## DECLARATION

I, Phiri Christopher Newton declare that this dissertation in its present form has not been submitted or accepted previously for the award of a degree or diploma in this or any other tertiary institution, I declare that this dissertation contains my own work except where specifically acknowledged. I further declare that I followed all the applicable ethical guidelines in the conduct of the research. This dissertation has been prepared in accordance with the Master of Science in Clinical Pathology, University of Zambia guidelines.

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**CERTIFCATE OF APPROVAL**

The University of Zambia approves this Dissertation on ***"Prevalence of Human Papillomavirus in semen and semen parameter abnormalities in men presenting with infertility at the University Teaching Hospital, Lusaka, Zambia".***

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## ABSTRACT

**Background:** The Human Papillomavirus (HPV) has been recently demonstrated to have a negative influence on sperm parameters, fertilization process and abortion rate. Several studies have identified HPV in human semen, mainly from men recruited at fertility or maternity clinics and also in semen from sperm donors with a prevalence ranging from 1–82%. Studies suggest that HPV induces abnormalities such as alteration of sperm motility and thus may play a major role in cases of idiopathic asthenozoospermia and male infertility. The prevalence of HPV in semen and semen abnormalities at the University Teaching Hospital (UTH) is not known and hence the goal of this study is to generate baseline data with a view to promoting further research on the effects of HPV infection on male fertility and the possibility of female HPV infection through semen.

**Aim:** The study was aimed at determining the prevalence of HPV in semen and semen parameter abnormalities of men presenting with infertility at the UTH.

**Methods:** This was a cross section study involving 78 semen samples from males seeking fertility medical assistance. The samples were obtained by masturbation after 3 days of sexual abstinence. After liquefaction at room temperature, volume, pH, sperm concentration, vitality, motility, and normal morphology were determined following World Health Organization Guidelines for semen analysis. DNA extraction was done following the protocol on isolation of Viral DNA by the manufacturer of the kit (ZR Viral DNA Kit) ZYMO RESEARCH. The presence of HPV infection was detected by PCR amplification of the extracted DNA with HPV specific primers (MY09/MY11). These primers target the conserved region of the L1 capsid genes.

**Results and Discussion:** The prevalence of HPV in semen was found to be 4%. Five abnormalities were demonstrated in our study; asthenozoospermia was observed in 49% (38/78) of the samples followed by teratozoospermia with 28% (22/78). Oligozoospermia was 12% (9/78) and 6% (5/78) of the samples presented with necrozoospermia. Lastly, 3% (2/78) of the samples presented with azoospermia. The presence of HPV in semen may suggest its involvement in inducing semen parameter abnormalities.

**Conclusion:** The prevalence of HPV is 4% in this study. The most prevalent semen abnormality was found to be asthenozoospermia (49%) whilst the least prevalent was azoospermia (3%). The presence of HPV in semen, its possible role in causing semen abnormalities and its sexual transmission needs further investigation.

**Key words:** *human papillomavirus, Zambia, semen, university teaching hospital*

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| ART | Assisted Reproduction Technology |
| DNA | Deoxyribonucleic Acid |
| dsDNA | Double Stranded Deoxyribonucleic Acid |
| EDTA | Ethylenediaminetetraacetic Acid |
| H & E | Haematoxylin and Eosin |
| HPV | Human Papillomavirus |
| IVF | In-Vitro Fertilization |
| LAMU | Lusaka Apex Medical University |
| NaCl | Sodium Chloride |
| OD | Optical Density |
| PCR | Polymerase Chain Reaction |
| RNA | Ribonucleic Acid |
| SS | Semen Specimen |
| STDs | Sexually Transmitted Diseases |
| UNZA | University of Zambia |
| UNZABREC | University of Zambia Biomedical Research Ethics Committee |
| UTH | University Teaching Hospital |
| WHO | World Health Organization |
| KS-HHV8 | Kaposi Sarcoma- Human Herpesvirus 8 |

# CHAPTER 1: INTRODUCTION

## 1.1 Background

Human papillomavirus (HPV) is one of the most common sexually transmitted viruses. They comprise a group of small, circular, non-enveloped, double stranded DNA viruses that infect both cutaneous and mucous squamous epithelia. They are a member of the papovaviridae family. The virus can be transmitted through skin contact or any other surface contact related to the genital area. Human Papillomavirus infection is commonly associated with genital and non-genital warts or cervical cancer. Human Papillomavirus genotypes are divided into high-risk types and low-risk types according to the correlation between HPV genotypes and causation of cancer (de Villiers *et al*, 2009). More than 120 HPV genotypes are known to infect the skin and mucous membranes. This includes the pubic area, oral cavity and perianal region (Munoz,*et al*., 2003). Both males and females from different regions are generally susceptible (Palefsky, 2010, Giuliano *et al*., 2011). There is no global or regional statistics on HPV semen infection.

Male HPV infection has rarely been of concern in Zambia. HPV semen infection among Zambian males has not been reported before. Although HPV infection is highly linked to males and its characteristics of infection types and histological distributions are similar to those in females, most HPV infections among males are benign (e.g., genital warts) with a rare tendency for malignancy. Human Papillomavirus DNA may lie not only in the perianal region and external genitalia, including the penis foreskin, scrotum and glans penis but also in the urethra, ductus deferens, epididymis and testis (Weidner *et al*., 1999).

This study was aimed at investigating the prevalence of HPV infection in semen and semen parameter abnormalities from males submitting specimens for semen analysis in parasitology laboratory at the UTH. The results will promote further research on the effects of HPV semen infection on male fertility and female infection through semen. The results will also promote further research into whether HPV infected sperm may fertilize, transfer viral DNA to oocytes, and if infected oocytes may be able to produce normal embryos. The study has also added to the board of knowledge, new data supporting that, HPV male vaccination could represent a possible strategy for male fertility preservation and for Assisted Reproduction Technology (ART) success rate improvement.

## 1.2 Statement of The Problem

Human Papilloma Virus (HPV) infection is one of the most common sexually transmitted infections worldwide. In 1993 the World Health Organization (WHO) established the role of genital tract infections in human infertility (WHO, 1993). Most male genital tract infections may induce infertility and previous studies reported that 15–20% of infertile subjects are affected by semen infections (Weidner, *et al*., 1999). Several studies have identified HPV in human semen, mainly from men recruited at fertility or maternity clinics and also in semen from sperm donors with a prevalence ranging from 1–82 % (Caliskan *et al*, Didelot *et al.*, 2007, Giuliano *et al.*, 2007, Olatunbosun *et al.*, 2001 and Rintala *et al.*, 2004**).** Recent evidence associates HPV infected semen with infertility in men (Garolla et al,2013). More studies are required to define the role of HPV sperm infection in clinical practice and this may be achieved by first knowing the prevalence and then characteristics of the HPV genotypes that are associated with human semen. In Zambia few studies if any have been done to determine HPV semen infection at molecular level and its possible consequences on male fertility as well as its potential to be transmitted to sexual partners.

## 1.3 Justification Of The Study

Little attention has been given to the transmission of HPV through semen, let alone the effect of the infection on the fertility of males whose semen is infected. This is despite the clinical importance of HPV in lower genital tract cancers**.** Data from other authors suggests that HPV, by inducing an alteration of sperm motility, may play a major role in cases of idiopathic asthenozoospermia and thus in male infertility (Garolla et al, 2013).

Currently two vaccines are available targeting specific HPV types but these are only administered to females excluding males in most parts of the world including Zambia. Circumcision is the prophylactic method being used in men in Zambia but if the virus can be transmitted through semen then the reduction in those cancers as a result of males being circumcised is minimal. Therefore, knowing the prevalence of HPV associated with human semen in the Zambian setting will help us determine whether HPV male vaccination could represent a possible strategy for male fertility preservation and prevention of penile and cervical cancers.

## CHAPTER 2: LITERATURE REVIEW

### 2.1 HPV Semen Infection and its complications

Sexually transmitted diseases (STDs) represent major health, social, and economic problems worldwide. Despite the availability of antibiotics and vaccines, and the development of disease prevention and control programs, STDs remain a significant cause of acute and chronic diseases with possible involvement in pregnancy complications and infertility (Ochsendorf, 2008).

