

**Factors associated with HIV Seroconversion during pregnancy in
Manzini region, Swaziland in 2012**

A dissertation submitted to the

SCHOOL OF NURSING AND PUBLIC HEALTH

COLLEGE OF HEALTH SCIENCES

UNIVERSITY OF KWAZULU-NATAL

DURBAN, SOUTH AFRICA

**In partial fulfilment of the requirements for the
Master of Public Health**

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29 November 2013

“As the candidate’s supervisor I agree/do not agree to the submission of this dissertation for examination”

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Date: 28 November 2013

DEDICATION

I dedicate this dissertation to all the women who participated in this study. Without your contribution, this dissertation would not have been possible.

ACKNOWLEDGEMENTS

Firstly, I would like to extend my sincere gratitude to my supervisor Dr Anna Voce, who was with me from the beginning to the completion of the dissertation. Thank you for guiding and showing me direction throughout the dissertation.

I am also grateful to my friends and family who gave me support and their unlimited assistance.

This dissertation would not have been completed without the most important person in my life, my husband Shepherd. Thank you love for the support, guidance and assistance you gave me especially during the writing of the dissertation.

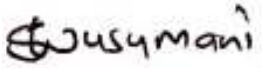
A special acknowledgement goes to the women who participated in this study, I would like to thank them for their time and the information they contributed.

Above all, I would like to thank God for being with me through the writing of this dissertation.

DECLARATION

I Sibongile Wusumani declare that:

- (i) The research reported in this dissertation, except where otherwise indicated, is my original work.
- (ii) This dissertation has not been submitted for any degree or examination at any other university.
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ABSTRACT

Background: The HIV epidemic has greatly affected sub-Saharan Africa, with the highest prevalence in the world found in Swaziland. One in three pregnant women in Swaziland has HIV. One of the PMTCT strategies is primary prevention of HIV among women who are uninfected. Understanding the reasons why pregnant women continue to seroconvert is the key in meeting this strategy.

Purpose: The purpose of this study is to determine the factors associated with seroconversion among pregnant women utilizing Raleigh Fitkin Memorial Hospital in 2012.

Objectives: The objectives of this study are to: determine the proportion of HIV non-infected pregnant women who are retested for HIV during pregnancy; determine the gestational age at which pregnant women are retested for HIV; establish the proportion of women who were initially HIV non-infected and seroconverted during pregnancy; and establish the factors associated with seroconversion during pregnancy.

Methods: An observational cross-sectional study design with both descriptive and analytic components was carried out at Raleigh Fitkin Memorial Hospital. Systematic sampling was used for the recruitment of 381 pregnant women who were initially HIV non-infected. An interviewer-administered questionnaire and chart review were used to collect demographic and clinical data. The data was analyzed using descriptive and analytic statistics.

Results: The results of the study show that demographic factors such as age and educational level are associated with HIV seroconversion during pregnancy. The findings also highlight how partner factors play a role in HIV seroconversion. The results indicate that sexual behaviours 333of the pregnant women contribute greatly to HIV seroconversion.

Conclusion: Pregnant women continue to engage in risky sexual behaviours during pregnancy and there is need to strengthen counseling on preventive measures throughout the antenatal care period. There is also need for programs to explore possibility of providing antiretroviral drugs for pre-exposure prophylaxis to all HIV negative women during pregnancy.

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DEFINITION OF TERMS

HIV non-infected	- a person who shows no evidence of HIV infection on an antibody blood test.
Living with HIV	-a person who has antibodies against HIV detected on a blood test.
Retest	- is repeating the HIV test following no detection of HIV antibodies.
Seroconversion	- is converting from sero-negative to sero-positive for HIV antibody test.

LIST OF ABBREVIATIONS

AIDS	- Acquired Immunodeficiency Syndrome
AHI	- Acute HIV infection
ANC	- Antenatal Care
ARV	- Antiretroviral
α	- alpha
β	- beta
CCR5	- Cysteine-Cysteine Chemokine Receptor 5
DHS	-Demographic and Health Survey
HIV	- Human Immune Virus
HSV2	- Herpes Simplex Virus 2 or Human Herpes Virus 2
MDGs	- Millennium Development Goals
MTCT	- Mother-to-Child Transmission
PMTCT	- Prevention of Mother-to-Child Transmission
p24	- A component of the HIV particle capsid
RFM	-Raleigh Fitkin Memorial
RNA	- Ribonucleic acid
SDHS	-Swaziland Demographic and Health Survey
STIs	- Sexually Transmitted Infections
TDF	- Tenofovir Disoproxil Fumarate
WHO	- World Health Organization
ZVITAMBO	-Zimbabwe Vitamin A for Mothers and Babies

CHAPTER 1: INTRODUCTION

1.1 Background of the study

This dissertation presents an investigation of factors associated with Human Immunodeficiency Virus (HIV) seroconversion during pregnancy among women in Swaziland. The HIV epidemic has posed a big challenge in sub-Saharan Africa with two thirds of people living with HIV found in this region [1].

