Stnd. error (pooled)*	*Lower limit*	*Upper limit*
Vitality $_{G5}$	29	92.52^{a}	3.2234	88.0059	97.0286
Vitality $_{G3}$	25	$76.68^{b,c}$	3.4717	71.8211	81.5389
Vitality $_{G4}$	25	70.72^{c}	3.4717	65.8611	75.5789
Vitality $_{G1}$	25	58.12^{d}	3.4717	53.2611	62.9789
Vitality $_{G2}$	25	$84.32^{a,b}$	3.4717	79.4611	89.1789

This is a table shows the mean for each column of data. It also shows the standard error of each mean, which is a measure of its sampling variability.Means with the different superscript are significantly different at 5% significant level.

Semen Volumes and comparison between the groups

Table 13a: Summary Statistics

Groups	*Count*	*Average*	*Standard deviation*	*Coeff. of variation*	*Minimum*	*Maximum*	*Range*	*Stnd. skewness*	*Stnd. kurtosis*
Volume $_{G5}$	29	2.87241	1.43798	50.0617%	1.0	7.0	6.0	2.0013	1.1944
Volume $_{G3}$	25	1.748	0.962254	55.0489%	0.2	3.8	3.6	0.2323	-0.7370
Volume $_{G4}$	25	1.788	0.844748	47.2454%	0.4	3.8	3.4	1.0115	-0.0108
Volume $_{G1}$	25	1.552	1.09244	70.3894%	0.2	4.3	4.1	1.0568	-0.0994
Volume $_{G2}$	25	1.632	0.8854	54.2525%	0.2	2.9	2.7	-0.5713	-1.0345

Table 13b: ANOVA Table

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	32.8385	4	8.20962	7.04	0.00
Within groups	144.704	124	1.16696		
Total (Corr.)	177.542	128			

Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the means of the 5 variables at the 95.0% confidence level. Multiple Range Test (Fischer LSD) was thus used to determine which means are significantly different from which others.

Table 13c: Means with 95.0 percent LSD intervals

Groups	*Count*	*Mean*	*Stnd. error (pooleds)*	*Lower limit*	*Upper limit*
Volume $_{G5}$	29	2.872[a]	0.200599	2.59166	3.1532
Volume $_{G3}$	25	1.748[b]	0.216052	1.44562	2.0504
Volume $_{G4}$	25	1.788[b]	0.216052	1.48562	2.0904
Volume $_{G1}$	25	1.552[b]	0.216052	1.24962	1.8544
Volume $_{G2}$	25	1.632[b]	0.216052	1.32962	1.9344

This is a table shows the mean for each column of data. It also shows the standard error of each mean, which is a measure of its sampling variability.Means with the different superscript are significantly different at 5% significant level.

Sexual Abstinence Periods and comparison between the groups

Table 14a: Summary Statistics

Groups	*Count*	*Average*	*Standard deviation*	*Coeff. of variation*	*Minimum*	*Maximum*	*Range*	*Stnd. skewness*	*Stnd. kurtosis*
Abstinence period $_{G5}$	29	7.89655	4.15198	52.5796%	3.0	21.0	18.0	3.3543	2.5249
Abstinence period $_{G3}$	25	9.44	6.69627	70.935%	3.0	30.0	27.0	5.2496	6.3342
Abstinence period $_{G4}$	25	12.6	9.88264	78.4337%	3.0	35.0	32.0	2.69089	0.2324
Abstinence period $_{G1}$	25	26.92	11.1277	41.3363%	7.0	50.0	43.0	-0.4543	0.0713
Abstinence period $_{G2}$	25	12.6	10.0	79.3651%	6.0	30.0	24.0	2.6179	-0.3791

Table 14b: ANOVA Table

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	5860.12	4	1465.03	19.59	0.00
Within groups	9274.69	124	74.7959		
Total (Corr.)	15134.8	128			

Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the means of the 5 variables at the 95.0% confidence interval. Multiple Range Test (Fischer LSD) was thus used to determine which means are significantly different.

Table 14c: Means with 95.0 percent LSD intervals

Groups	*Count*	*Mean*	*Stnd. error (pooled)*
Abstinence period $_{G5}$	29	7.90[a]	1.6059
Abstinence period $_{G3}$	25	9.44[a,b]	1.7296
Abstinence period $_{G4}$	25	12.60[b]	1.7296
Abstinence period $_{G1}$	25	26.92[c]	1.7296
Abstinence period $_{G2}$	25	12.60[b]	1.7296

This is a table shows the mean for each column of data. It also shows the standard error of each mean, which is a measure of its sampling variability.Means with the different superscript are significantly different at the 5% significant level.