It is proven that sexually transmitted infections (STIs) represent an important cause of male infertility since they can induce urethral strictures, epididymorchitis and testicular failure (Ochsendorf, 2008).In 1993 the World Health Organization (WHO) established the role of genital tract infections in human infertility (WHO, 1993). Most male genital tract infections may induce infertility and previous studies reported that 15–20% of infertile subjects are affected by semen infection (Weidner,*et al*., 1999).

In 2001, Dejucq and Jégou stressed the need to study and understand the role of viruses in fertility and since then progress has been made in this direction. Depending on sites of infection, various pathogenic mechanisms have been described: systemic, acute or chronic infections can result in transient or permanent infertility, impairing hormones, testicular function and spermatogenesis. Male accessory gland (epididymis, prostate, and seminal vesicles) and urethral infections have been identified as playing a negative role in male reproductive function and fertility owing to obstruction or sub-obstruction, altered secretory function, and release of inflammatory mediators (La Vignera, *et al*., 2011).

Semen viral infections are frequently present even in asymptomatic males, and they are often associated with poor sperm quality (Bezold *et al*., 2007). In 2008 a study was done that showed an association between viral semen infection and an increased frequency of sperm aneuploidy and DNA fragmentation (Moretti *et al*., 2008). Little is known about HPV semen infection; however, growing evidence suggests that this virus may play a major role in male infertility (Perino *et al*., 2011). Perino reported that couples who underwent assisted reproductive technology cycles experienced an increased risk of pregnancy loss when HPV DNA testing was positive in the male partner, compared with non-infected patients. In fact, HPV has been recently demonstrated to have a negative influence on sperm parameters, the fertilization process and the abortion rate (Foresta *et al*., 2010a, Foresta *et al*., 2010b, Foresta

*et al*., 2011a, Pérez-Andino *et al*., 2009 and Perino *et al*., 2011). Both the possible presence of viral DNA or RNA at the sperm level and the treatment of patients with antiviral and antiretroviral therapies able to induce testicular damage may have a further detrimental effect on sperm parameters (Lorusso *et al*., 2010).

In the past, HPV semen infection was always considered transient and without clinical consequences. In a recent study, nested Polymerase Chain Reaction (PCR) showed the presence of HPV DNA sequences in 10% of semen samples from asymptomatic young adult males who had had unprotected sexual intercourse (Foresta *et al*., 2010a). Another study, considering the prevalence of HPV semen infection in patients with risk factors for the virus (subjects with genital warts, partners of HPV-positive females, and infertile patients) showed that in infected semen samples, HPV can be localized at different levels: in spermatozoa, in exfoliated cells or in both.

Human Papillomavirus infection may have the same mechanism as other viruses that infect sperm. They localize at the equatorial region of the sperm head or tail to decrease function and ability of the acrosome, thus affecting the fusion of gametes. It was demonstrated that infertile patients had both a prevalent infection in sperm and a higher percentage of infected sperm, while exfoliated cells were significantly more affected in patients with other risk factors. In fertile control subjects HPV infection was never found in sperm cells (Foresta *et al*., 2010b). A recent study highlighted that HPV can be detected in the placenta and that this infection may occur not only through ascending infection from the cervix, but also via infected sperm at fecundation (Weyn *et al*., 2006).

Additionally, a clinical study performed in women undergoing in vitro fertilization (IVF) reported a significant reduction of pregnancies in the presence of HPV cervical infection compared with no infection (Spandorfer *et al*., 2006). However, it is not known whether HPV-infected sperm are able to fertilize, to transfer viral DNA to oocytes, and if infected oocytes are able to produce normal embryos.

## 2.2 **Semen Parameter Abnormalities**

The semen analysis provides information on semen volume, sperm count, vitality, motility and sperm morphology. Sperm vitality is done to determine whether non motile sperm are viable by identifying which sperm has intact cell membranes. The total number of spermatozoa per ejaculation and the sperm concentration are related to both time to pregnancy (Slama *et al*., 2002) and pregnancy rates (WHO, 1996; Zinaman *et al*., 2000) and are predictors of conception (Bonde *et al*., 1998; Larsen *et al*., 2000).

Infertility is defined as a failure to conceive within one or more years of regular unprotected coitus. It can be categorised into primary and secondary infertility. Primary infertility is applied to those individuals who have never conceived before, while secondary infertility indicates previous pregnancy but failure to conceive subsequently (Dutta, 2008).

The infertility state is dependent on the female factor as well as the male factor; an altered male factor is designated when any cause or causes of infertility reside in the male. The male factor as a cause of infertility is present in 40-50% of cases hence the importance of an integral evaluation of semen (Cooper, 2010).

The World Health Organisation in 2010 put up guidelines for the accepted ranges for semen parameters. Semen volume more than 1.5 ml per ejaculation is considered normal. The sperm count greater than or equal to 20 million per ml is considered normal. Motility is considered normal when the total sum of progressive motility and sluggish progressive motility is greater than or equal to 50%. Semen is considered vital or viable when more than 50% of the spermatozoa are viable. Semen is considered normal when 15% or more of the spermatozoa are morphologically normal (Table 1).

Semen abnormalities are often labelled as follows:

**Oligozoospermia** - decreased number of spermatozoa in semen

**Aspermia** - complete lack of semen

**Hypospermia** - reduced seminal volume of less than 1.5 ml

**Azoospermia** - absence of sperm cells in semen

**Teratozoospermia** - increase in sperm with abnormal morphology

**Necrozoospermia-** the spermatozoa present are dead

**Asthenozoospermia** - reduced sperm motility of less than 50%

There are various combinations of these depending on the abnormalities found in the semen, for instance *Teratoasthenozoospermia*, which is increased abnormal morphology and reduced sperm motility (Burney *et al*, 2007).

**Table 1: WHO Guidelines for Normal Seminal Fluid Analysis**

| Parameter | Accepted Rage |
|---|---|
| Semen Volume | ≥ 1.5 ml. |
| Sperm Count | ≥20 million/ml |
| Sperm Motility | ≥ 50% (Progressive motility + Sluggish progressive motility) |
| Sperm Vitality | > 50% vital |
| Sperm Normal Morphology | ≥ 15% normal forms |

NOTE: Data from World Health Organisation (WHO, 2010).

## 2.3 RESEARCH QUESTION

- What is the prevalence of HPV in semen and semen parameter abnormalities in men presenting with infertility at the University Teaching Hospital?

## 2.4 OBJECTIVES

### 2.5 General Objective

1. To determine the prevalence of Human Papillomavirus (HPV) in semen and semen parameter abnormalities in men presenting with infertility at the University Teaching Hospital.

### 2.6 Specific Objectives

1. To detect Human Papillomavirus (HPV) infection in semen using Polymerase Chain Reaction (PCR) amplification of the isolated DNA.
2. To detect abnormalities on semen parameters using staining techniques and microscopy.

# CHAPTER 3: MATERIALS AND METHODS

## 3.1 Study Design

This study had a cross-sectional design involving semen samples that were submitted to the Parasitology Laboratory at UTH.

## 3.2 Study Setting

The study was conducted at the University Teaching Hospital (UTH), parasitology laboratory and Kaposi Sarcoma- Human Herpesvirus 8 (KS-HHV8) laboratory in the Department of Paediatrics and Child health. Collection of semen samples for detection of HPV infection from the samples submitted for semen analysis in the Parasitology Laboratory was done. Semen parameter abnormality detection was done in parasitology laboratory and PCR for HPV DNA detection was done at KS-HHV8 laboratory.

## 3.3 Study And Target Populations

Study population comprised all those that met the eligibility criteria from the requesting sites and had submitted the specimen to parasitology laboratory. The target population comprised all males who are sexually active.