Swaziland's HIV prevalence has been among the highest in the world. The antenatal HIV surveillance in 2010 estimated prevalence among pregnant women to be 41% [2]. Previous studies have shown that women are greatly affected by the HIV epidemic because of gender inequalities which deprive them of negotiating power for safer sex, and because of poverty which also increases their vulnerability to HIV infection because of low socioeconomic status [3, 4]. In Uganda the HIV incidence is shown to be higher among pregnant women compared to their non-pregnant counterparts [5]. Various biological and behavioral factors that occur during pregnancy have been implicated as the causes of this high incidence [5, 6].

According to the Swaziland Demographic and Health Survey (SDHS) [7], the antenatal coverage in Swaziland is above 97% with 74% of pregnant women attending antenatal care (ANC) for at least four visits. The median gestational age at initial ANC is 20 weeks. Antenatal care therefore provides an opportunity to care for pregnant women living with HIV, and to implement interventions for the prevention of mother-to-child transmission (PMTCT). However, 5% of pregnant women in Swaziland, the highest percentage in the world, seroconvert during pregnancy [8].

1.2 Problem Statement

Seroconversion during pregnancy is associated with high mother-to-child transmission of HIV. In the Zimbabwe Vitamin A for Mothers and Babies (ZVITAMBO) study [9], the transmission of HIV was high in children born to mothers who were initially HIV negative and seroconverted during pregnancy (35 infants per 100 child years) compared to exposed babies whose mothers were diagnosed with HIV at initiation of ANC (9 infants per 100 child years). Acquiring HIV infection during pregnancy increases mother-to-child transmission because the acute HIV infection period is characterized by very high viral load, which facilitates transmission [10, 11]. Pregnant women are at higher risk of acquiring HIV compared to non-pregnant women because of hormonal changes during pregnancy which are a mechanism for efficient transmission of HIV [12].

Pregnant women who are in discordant relationships, where the woman is non-infected and the partner is living with HIV, are at high risk of acquiring HIV through unprotected sex [13, 14].

Behavioural factors can also explain the reasons why there is a greater risk of getting infected with HIV during pregnancy. In west Africa, previous studies have shown that during pregnancy and breastfeeding periods sexual activity decreases in women, with male partners engaging in risky sexual behaviours [6, 15]. In contrast, sexual behaviors among pregnant and lactating women in southern Africa are less known. However, a recent study in Malawi by Keating *et al.* [16] showed that many pregnant women became less sexually active in the last trimester. This decrease in sexual activity can put pregnant women at risk where the partner is not able to abstain and engages in high risk sexual activity.

Most women who seroconvert during pregnancy contribute greatly to the burden of mother-to-child transmission because most HIV antibody tests cannot detect antibodies during the window period. Standard serologic tests used in most developing countries for diagnosing HIV will not identify all infected pregnant women [10, 17]. Hence women who acquire HIV during pregnancy are not diagnosed as being infected, resulting in a high proportion of new infections in infants which could have been prevented if HIV had been detected early.

The particular concern of this dissertation is women who start their pregnancy HIV free and are infected during pregnancy. The factors associated with the acquisition of new HIV infection during pregnancy needs to be known in order to implement intervention programs aimed at preventing seroconversion during pregnancy [8, 18].

1.3. Aim

The research undertaken sought to determine factors associated with seroconversion during pregnancy among women delivering at Raleigh Fitkin Memorial Hospital in 2012.

1.4. Objectives

The objectives of this study were to:

1. Determine the proportion of HIV negative pregnant women who are retested for HIV during pregnancy;
2. Determine the gestational age at which pregnant women are retested for HIV;
3. Establish the proportion of women who were HIV negative and seroconverted during pregnancy; and

4. Establish the factors associated with seroconversion during pregnancy.

1.5. Significance of study

Most studies have looked at factors associated with HIV seroconversion among lactating women [19-21]. However, few studies have explored why women seroconvert during pregnancy, despite evidence showing that there is greatest risk of vertical transmission when women seroconvert during pregnancy. Studies have shown that many infants who are HIV infected acquired HIV from mothers initially found to be HIV negative in pregnancy [22-24, 23]]. Vertical transmission could have been prevented if the mother had remained non-infected.

Kieffer *et al.* [8] has demonstrated high seroconversion rate during pregnancy in Swaziland. However, it is unclear what could be the factors contributing to the risk of acquiring HIV during pregnancy in Swaziland. Finding out the characteristics, behaviours and practices of the initially HIV negative pregnant women is very important. Having a better understanding of what happens whilst the women are pregnant can contribute to the development of interventions to avert much vertical transmission that occurs as a result of women seroconverting. One of the strategies for the PMTCT program in Swaziland is the prevention of primary HIV infection in pregnant women.

1.6. Overview of the dissertation

This chapter has provided the background to this dissertation, the problem statement and the aim and objectives of the dissertation.