Semen pH comparison between the groups

Table 15a: Summary Statistics

Groups	*Count*	*Average*	*Standard deviation*	*Coeff. of variation*	*Minimum*	*Maximum*	*Range*	*Stnd. skewness*	*Stnd. kurtosis*
pH $_{G5}$	29	7.19	0.431231	5.99794%	6.5	8.5	2.0	2.06608	2.1711
pH $_{G3}$	25	8.12	0.439697	5.41499%	7.0	9.0	2.0	0.583343	1.7562
pH $_{G4}$	25	8.08	0.533854	6.6071%	7.0	9.0	2.0	-0.70143	0.7891
pH $_{G1}$	25	8.16	0.374166	4.58536%	7.5	9.0	1.5	2.70866	1.3510
pH $_{G2}$	25	8.14	0.445346	5.47108%	7.0	9.0	2.0	0.321531	1.4739

Table 15b: ANOVA Table

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	19.7551	4	4.93878	24.69	0.00
Within groups	24.8069	124	0.200056		
Total (Corr.)	44.562	128			

Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the means of the 5 variables at the 95.0% confidence level.

Table 15c: Means with 95.0 percent LSD intervals

Groups	*Count*	*Mean*	*Stnd. error (pooled)*
pH $_{G5}$	29	7.19^{a}	0.083057
pH $_{G3}$	25	8.12^{b}	0.089455
pH $_{G4}$	25	8.08^{b}	0.089455
pH $_{G1}$	25	8.16^{b}	0.089455
pH $_{G2}$	25	8.14^{b}	0.089455

This is a table shows the mean for each column of data. It also shows the standard error of each mean, which is a measure of its sampling variability. Differences exist between means with different superscript at 5% level.

Absolute $CD4^+$ count comparison between the groups

Table 16a: Summary Statistics

Groups	*Count*	*Average*	*Standard deviation*	*Coeff. of variation*	*Minimum*	*Maximum*	*Range*	*Stnd. skewness*	*Stnd. kurtosis*
Absolute $CD4_{G5}$	29	991.52	516.3760	52.0794%	454.0	3123.0	2669.0	6.3044	11.9164
Absolute $CD4_{G3}$	25	371.72	64.4409	17.3359%	257.0	492.0	235.0	-0.2280	-1.0844
Absolute $CD4_{G4}$	25	338.32	83.1313	24.5718%	200.0	491.0	291.0	-0.1988	-0.8874
Absolute $CD4_{G1}$	25	311.24	55.9585	17.9792%	251.0	429.0	178.0	1.5795	-0.6396
Absolute $CD4_{G2}$	25	564.12	75.1777	13.3266%	502.0	733.0	231.0	2.4648	0.3454

Table 16b: ANOVA Table

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	8.51489E6	4	2.12872E6	35.05	0.00
Within groups	7.40908E6	122	60730.1		
Total (Corr.)	1.5924E7	126			

Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the means of the 5 variables at the 95.0% confidence level. Multiple Range Test (Fischer LSD) was thus used to determine which means are significantly different.

Table 16c: Means with 95.0 percent LSD intervals

Groups	*Count*	*Mean*	*Stnd. error (pooled)*
Absolute $CD4_{G5}$	29	991.52^a	47.426
Absolute $CD4_{G3}$	25	371.72^b	49.287
Absolute $CD4_{G4}$	25	338.32^b	49.287
Absolute $CD4_{G1}$	25	311.24^b	49.287
Absolute $CD4_{G2}$	25	564.12^c	49.287

This is a table shows the mean for each column of data. It also shows the standard error of each mean, which is a measure of its sampling variability. Means with the different superscript are significantly different at the 95% confidence Interval.

Percentate Distribution of the various colours of semen samples in the different groups

Semen Colour	G1	G2	G3	G4	G5
Grey	32	45	36.67	40	80
Greyish-White	4	23	4.17	4	16
Brown	64	24	50.83	38	0
Low Blood stain	0	4	4.17	4	0
Yellow	0	4	4.17	14	4

A Distribution showing the viscosity of various semen samples in different groups

Semen Viscosity	G1	G2	G3	G4	G5
High	19	12	14	8	12
Slightly higher	44	50	34	36	14
Normal	27	30	30	41	66
Low	10	8	22	15	8

Meaning of abbreviations used on the tables:

Mph- Morphology

NPM-Non-progressive motility

RPM- Rapid progressive motility

SPM- Slow progressive motility

IGC- Immature germ cells

Conc - Concentration

RBC- Red Blood Cell

WBC- White Blood Cells

The subscripts denote the groups

Solutions Used for Semen Analysis

Physiological Saline (0.85% w/v)

- 8.5 grams of sodium chloride was weighed and transferred to a leak-proof bottle premarked 1 litre flat bottom flask.
- Distilled water was added to the salt and mixed until the salt was fully dissolved.
- The bottle was the labeled and stored for use.