### 3.4 Sample Size And Sample Selection Method

A convenience sampling method in which consecutive samples with infertility that met the inclusion criteria was used in this study. As the submission of samples is done once per week the sample size is limited to 10 samples per week. Data collection was done in three months period. This gave us 120 samples (population) at most in three months. All the samples meeting eligibility criteria were considered in this study. The calculated sample size was as shown below,

Prevalence of HPV in semen at the UTH is unknown. Since the prevalence is unknown, the prevalence of 64.3% sited earlier in the statement of the problem was used. In order to estimate the prevalence within 5% (or 0.05) and considering 95% confidence level, a minimum sample size of 91 was analysed, as shown by the calculation:-

$n = \frac{p(1-p)}{e^2}$ , where $p = 64.3\%$ (or 0.643) is the sample proportion and

$e^2 = \left(\frac{0.05}{1.96}\right)^2 \approx 0.000625$

n = 367

Applying correction for finite population size formula (Dell *et al*, 2002), the new sample size was:

$$\text{New ss} = \frac{n}{1+\frac{n-1}{pop}}$$

Where, ss= sample size; pop = population,

We calculate the new n= $\frac{n}{1+\frac{n-1}{N}}$, = $\frac{367}{1+\frac{367-1}{120}}$ = 90.6= 91

n= sample size 367 as calculated above, N=Total number of semen specimens stored for three consecutive months which is the study data collection period.

Therefore, the minimum sample size (n) was 91.

### 3.5 Specimen Labelling

Each semen specimen was given a new identification code and number for easier identification and ensuring confidentiality. The letters "SS" (semen specimen) plus a three digit numbering system (i.e.000) was used. Therefore, specimen numbers 1, 5, 35 and 120 was labelled as SS001, SS005, SS035 and SS120, respectively.

### 3.6 Eligibility Criteria

#### *3.6.1 Inclusion Criteria*

All semen submitted for analysis with infertility was eligible for the study.

#### *3.6.2 Exclusion Criteria*

Semen with low volume that could not allow extra analysis from the normal routine parameters was excluded from the study. Also semen submitted for other reasons other than infertility was excluded (e.g. Haematospermia, vasectomy).

### 3.7 Study Procedures

Two pathways were followed in the laboratory for sample analysis. They both started with the patients' samples; the first one started with macroscopic examination where the pH, viscosity, colour and volume were measured. This was then followed by microscopic examination which involved both wet and stained preparations. Wet preparations measured sperm concentration, motility, aggregation of spermatozoa, and agglutination of spermatozoa and cellular elements other than spermatozoa. Stained preparations measured sperm vitality, normal and abnormal morphology. The second pathway involved DNA extraction from the patients' samples. At this stage, PCR was used to detect HPV DNA presence (figure 1).

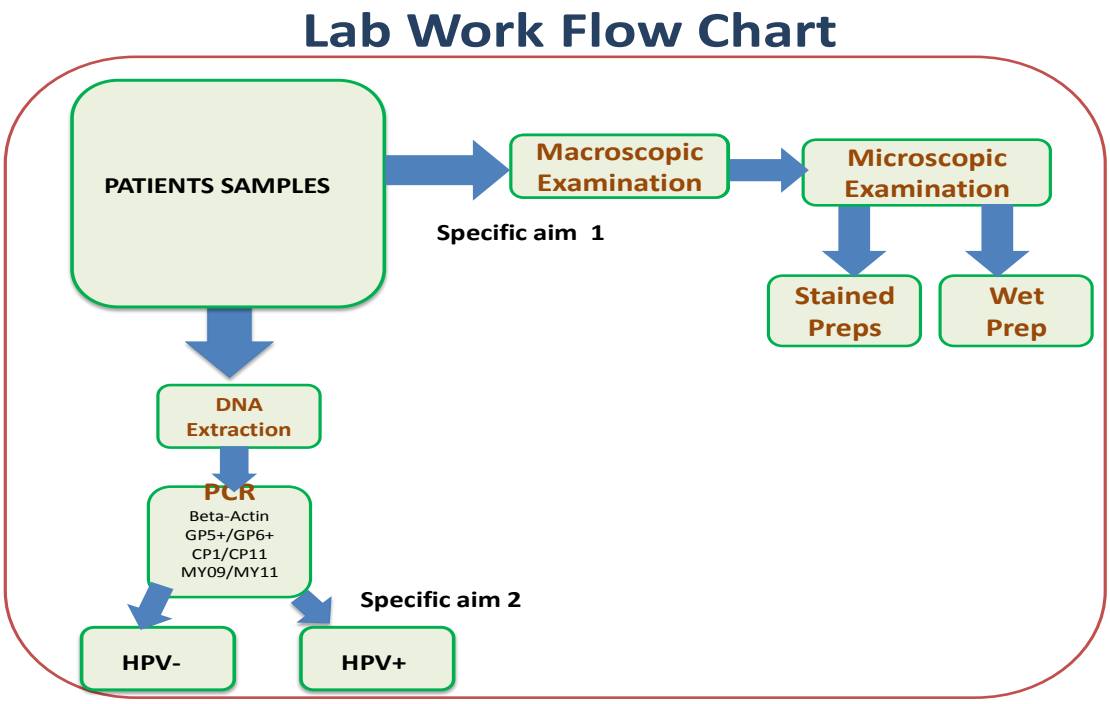


Figure 1showing laboratory work flow chart with two pathways both starting with patient samples. The two pathways answered the two specific objectives in the study.

### 3.8 Sample Collection

Each sample was collected directly into a graduated measuring container with a wide mouth and by masturbation after 3 days of sexual abstinence. After liquefaction at room temperature, semen colour, volume, pH, sperm concentration, viability, motility, and normal morphology was determined following the World Health Organization guidelines for semen analysis (WHO, 2010). An aliquot was made for further analysis.

### 3.9 Macroscopic Examinations

The volume was read directly from the graduations (0.1 ml accuracy) of the measuring container and it was considered normal if within the range of ≥ 1.5 ml. Immediately after ejaculation into the collection vessel, semen is typically a semi- solid coagulated mass. Within a few minutes at room temperature, the semen usually begins to liquefy, If the semen did not liquefy within 30 minutes, we did not proceed with semen analysis but waited for another 30 minutes. A normal liquefied semen sample has a homogeneous, grey-opalescent appearance. A pH paper in the range 6.0 to 10.0 was used to measure pH. The semen samples

were well mixed, after which a drop was spread evenly onto the pH paper and waited for the colour of the impregnated zone to become uniform. This colour was compared to that of the calibration strip to read the pH. A pH of 7.2 and 8.0 was taken as a lower and upper threshold value respectively as guided by WHO guidelines. The viscosity was evaluated by introducing a glass rod into the sample and observing the length of the thread that forms upon withdrawal of the rod. The viscosity was recorded as abnormal when the thread exceeded 1 cm.

### 3.10 Microscopic Examinations (Wet Prep)

***Sperm Numbers***

Semen was mixed and dilutions with fixative were prepared. The haemocytometer chamber was loaded and spermatozoa were allowed to settle in a humid chamber. The samples were assessed within 15 minutes (after which evaporation has noticeable effects on sperm position within the chamber). At least 200 spermatozoa per replicate were counted. Replicate counts were compared to see if they are acceptably close. If so, we proceeded with calculations; if not, new dilutions were prepared. The concentration in spermatozoa per ml was calculated as well as the total number of spermatozoa per ejaculation. The normal total sperm count was considered to be greater than or equal to 20 million spermatozoa per ml.

***Semen Motility***

The semen sample was well mixed after which an aliquot of semen was removed immediately allowing no time for the spermatozoa to settle out of suspension. The semen sample was remixed before removing a replicate aliquot. For each replicate, we prepared a wet preparation approximately 20 um deep. We waited for the sample to stop drifting (within 60 seconds). The slide was examined with phase-contrast optics at ×200 or × 400 magnifications. Approximately 200 spermatozoa per replicate were assessed for the percentage of different motile categories. The replicate values were compared to check if they are acceptably close. If so, we proceeded with calculations; if not, we prepared new samples. The motility of each spermatozoon was graded as follows: Progressive motility (PR): spermatozoa moving actively, either linearly or in a large circle, regardless of speed. Non-progressive motility (NP): all other patterns of motility with an absence of progression, e.g. swimming in small circles, the flagellar force hardly displacing the head, or when only a

flagellar beat can be observed. Immotility (IM) or non motile (NM): no movement. The sample was considered motile when more than or equal to 50% of the spermatozoa had PR.

### 3.10.1 Microscopic Examination (Wet-Stained Prep)

***Vitality Test using Eosin–Nigrosin***

The semen sample was well mixed. A 50 ul aliquot of semen was removed and mixed with an equal volume of eosin– nigrosin suspension in a test-tube, allowing them to mix thoroughly for 30 seconds. The semen sample was remixed before removing a replicate aliquot and then mixed with eosin–nigrosin and treated as in the step above. For each suspension, a smear was made on a glass slide and allowed to air dry. The smear was then examined immediately after drying, or later after mounting with a permanent non-aqueous mounting medium. Each slide was examined with brightfield optics at ×1000 magnification and oil immersion. The number of stained (dead) or unstained (vital) cells was tallied with the aid of a laboratory counter. Two hundred spermatozoa in each replicate were evaluated, in order to achieve an acceptably low sampling error. The average and difference of the two percentages of vital cells from the replicate slides were calculated. If the difference between the percentages is acceptable, the average percentage of vital spermatozoa was reported. If the difference is too high, two new preparations from two fresh aliquots of the semen sample were made and the assessment was repeated. The average percentage of vital spermatozoa was reported to the nearest whole number. The sample was considered vital when more than or equal to 50% spermatozoa was vital.

### 3.10.2 Microscopic Examination (Stained Prep)

***Sperm Morphology***

A smear of semen on a slide was prepared. It was then air-dried, fixed and stained. The slide was mounted with a cover slip as it was to be kept for a long time. The slide was then examined with bright field optics at ×1000 magnification under oil immersion. Approximately 200 spermatozoa per replicate were assessed for the percentage of normal and abnormal forms. Replicate values were compared to see if they were acceptably close: if so we proceeded with calculations; if not, we re-read the slides.

Since air- drying of semen smears is associated with changes in sperm dimensions such as reduction in size of dried, fixed and stained spermatozoa than live spermatozoa visualized in semen (Katz *et al*., 1986); as well as expansion of immature sperm heads (Soler*et al*., 2000), and loss of osmotically sensitive cytoplasmic droplets (Abraham-Peskir*et al*., 2002; Cooper et al., 2004).Two or more smears were made from the fresh semen sample in case there were problems with staining or one slide was broken. Assessment was performed in replicate, preferably on each of the two slides, because there may be significant variations in sperm morphology between-slides

### 3.10.3 Papanicolaou Staining Procedure for Sperm Morphology

The air-dried semen smears were fixed by immersing the slides in 95% (v/v) ethanol and ether for 15 minutes. Staining the fixed semen smears was done sequentially by immersing the slides in decreasing concentrations of 80%, 70% and 50% ethanol for 10 seconds in each jar and then 10 seconds in distilled water. The slides were then dipped in Harris's or Mayer's haematoxylin for 3 minutes and then rinsed in running tap water for 3 minutes. The slides were dipped in 0.5% hydrochloric acid for 2 seconds and rinsed in running tap water for 3 minutes. This was then followed by 4 minutes of dipping in Scott's solution and then 1 second in distilled water. At this stage the slides were immersed in increasing concentrations of 50%, 70%, 80% and 90% ethanol for 10 seconds in each jar and then in G-6 orange stain for 2 minute. The slides were at this stage immersed in two consecutive jars of 95% each of ethanol for 10 seconds in each jar and later in EA-50 or 65 green stain for 5 minutes. The slides were then immersed in three consecutive jars of again 95% each of ethanol for 5

seconds in each jar then in 99.5% of ethanol for 2 minutes. Lastly the slides were immersed in three consecutive jars of xylene for 1 minute in each jar.

### 3.11 DNA Extraction

This was done using the ZR Viral DNA Kit™ from Zymo Research. Prior to use 24ml of anhydrous (100%) ethanol was added to each 6ml of DNA Wash Buffer concentrate to obtain the final wash buffer solution and as per recommendation by the manufacturer of the extraction kit, 250$\mu l$ of beta-mercaptoethanol was added to each 50 ml of ZR Viral DNA Buffer to make the final dilution of 0.5% (V/V). All centrifugation steps were performed at 12000x g.

200$\mu l$ of semen sample were put in a 1.5 ml microcentrifuge tube and 800$\mu l$ of ZR Viral DNA Buffer were added. The mixture was than vortexed and incubated at room temperature for 1 hour. After incubation, the sample /ZR Viral DNA Buffer mixture was centrifuged to remove particulate matter that could clog the column and transferred to the Zymo-spin™ IC Column in a collection tube. The column in the collection tube was then centrifuged for 1 minute and the flow through was discarded from the collection tube.

At this stage, 300$\mu l$ of DNA Wash Buffer was added to the column and centrifuged for 1 minute. The flow through was then discarded from the collection tube. This step was repeated to increase the purity of the extracted DNA.

Lastly the Zymo-Spin™ IC Column was placed into the new microcentrifuge tube and 30$\mu l$ 0f DNA Elution Buffer was added. The mixture was incubated at room temperature for 5 minutes and later centrifuged for 1 minute to elute the DNA.

### 3.12 DNA Estimation

The concentration of DNA was estimated by ultraviolet spectroscopy at 260nm using NANO drop spectrophotometer 2000 (thermo scientific, USA). A DNA sample with an optical density (OD) of 1 at 260nm corresponded to a DNA concentration of 50μg/ml of double-stranded DNA. The purity of the DNA was determined by a DNA/protein absorbance ratio of 260nm/280nm and DNA was considered pure at >1.8. The DNA was stored at -20°C until required.

### 3.13 HPV DNA Detection

The presence of HPV infection was detected by PCR amplification of the extracted DNA with HPV specific primers using the Applied Biosystems 2720 Thermal Cycler version 2.09. Three redundant primers were used in this research, My09/My11, GP5+/GP6+ and CPI/CPII. (**Forward Primer**: **My09**:5'-CGT CCM ARR GGA WAC TGA TC-3' and **Reverse Primer**: **My11**:5' -GCM CAG GGW CAT AAY AAT GG-3') that amplify 450 base pairs of the L1 region (nt 6583-7034) was used to detect HPV in the sample. Another pair of redundant primers (**Forward Primer**:**GP5+**:5'-TTT GTT ACT GTG GTA GAT ACT AC-3' and **Reverse Primer**: **GP6+**:5'-GAA AAA TAA ATG TAA ATC ATA TTC-3') that amplify 150 base pairs of the L1 region ( nt 6624-6746)of the HPV genome was used to detect HPV in the samples. Another pair of redundant primers (**Forward Primer**: **CPI**: 5'-TTA TCWTAT GCC CAY TGT ACC AT-3'-and **Reverse Primer**: **CPII**: 5'-ATG TTA ATW SAG CCW CCA AAA TT-3') which are targeted to the E1 region (nt 1777-1964) and amplify 188 bp of the HPV genome were also used to detect HPV in the samples. The β-Actin primers (**Forward Primer**: 5'-GCC ATG TAC GTT GCT ATC C-3' and **Reverse Primer**: 5'CCG CGC TCG GTG AGG ATC-3') was used as internal quality control. The use of the β-Actin primers on our samples and the detection of HPV using three sets of HPV specific primers provided a robust set of results for our analysis. Briefly the PCR thermal profile reaction conditions were set as follows; 94°C for 1 minute (94°C for 30 seconds, 55°C for 1 minute, 72°C for 90 seconds and 72°C for10 minutes) for 30 cycles and then hold at 4°C . The amplified products were applied to 2% agarose gel which was stained with 10$\mu l$ ethidium bromide and then gel electrophoresis was performed at 60 volts for 60 minutes using the Fisher Scientific Electrophoresis. The bands were visualized under UV illumination at 302 nm by photography using the UV Transilluminator (BioDoc-It™ Imaging System. Upland. CA, USA).