Sodium bicarbonate-formalin semen diluting fluid

- 5 grams of Sodium bicarbonate was measured and transferred to a clean bottle.
- 100 ml of distilled water was added to the salts and mixed well to dissolve.
- 1 ml of concentrated formaldehyde solution was then added and mixed well. The bottle was then labeled and marked toxic and stored well for use.

Eosin Solution

- 5 grams of the eosin was dissolved in a liter of physiological saline prepared above to obtain the eosin solution for sperm vitality assessment.

Preparation of Staining Solution sperm morphology (Gram stain)

Crystal Violet Solution (Solution A)

- 0.5 grams of pure crystal violet was weighed and dissolved in a 100 ml of distilled water in 200 ml clean glass beaker and stirred gently to obtain a uniform solution.

Lugol's Iodine Solution (Solution B)

- Solution B, called the mordant was prepared by dissolving 2 grams of Potassium iodide (KI) in 100 ml of distilled water and swirled gently to dissolve completely.
- 1 gram of Iodide crystal was then added to the solution and swirled for complete dissolution to take place.

Acetone-alcohol Decolourizer

470 ml of absolute ethanol was measured and transferred into a bottle of 1 litre capacity. 25ml of distilled water was then added to the alcohol.

500 ml of acetone was measured and added to the alcohol solution and mixed thoroughly to obtain a uniform solution.

The reagent was labeled stored till ready to be used.

Safranin Solution (Solution C)

- The counter stain was prepared by first dissolving 2.5 grams safranin stock solution in 100 ml of 96% ethanol.
- Next, 10 ml of the resultant solution was pipetted into 90 ml of distilled water and mixed thoroughly until a uniform solution was obtained as the working solution C.

Record form for semen analysis

Date of Sample Collection	**Sample 1**	**Sample 2**
Duration of Abstinence (Days)		
Time(s) of specimen production and specimen analysis		
Interval between ejaculation and start of Analysis(Min)		
Appearance (1-Normal, 2- Abnormal)		
Type of Abnormality(if any)		
Liquefaction (1-Normal, 2- Abnormal)		
Liquefaction Time		
Consistency (1-Normal, 2- Abnormal)		
Volume (mL)		
pH		
Motility (% Spermatozoa) A. Rapid progression B. Slow progression C. Non-progressive D. Immotile		
Agglutination (%)		
Vitality (% Live)		
Concentration (10^6 /ml)		

Morphology (%) - Normal - Head defects - Neck or midpiece defects - Tail defects - Cytoplasmic droplets		
White blood cells (10^6/ ml)		
Immature germ cells (10^6/ ml)		

INTERVIEW GUIDE TO ASCERTAIN THE EFFECT OF HIV INFECTION AND ANTIRETROVIRAL TREATMENT ON SEMEN QUALITY OF SEROPOSITIVE MALE PATIENTS

1	Code Number	
2	How old are you?	20-25, 26-30, 31-35, 36-40, 41-50, 51-55 Does not know
3	Where do you live?	District……Region……. Metropolis……..
4	Are you currently employed? What work do you do?	Yes/ No
5	What is the highest level of school you attained?	Basic Education, Secondary, Tertiary, None.
6	What is your marital status?	Single/ Married/ Living in union/ Widowed/ Divorced/ Separated
7	Do you have children? Have you ever fathered a child (dead or alive including babies who were aborted)?	Yes/ No
8	If yes, how many?	1, 2, 3,4
9	If childless, do you wish to have a child? Or do you want to have more children?	Yes/ No/ May be
10	Have you heard of HIV/AIDS?	Yes/ No
11	If yes, what are the ways through which one can be infected with the virus?	Having an unprotected sex with an infected person/ Eating with an infected person/ Other, specify
12	How then can one prevent HIV infection?	Abstinence/ Faithfulness to a single uninfected partner/ Other, specify
13	Is it possible for a healthy-looking person to be infected with the HIV virus?	Yes/ No/ Does not know
14	Can HIV be transmitted from a mother to a child?	Yes/ No/ Does not know
15	How long have you been diagnosed with HIV?	< 6MOTHS < 1YR >1YR Other, Specify
16	What is the HIV status of your partner, if	Infected/ Not infected/ Does not know