### 3.14 STATISTICAL ANALYSIS

**Table 2: Dependent and Independent Variables**

| **Independent variable** | **Presence/absence of HPV** |
|---|---|
| **Dependent variable ( continuous)** | pH<br>volume<br>sperm count |
| **Dependent variable (categorical or nominal or ordinal)** | Viscosity<br>Motility<br>Vitality<br>Morphology<br>Colour |

Both descriptive and inferential statistics was used to analyse the data. For associations between categorical variables, unpaired t-test was used and spearman rank correlation test was used to determine the relationship between continuous variables. Bivariate linear regression and correlation coefficients were used to assess correlation between Age and Sperm Count, Volume and Sperm Count, and pH and Age. The Bivariate linear regression data was then plotted and presented on scatter graphs. All statistical tests were performed at 5% significance level or 95% confidence interval with p-value of $<0.05$ to determine statistical significance. SAS statistical software version 5.0.1 (SAS Institute Inc, Cary, NC, USA) was used for all analysis.

### 3.15 ETHICAL CONSIDERATIONS

Approval was requested from the University of Zambia Biomedical Research Ethics Committee (UNZABREC) to carry out the study using semen specimens submitted for routine analysis in parasitology laboratory after the requested for tests were done. Permission to conduct the study at UTH was obtained from the hospital management. The main ethical issue surrounding this project was confidentiality and to guarantee privacy, each semen specimen was given a new identification code and number as explained in 4.5. All the results routinely reported in semenogram were sent back to clinicians for patient management consideration. Approval to carry out this study was granted by UNZABREC, Ref. No.009-11-15 obtained on 3rd February, 2016.

## CHAPTER 4: RESULTS

The calculated sample size for the study was 91 but only 78 qualified for data analysis because 13 samples did not have viable DNA after extraction. Out of the total 78 patients, 40 had primary infertility and 38 had secondary infertility. The mean age for primary infertility was 32 years and secondary infertility was 39 years. Thirty five years was the mean age for the total population (table 3).

**Table 3: Comparisons between Primary and Secondary Infertility Vs Age**

| Group | Observation | Mean | Std Deviation | Variance |
|---|---|---|---|---|
| Primary Infertility | 40 | 32.475 | 5.684 | 32.307 |
| Secondary Infertility | 38 | 38.579 | 6.591 | 43.440 |
| Combination | 78 | 35.449 | 6.831 | |

Table 3 shows mean ages for primary infertility (32 years) and secondary infertility (39 years). It also shows the mean age for the total population (35 years).

The first specific aim was to detect Human Papillomavirus (HPV) infection in semen using Polymerase Chain Reaction (PCR) amplification of the isolated DNA. This was achieved by determining HPV infection by the presence of the approximately 450bp HPV DNA in the pool of extracted DNA from semen samples. Out of the total 78 samples, 3 samples amplified with the MY09/MY11 primers (figure 2 A and B).

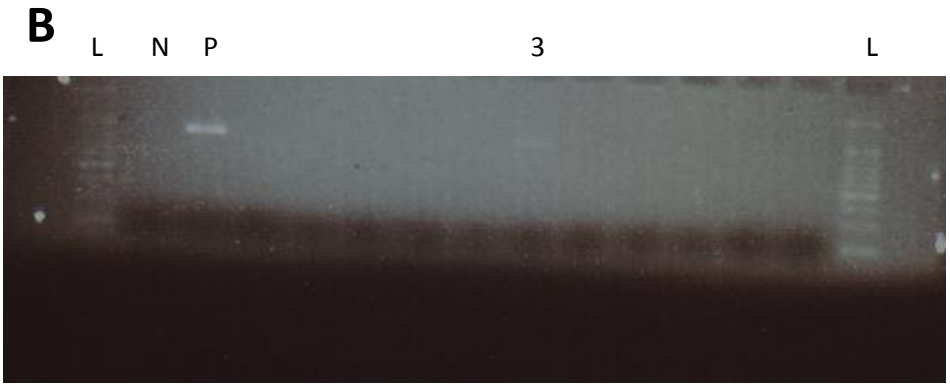


**Figure 2; Images of Gel Electrophoresis**: A and B Images of a 2% agarose gel with bands of the ladder (L), positive control (P), three positive samples (1, 2 and 3) and negative control (N).

To answer specific objectives number two and three, the samples were handled as described in the methodology numbers 4.9 to 4.10.3. Out of the total 78 samples, 38 presented with reduced sperm motility of which 20 were from those patients with primary infertility and 18 from secondary infertility. Twenty two samples presented with abnormal morphology of which 5 were from primary infertility patients and 17 from secondary infertility patients. Reduced sperm count was observed in 9 samples with the proportion of 4 from primary and 5 from secondary infertility. Five samples were not viable or vital with the proportion of 3 from primary infertility and 2 from secondary infertility. The absence of spermatozoa in semen was observed in 2 samples both from secondary infertility (Table 4).

**Table 4: Proportions of Semen Parameter Abnormalities**

| Abnormality | Primary Infertility | Secondary Infertility | Total Proportion | Total % |
|---|---|---|---|---|
| Asthenozoospermia | 20 | 18 | 38 | 49 |
| Teratozoospermia | 5 | 17 | 22 | 28 |
| Oligozoospermia | 4 | 5 | 9 | 12 |
| Azoospermia | 0 | 2 | 2 | 3 |
| Necrozoospermia | 3 | 2 | 5 | 6 |

Table 4 is showing proportions of different semen parameter abnormalities grouped in primary and secondary infertility. The table also shows the total proportions and percentages.

Correlations between semen abnormalities and with clinical data were performed. There was no relationship observed between age and volume with p-Value of 0.36 (table 5). The Spearman rank correlation and the Bivariate linear regression analysis of age vs sperm count showed a weak negative correlation but statistically insignificant with r of -0.04 and p-value of 0.70 (figure 3). However a weak but statistically significant correlation (r=0.37, p = 0.003) between age and pH was observed in this study (figure 4). The analysis of Volume and Sperm count indicated a weak inverse correlation but statistically insignificant with r= -0.007 and p-value of 0.95 (figure 5).

**Table 5: Age Vs Volume**

| | Correlation | Count | Z- Value | P- Value | 95% Lower | 95% Upper |
|---|---|---|---|---|---|---|
| AGE,VOLUME | .105 | 78 | .915 | .3600 | -120 | .320 |

The table above shows the relationship between age and volume with p-Value > 0.05(0.36). This means there is no correlation between age and volume as the p-Value is greater than 0.05.