	applicable?	
17	Do you still have sex with your partner, if you have?	Yes/ No
18	If yes, do you use condom? Do you think condom use can give protection from reinfection?	Yes/ No Yes/ No/ Does not know
19	What is your CD4 count now?	
20	Have you started Antiretroviral treatment?	Yes/ No
21	If yes, how long have you been on Antiretroviral Therapy	
22	What drug regimen are you on?	(ZDT/ 3TC/ NVP),(ZDT/ 3TC/ EFV),(D4T/ 3TC/ NVP),(D4T/ 3TC/ EFV),(D4T/ 3TC/ NFV),D4T/3TC/ALUVIA), (CBV/ALUVIA), (CBV/NFV)
23	Have you ever used herbal medication since you were diagnosed with HIV?	Yes, No If Yes, what is its name?
24	What did you use it for?	
25	Are you currently using any herbal medication?	
26	Now that you are infected, do you plan of getting children with your wife?	Yes/ No
27	Do you know of the implications of such an action?	Yes/ No
28	Do you think the ART is working well for you?	Yes/ No
29	If yes, what improvement have you seen after commencing treatment?	
30	Have you noticed any physical changes in your body?	Yes/ No
31	If yes, what has changed?	
32	Have you noticed any testicular size change after HIV infection?	Yes/ No
33	Have you also noticed any testicular size change after commencing the therapy?	Yes/ No

34	If your Doctor recommends semen investigation to ascertain the effect of HIV (and/or Antiretroviral therapy) on your semen quality, would you be willing to provide semen samples for the test?	Yes/ No

Ethics Approval LetterForThe Study

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
COLLEGE OF HEALTH SCIENCES
SCHOOL OF MEDICAL SCIENCES
COMMITTEE ON HUMAN RESEARCH PUBLICATION AND ETHICS

Our Ref: CHRPE/105/09

December 15, 2009

Dr. Kweku Bedu-Addo
Department of Physiology
SMS-KNUST

Dear Sir,

LETTER OF APPROVAL
Protocol Title: *"The Effect of HIV Infection and Antiretroviral Therapy (ART) on Semen Quality"*

Sponsor: Principal Investigator

Your submission to the Committee on Human Research Publication and Ethics on the above named protocol refers.

The Committee has considered the ethical merit of your submission and approved the protocol. The approval is for a fixed period of one year. The committee may however, suspend or withdraw ethical approval at anytime if your study is found to contravene the approved protocol.

Data gathered for the study should be used for the approved purposes only. Permission should be sought from the committee if any amendment to the protocol or use, other than submitted, is made of your research data.

The Committee should be notified of the actual start date of the project and would expect a report on your study, annually or at close of the project, whichever one comes first. It should also be informed of any publication arising from the study.

Please call us on 0205453785 or email at chrpe.knust.kath@gmail.com if you have questions.

Many thanks for your application.

Yours faithfully,

Osomfuor Prof. Sir J.W. Acheampong
Chairman

Participant Information Leaflet and Consent Form

What every prospective participant should know before deciding to or not to participate

Title of Research: The effect of HIV/ AIDS and ART on semen quality.

Name(s) and affiliation(s) of researcher(s): Dr. K. Bedu-Addo (SMS, KNUST), Alberta BiritumKyarko (Kumasi South Regional Hospital), Emmanuel AmankwahNtim (SMS, KNUST).

Purpose(s) of research: The purpose of the study is to find the effect of HIV infection and ART on semen quality of HIV-infected men.

Procedure of the research, what shall be required of each participant and approximate total number of participants that would be involved in the research: Each participant would be expected to present two semen sample. Participants will be taken through the procedure on how to produce the semen samples, preferably, by masturbation into sterile containers that would be provided. Participants would be divided into those who are on ART treatment and those who are not on ART treatment. Those on ART would also be subdivided into clients who have received ART for less than a year and those who have received ART for a year and/ or more. Furthermore, those not on treatment would be subdivided based on whether the client is asymptomatic or exhibits the some degree of symptomatology of AIDS.

Risk(s): The only risk that has been identified by the study which may be caused to participants are 48 hour sexual abstinence and the difficulty of providing some information regarding the time the client was diagnosed and whether or not his partner(s) (wife or otherwise) has also been infected.The latter are sensitive issues which some of the clients may find it somehow uncomfortable to disclose.