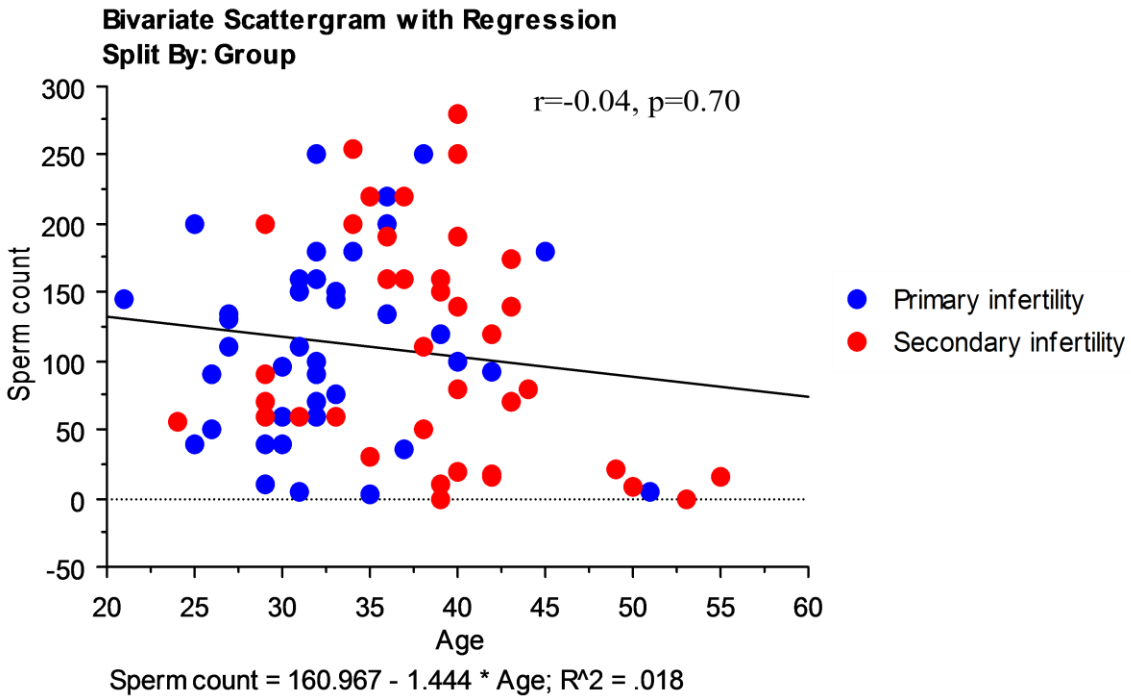


**Figure 3; Age Vs Sperm Count:** The Spearman rank correlation and the Bivariate linear regression analysis of age vs sperm count shows a weak negative correlation but statistically insignificant with r = -0.04 and p-value more than 0.05(0.70).

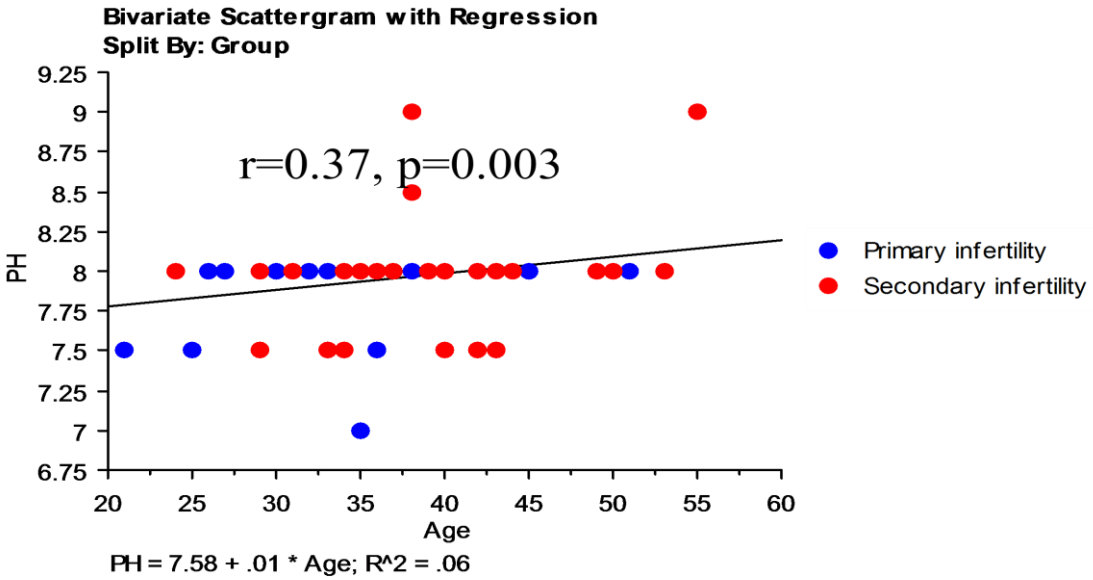


**Figure 4; Age Vs pH:** Spearman rank correlation coefficient and the Bivariate linear regression analysis of age vs pH indicated a weak but statistically significant correlation (r=0.37, p = 0.003).

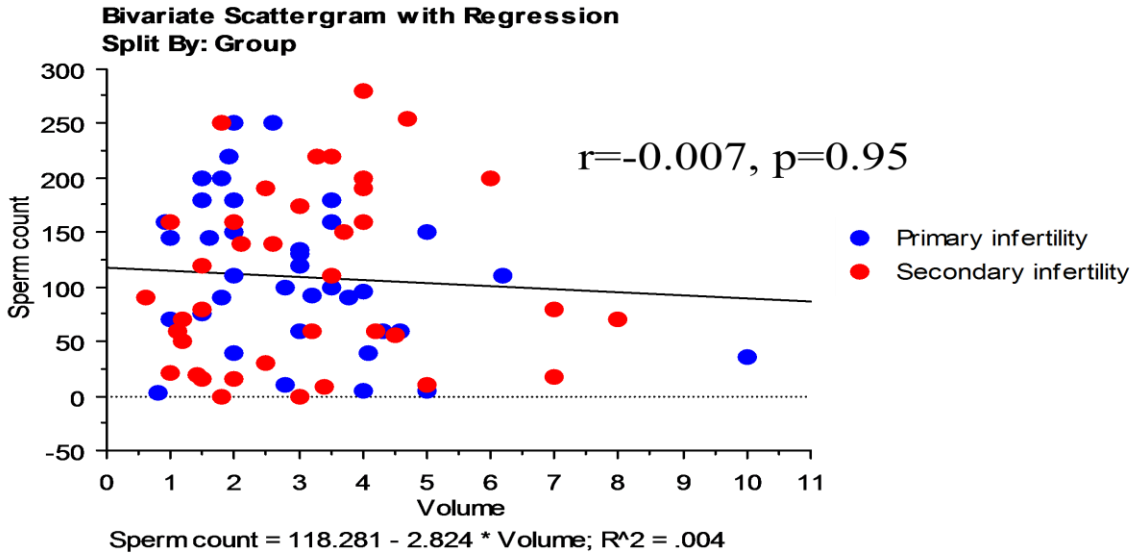


**Figure 5; Volume Vs Sperm Count:** Spearman rank correlation coefficient and the Bivariate linear regression analysis of Volume vs Sperm count indicated a weak inversely but statistically insignificant correlation (r= -0.007, p = 0.95).

## CHAPTER 5: DISSCUSION

### 5.1 HPV Prevalence

The presence of HPV in semen emphasizes the potential risk of this route of transmission, and underlines the need to determine its prevalence in asymptomatic male partners of infertile couples. There is no study on the prevalence of HPV in semen of asymptomatic sexually active Zambian men in the literature. HPV is prevalent in all sexually active populations and frequently presented clinically as anogenital warts in both males and females. High-risk, oncogenic, HPV types are associated with 99.7% of all cervical cancers; low-risk HPV types are responsible for almost all cases of genital warts (Caliskan *et al,* 2010).

HPV infection rates in men range from 1.0% to 82%. This wide range may be due to the variation in the clinical material analyzed such as penile surface, glans, scrotum, urethra, semen, and urine (Caliskan et al, 2010). Another reason for the wide rage is the status of the source of the clinical material such as from symptomatic i.e. (genital warts patients) or asymptomatic patients for HPV.

In 2010, Foresta *et al* reported HPV semen infection in 6.1% of the 98 cryopreserved samples from a cohort of patients who banked sperm as a result of testicular cancer. In 2011 she reported 3.3% prevalence in 60 healthy young males who were used as controls in the previous study. In another study by caliskan in 2010, HPV prevalence was reported to be 1.1% (2/175) of asymptomatic fertile and infertile men. The above results are in accordance with, and comparable to our results in this study. HPV prevalence in our study was found to be 4% (3/78) as shown in figures 2 A and B.

Sperm infection with HPV has been demonstrated to reduce sperm motility, sperm–ovum interaction, the pregnancy rate, and the increase in abortion rate (Weyn *et al*, 2006.Spandorfer *et al*, 2006. Foresta *et al*, 2011a and Perino *et al*, 2011).

In 1997, Lai *et al* reported the presence of HPV DNA and RNA in 24 sperm samples of subfertile men. HPV 16 DNA was found in 25% of the samples and RNA was found in 2% of samples. HPV 18 DNA was found in 46% of the samples and RNA was found in 21%. They reported 75% incidence of asthenozoospermia among patients infected with HPV and 8% in those without HPV in their sperm cells. Curvilinear velocity, straight-line velocity, and mean amplitude of lateral head displacement was performed and reported significantly lower in HPV infected specimens. The differences of linearity, beat cross frequency, and straightness

were not statistically significant. With these findings, they concluded that, 1) certain HPV specific genes are actively transcribed; 2) that the presence of HPV in sperm cells may affect sperm motility parameters; 3) and that asthenozoospermia may be associated with sperm HPV infection. In 2010, Foresta *et al* confirmed these findings. According to data from other authors such as cited above, our results of the presence of HPV DNA in semen suggest that HPV by inducing an alteration of sperm motility may play a major role in cases of idiopathic asthenozoospermia.

In HPV infected semen samples, it has been proven that HPV can be localised at different levels; in spermatozoa, in exfoliated cells or in both sites. This suggests that spermatozoa or indeed semen can act as the vector for HPV infection to sexual partners. In 2006, Weyn *et al* reported HPV in the placenta. They suggested that this infection may occur not only through ascending infection from the cervix but also via infected sperm at fertilization. The results of HPV in semen in this study also suggest that some of the cervical cancer cases may be caused by cervical HPV infection via infected semen.

Circumcision services in Zambia and indeed many other sub-Saharan countries represent an opportunity to reduce HPV infection as well as HIV infection in men. This is done with a view that women would benefit indirectly from a lower prevalence of HPV and HIV in their male partners. The effects of circumcision which are, reduced acquisition rate of penile HPV infections, and increased clearance rate of pre-existing HPV from the penis are well understood. However, more studies are needed to understand if these effects due to circumcision can be observed in semen.

The results of HPV in semen suggest that circumcision alone could not effectively reduce the burden of HPV infection in males and later on in females. Other strategies such as vaccination against specific strains of HPV in males and education on reduced number of sexual partners need to be employed. In our view, regular cervical cancer screening for all sexually active women and treatment of precancerous lesions remains the key strategy to prevent cervical cancer.

## 5.2 Semen Parameter Abnormalities

Nineteen samples were Normospermia out of the total 78. The male factor contributed to 76% (59/78) of infertility in this population. This study showed an inversely correlation between Volume and Sperm count but statistically insignificant as shown by the value of r which was -0.007 and p value of 0.95(figure 5). This means that, with the increase in volume, no statistical difference in sperm count was observed in this group.

As men age, there is a consistent decline in semen quality, and this decline appears to be due to DNA damage (Silva, 2012). Figure 4 indicated a statistically significant correlation between Age and pH with r value of 0.37and p value of 0.003. This means that, with increase in age, there is an increase in pH of semen in this group of men, however the range for pH in our study was within the optimal (7.0 – 9.0) for spermatozoa and hence clinically these results are insignificant. The study demonstrated that there was a non significant negative correlation between age and sperm count as shown in figure 3 with the r value of -0.04 and p value of 0.70. This study also showed in table 5 that there was no correlation between age and volume as the p value was greater than 0.05 (0.36).

In 2003, a study from University College Hospital Ibadan done by Adenijiv *et al*, demonstrated that asthenozoospermia was the most common seminal index found altered among males of infertile couples with 27.8% incidence whilst azoospermia was the least with 6.7% cases. Another study in 2010 by Ugboaja et al on the pattern of seminal fluid abnormalities in male partners of infertile couples in South-Eastern Nigeria over a period of 12 months demonstrated that out of 237 samples with semen fluid abnormalities, Asthenozoospermia (16.7%) was the single main abnormality. The above findings with respect to the most common semen parameter abnormality are in line with our findings as demonstrated in table 3.The reason for the difference in percentages may be due to the difference in geographical areas where different environmental factors affect seminal parameters differently.

Five abnormalities were demonstrated in our study. Asthenozoospermia was observed in 49% (38/78) of the samples. Second in magnitude was Teratozoospermia with 28% (22/78) of the samples. Oligozoospermia was third with 12% (9/78). 6% (5/78) of the samples presented with Necrozoospermia making it the fourth common abnormality in this group. Lastly, 3% (2/78) of the samples presented with Azoospermia making it the least common abnormality.

# CHAPTER 6: CONCLUSION

The prevalence of HPV in semen was found to be 4% in our study. This is comparable to other studies done both in Europe and Africa considering the status of the source of semen that was asymptomatic to HPV. Since the presence of HPV in semen may threaten the fertility of affected men and health of their partners, appropriate measures should be taken such as vaccination in addition to circumcision of males, as currently the vaccine is only available to females in Zambia and many sub Saharan countries. The most prevalent semen abnormality was found to be Asthenozoospermia (49%) whilst the least prevalent was Azoospermia (3%). The presence of HPV DNA in semen suggests that HPV by inducing an alteration of sperm motility may play a major role in cases of idiopathic asthenozoospermia. The presence of HPV in semen also suggests that male circumcision may prevent other Sexually Transmitted Infections such as HIV infection to males but females may still be at risk of cervical cancer and other diseases that are caused by the exposure to HPV through semen.

## 6.1 IMPLICATIONS AND RECOMMENDATION

We recommend that a study with a much larger sample size be carried out to verify our findings as this was the first study on this topic and type of samples in Zambia. Another recommendation is that, if a study was to be carried out on this topic, sample collection be done on site as time of delivery for samples may affect such parameters as motility. To avoid or minimize on the errors due to time of delivery, we instructed the patient to bring the samples to the laboratory within one hour of collection.

## 6.2 LIMITATIONS

The limitation of this study is the lack of genotyping of the isolated HPV DNA as that could have given more insight on the specific HPV genotype isolated from these samples. This could have given more guidance on whether the current available vaccines could work in this population or not as the vaccines are genotypes specific.

## 6.3 FUTURE DIRECTION

In future we intend to genotype all the amplified HPV DNA so we can have the data base of the genotypes associated with semen in this population. We also intend to investigate whether there is an association between the male factor and HPV in this population in Zambia. We intend to study the effects of circumcision on HPV semen infection and clearance. Lastly we intend to investigate whether spermatozoa infected with HPV DNA have the capacity to fertilize, transfer viral DNA to oocytes, and if infected oocytes may be able to produce normal embryos.

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## APPENDICES

## APPENDIX I: INFORMATION SHEET

My name is Phiri Christopher Newton studying for a Master of Science degree (MSc.) in Pathology (Clinical Pathology) at the University of Zambia, Ridgeway Campus. I am carrying out a research as a requirement for fulfilment of Master of Science (MSc) degree.

### About the Study

Human Papillomavirus (HPV) is a virus that is known to cause cervical cancer. It is categorised into high-risk and low-risk types based on their ability to cause malignant transformation. HPV has been recently demonstrated to have a negative influence on sperm parameters, the fertilization process and the abortion rate.

This study was aimed at investigating the prevalence of HPV infection in semen and semen parameter abnormalities from males submitting specimens for semen analysis in parasitology laboratory at the UTH. The results will promote further research on the effects of HPV infection on male infertility and female infection through semen. The results will also promote further research into whether HPV infected sperm may fertilize, transfer viral DNA to oocytes, and if infected oocytes may be able to produce normal embryos. The study will also add to the board of knowledge, new data supporting or disputing whether HPV male vaccination could represent a possible strategy for male fertility preservation and for Assisted Reproduction Technology (ART) success rate improvement..

### Participating in the Study

You have been invited to take part in this study because you are seeking medical fertility attention and meet the study inclusion criteria. However, you are under no obligation to take part in this study. If you decide to take part, you are free to withdraw from the study at any point, without having to give a reason. If you decide to take part in this study, you will be requested to consent by way of signing a consent form , allowing the research team to use part of your submitted semen specimen for routine analysis in this study without affecting your routine results.

If you do withdraw from the study your personal data will be destroyed and, therefore, excluded from any of the results reported. If you decide not to take part or withdraw from the

study it will not affect the type of care you receive in any way, nor will it affect your relationship with any of the staff at UTH.