Benefit(s): Some potential direct benefits to participants would be:

The infectiousness of men in serodiscordant relationship would be made known by their seminal viral loads. This would aid them to extra careful if they do not want their partner(s) to become infected. Also, for the participants who have been on ART for 3 months and above, in consultation with their medical officer, who is one of the principal investigators, would know whether or not the ART regimen a particular participant is on should be changed. Finally, participant would be given GHc5.00 to compensate them for their time and transportation. Furthermore, the potential benefit from this study science and health care is so enormous to both the study population, science and society as a whole for this simple reason. Sexual transmission of HIV in the developing nations has become aformidable burden which all governments and management of this pandemic have to contend with for all times in every part of the world. Due to the rate at which the HIV infection is spreading, it has become imperative for us the

developing nationals to consider pragmatic strategies to control the spread and effectively manage the HIV-infected patients who are in our midst. In the developing nations, routine management of seropositive patients basically is about administration of ART and monitoring of the immune system reconstitution by following the absolute $CD4^+$dynamics of the patients. The absolute $CD4^+$ count of patients give information about the health status of the patient, ie, whether the patient would develop opportunistic infections or not and how the patient health is improving. On the order hand, as we are prolonging their lives, a system should be in place to monitor the dynamics of their blood viral load and most importantly, the seminal viral loads of the males which account for the major percentage of the transmission of the virus. This is because, there would not be any success story to tell if, you prolong these people's lives but still remain very infectious. It only put the seronegative population at a higher risk! For now, it remains very expensive to run these viral loads tests and are not part of our routine lab test requested in the management of HIV patients in developing nations. This study however seeks to find the "weak-link" between seminal viral load and semen quality by first assessing the effect of HIV infection on semen quality as the main step. If that weak-link is found, it means that, routine semen analysis of HIV seropositive male patients could be used to predict the seminal viral loads of male patients. At least, this could help us for now that we don't have the technology and the money to run PCR test for the estimation of blood and seminal viral loads for routine effective management of those infected so as to contained the pandemic vigorously and prevent it spread to HIV naive populations which is at a very high risk now that those harbouring the virus are living longer.

Confidentiality: The answers that you give and the information that we collect from the questionnaire during this study will be kept private to the extent permitted by law. Research information that identifies you may be shared with the Ethics committee at the Kwame Nkrumah University of Science and Technology (KNUST) and others who are responsible for ensuring compliance with law and regulations. The compiled results of this survey may be published for scientific purposes; however, your identity will not be revealed. All data will be stored on secured computers and locked cabinets to which only research staff will have access. All information collected in this study will be given code numbers and no name will be recorded. This cannot be linked to you in anyway and your name or any identifier will not be used in any publication or reports from this study..

Voluntariness: Participation in this study is entirely voluntary.

Alternatives to participation: Alternate to participation is not to participate.

Withdrawal from the research: You may choose to withdraw from the research at anytime without having to explain yourself. You may also choose not to answer any question you find uncomfortable or private

Consequence of Withdrawal: You are free to withdraw your consent and to end your participation in this study at any time. This will have no effect on the service that you receive from your local health service.

Costs/Compensation: The study will be of no cost to you. However, GHc5.00 would be given to all participants for their time and transportation cost.

Contacts: If you have any questions about the research or a research related injury, Dr. Alberta BiritwumNyarko, attending physician at Kumasi South Hospital will be glad to answer them. Dr.Nyarko may be reached at 233-244-785-208 between the hours of 8:00 am and 5:00 pm, Monday through Friday. If you have questions about your rights as a research participant, or concerns or complaints about the research, you may contact either Dr. K. Bedu-Addo of Physiology Department, School of Medical Sciences, KNUST, on 233-242631589 OR Mr. Emmanuel A. Ntim, Demonstrator at the department of Physiology, School of Medical Sciences, KNUST.

Further, if you have any concern about the conduct of this study, your welfare or your rights as a research participant, you may contact:

The Chairman
Committee on Human Research and Publication Ethics
Kumasi
Tel: 22301-4 ext 1098 or 020 5453785

CONSENT FORM

Statement of person obtaining informed consent:

I have fully explained this research to _____ and have given sufficient information, including that about risks and benefits, to enable the prospective participant make an informed decision to or not to participate.

DATE: _____ SIGNATURE: _____

NAME: DR. K. BEDU-ADDO

Statement of person giving consent:

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

Name_____

DATE: _____ SIGNATURE/THUMB PRINT: _____

WITNESS' SIGNATURE: _____

WITNESS' NAME: _____

CHRPE/105/09