**Benefits for Participation**

Although there are no direct benefits from the results of this study to you as a participant, your data will help us determine whether HPV male vaccination could represent a possible strategy for male fertility preservation and prevention of penile and cervical cancers in future.

**Problems with the Study**

There are no problems expected to arise in this study as the procedure by participants to submit the specimen is non invasive and part of the same specimen for routine analysis will be used for the study without affecting the routine test results.

**Participant Confidentiality**

All information that is collected about you during the course of the research will be anonymous and stored securely and with strict confidentiality. All data collected during the course of the research will only be used for the purposes of the study. It will not be possible to identify you in any report or publication since study numbers will be used for each individual. All your personal medical records will be kept confidential.

**Deliverables**

The data generated from this project will be used for the MSc dissertation, disseminated by publication and presentation at scientific conferences.

**Contact Details**

In case you have any more questions about this study at any time, please feel free to contact any of the numbers below:-

1. **Phiri Christopher Newton**
   The Principle Investigator
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4. **The University of Zambia**
   **Biomedical Research Ethics Committee**
   Ridgeway campus
   P.O.Box 50110
   Lusaka, Zambia
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   Email: unzarec@unza.zm

## APPENDIX II: PERMISSION LETTERS FROM HOSPITAL MANAGEMENT

Phiri Christopher Newton
University of Zambia
School of Medicine
Department of Pathology and Microbiology
**LUSAKA,**

12 OCT 2015

12TH October 2015.

REPUBLIC OF ZAMBIA
SENIOR
MEDICAL SUPERINTENDENT
12 OCT 2015
UNIVERSITY TEACHING HOSPITAL
PRIVATE BAG RW1X, LUSAKA

The Senior Medical Superintendent
University Teaching Hospital
**LUSAKA.**

Dear Sir,

**RE: REQUEST FOR PERMISSION TO CONDUCT THE RESEARCH PROJECT AT THE INSTITUTION (UTH)**

I am a student pursuing a master of science in pathology (clinical pathology) at the University of Zambia, school of medicine.

I have written to request for permission to conduct my research at your institution titled "**Molecular Characterisation of Human Papilloma Virus (HPV) in semen from males attending the Fertility Clinic at the University Teaching Hospital**".

Please find attached a copy of my project proposal containing all the necessary information. The school has approved the research proposal and Ethical approval will be sought from UNZABREC upon your consideration of my request.

Yours sincerely

**Phiri Christopher Newton.**

University of Zambia
School of Medicine
Department of Pathology and Microbiology
**LUSAKA, ZAMBIA.**

16TH February 2016.

The Principal Investigator
KS-HHV8 Laboratory,
P.O Box 50223
**RIDGEWAY, LUSAKA.**
**ZAMBIA.**

Dear Madam,

**RE: REQUEST FOR PERMISSION TO CONDUCT THE RESEARCH PROJECT AT YOUR LABORATORY.**

Reference is made to the above captioned matter. I am a student pursuing a master of science in pathology (clinical pathology) at the University of Zambia, school of medicine.

I have written to request for permission to conduct my research at your laboratory titled "**Molecular Characterisation of Human Papilloma Virus (HPV) in semen and semen abnormalities at the University Teaching Hospital (UTH), Lusaka, Zambia.**".

The school, UTH and UNZABREC has already approved the research proposal. Please find attached a copy of my project proposal containing all the necessary information.

Your favourable consideration of my request will be highly appreciated.

Yours sincerely

**Phiri Christopher Newton.**

**(0969288036)**

## APPENDIX III: AUTHORIZATION LETTERS FROM SCHOOL MANAGEMENT

# THE UNIVERSITY OF ZAMBIA

SCHOOL OF MEDICINE

**PATHOLOGY AND MICROBIOLOGY DEPARTMENT**

***INTERNAL MEMORANDUM***

| | | |
|---|---|---|
| **TO** | : | Assistant Dean, Postgraduate - School of Medicine |
| **FROM** | : | Head, Pathology and Microbiology Department |
| **DATE** | : | 10th August, 2015 |
| **SUBJECT** | : | **SUBMISSION OF MR PHIRI CHRISTOPHER NEWTON: RESEARCH PROPOSAL TO UNZABREC** |

I write to confirm that the corrections have been made to the proposal and is now ready for clearance by **UNZABREC**. The supervisors are satisfied that the research proposal entitled **"Molecular Characterization of Human Papilloma Virus (HPV) in Semen from Males Attending the Fertility Clinic at the University Teaching Hospital, Lusaka"** meets the requirements set by the Directorate of Research and Graduate Studies (DRGS) and is therefore ready for submission to **UNZABREC**.

Dr T. Kaile
**HOD/MSc PATHOLOGY COORDINATOR**

# THE UNIVERSITY OF ZAMBIA

SCHOOL OF MEDICINE

Telephone : +260211252641
Telegram: UNZA, Lusaka
Telex: UNZALU ZA 44370
Email: assistantdeanpgmedicine@unza.zm

P.O Box 50110
Lusaka, Zambia

11th August, 2015

Mr. Phiri Christopher Newton
Department of Pathology & Microbiology
School of Medicine
UNZA
**LUSAKA**

Dear Mr. Phiri,

**RE: GRADUATE PROPOSAL PRESENTATION FORUM**

Following the presentation of your dissertation entitled **"Molecular Characterization of Human Papiloma Virus (HPV) in Semen from Males Attending the Fertility Clinic at the University Teaching Hospital, Lusaka"** your supervisor has confirmed that the necessary corrections to your research proposal have been done.

You can proceed and present to the Research Ethics.

Yours faithfully,

UNIVERSITY OF ZAMBIA
SCHOOL OF MEDICINE
12/ AUG 2015
ASSISTANT DEAN (PG)
P.O. BOX 50110, LUSAKA

Dr. S.H. Nzala
**ASSISTANT DEAN, POSTGRADUATE**

CC: HOD, Pathology & Microbiology

## APPENDIX IV: LETTER OF APPROVAL FROM UNZABREC

# THE UNIVERSITY OF ZAMBIA

## BIOMEDICAL RESEARCH ETHICS COMMITTEE

Telephone: 260-1-256067
Telegrams: UNZA, LUSAKA
Telex: UNZALU ZA 44370
Fax: +260-1-250753
E-mail: unzarec@unza.zm

Ridgeway Campus
P.O. Box 50110
Lusaka, Zambia

**Assurance No. FWA00000338**
**IRB00001131 of IORG0000774**

3rd February, 2016.

Our Ref: 009-11-15.

Mr. Christopher N. Phiri,
University of Zambia,
School of Medicine,
Department of Pathology and Microbiology,
P.O Box 50110,
**Lusaka.**

Dear Mr. Phiri,

**RE:** RESUBMITTED RESEARCH PROPOSAL: **"MOLECULAR CHARACTERIZATION OF HUMAN PAPILLOMA VIRUS (HPV) IN SEMEN AND SEMEN PARAMETER ABNORMALITIES AT THE UNIVERSITY TEACHING HOSPITAL (UTH), LUSAKA, ZAMBIA" (REF. No. 009-11-15)**

The above-mentioned research proposal was presented to the Biomedical Research Ethics Committee on 3rd February, 2016. The proposal is approved.

**CONDITIONS:**

- This approval is based strictly on your submitted proposal. Should there be need for you to modify or change the study design or methodology, you will need to seek clearance from the Research Ethics Committee.
- If you have need for further clarification please consult this office. Please note that it is mandatory that you submit a detailed progress report of your study to this Committee every six months and a final copy of your report at the end of the study.
- Any serious adverse events must be reported at once to this Committee.
- Please note that when your approval expires you may need to request for renewal. The request should be accompanied by a Progress Report (Progress Report Forms can be obtained from the Secretariat).
- **Ensure that a final copy of the results is submitted to this Committee.**

Yours sincerely,

M.C Maimbolwa PhD
**CHAIRPERSON**

**Date of approval:** 3rd February, 2016. **Date of expiry:** 2nd February, 2017.