Chapter 2 presents the theoretical and empirical framework for the dissertation through a review of the literature on factors associated with HIV seroconversion during pregnancy, mother-to-child transmission and prevention of mother-to-child transmission of HIV.

Chapter 3 describes the methods that were used in conducting this study. It describes the study site, study design, target population, sampling framework, data collection techniques, data instruments, data analysis process, and data storage. Furthermore, it details the ethical considerations underpinning this study.

Chapter 4 reports on the results of the study. It summarizes the data using descriptive statistics. The outcomes of bivariate and multivariate analysis are presented.

Chapter 5 discusses the findings of the research study and provides conclusions which may be drawn from these results. It further highlights some of the implications of the findings and makes proposals for further research and for practice.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

This chapter presents a review of the literature on HIV seroconversion in pregnant women. The first part of the chapter describes the status of the HIV epidemic in Swaziland in the general population. HIV in pregnancy is discussed in the second part of the review. The third part discusses seroconversion, including factors associated with seroconversion as identified through the literature. The fourth part highlights mother-to-child transmission amongst women living with HIV at onset of pregnancy and those who seroconvert during pregnancy. Prevention of mother-to-child transmission is also discussed in this section. Lastly the conceptual framework guiding this study is described in detail.

2.2 The status of the HIV epidemic in Swaziland

Sub-Saharan Africa, including Swaziland, is greatly affected by the HIV epidemic [1]. The National HIV prevalence survey shows that Swaziland has the highest HIV prevalence in the world, which is estimated to be 26% among men and women 15 to 49 years of age [7]. The way HIV impacts on women in sub-Saharan Africa is disproportionate: in Swaziland female prevalence is 31% compared to 21% in males [1, 7]. The Swaziland Demographic Health Survey (SDHS) [7] shows a variation in HIV prevalence by age and sex; prevalence is highest at 49% among women aged 25 to 29 years old and at 45% in men aged 35 to 39 years old.

According to UNAIDS [1], more than half of the people living with HIV in the world are women. Earlier in the epidemic, in the early 80s, only 35% of women were infected [1]. The shift in the epidemic saw a greater proportion women infected compared to men as a result of heterosexual transmission. Rodrigo *et al* [25] reported the phenomenon of the feminization of HIV and highlighted the relationship between women, poverty and HIV. Most heterosexual infections are a result of multiple partners, either serial or concurrent. Intergenerational sex, which is very common in sub-Saharan Africa, has also been shown to be associated with an increase in risk of HIV acquisition in women. Other factors include socio-cultural factors such as gender norms which result in gender inequality [26]. As a result, women do not feel they have the liberty to choose partners and control sexual activity, including the negotiation of condom use [27]. Polygamy and wife inheritance are some of the cultural practices that put women at risk of getting HIV [28], including in Swaziland.

2.3 HIV in pregnancy

The antenatal prevalence in Swaziland is estimated to be 41%, the highest among pregnant women in the world [29]. Despite the prevalence being high among pregnant women, the antenatal seroprevalence survey has shown that HIV has stabilized when compared to the previous ANC sentinel surveillance. The antenatal survey highlights that the prevalence of HIV among pregnant women is high in the 30-34 age group (54%) and low in the 15-19 age group (20%) [29]. Approximately 13500 exposed infants are born in Swaziland annually and, without intervention, 15 infants will be infected each day, representing a transmission rate of 41% [30]. Most pediatric HIV infections are due to mother-to-child transmission (MTCT). Globally 390 000 new infections occur in children through vertical transmission and of these 360 000 are in sub-Saharan Africa [1]. Vertical transmission occurs mainly through three stages: in utero, during childbirth and during breastfeeding. The rates of MTCT differ in each stage: if there are no interventions, in utero the transmission rate is 5-10%, at childbirth it is 10-15% and during breastfeeding it is 10-15% [31]. When comparing the total rates of vertical transmission without intervention, it is approximately lower in developed countries, at 15-25% versus 25-35% in the developing countries [1].

One of the factors associated with MTCT is seroconversion during pregnancy. HIV seroconversion in sub-Saharan Africa, including Swaziland, is higher among pregnant women compared to non-pregnant women.

2.4 HIV seroconversion during pregnancy

2.4.1 HIV seroconversion and pregnancy

HIV seroconversion and acute HIV infection (AHI) play a major role in MTCT of HIV. AHI is the time from which a person acquires HIV until seroconversion, which is the early stages of HIV infection before the antibodies can be detected [32]. Studies have shown that acute HIV infection in pregnancy increases the risk of MTCT of HIV. The timing of AHI during pregnancy also determines the chances of MTCT. Wertz *et al.* [33] highlight that perinatal transmission is greater when acute HIV infection occurs at time of delivery. This is because the HIV shedding into the cervicovaginal fluid is very high during the time of delivery as observed by Pilcher *et al.* [34].

2.4.2 Factors associated with HIV seroconversion during pregnancy

In sub-Saharan Africa HIV seroconversion during pregnancy has ranged from 1.3 per 100 person years to 5.2 per 100 person years [5, 21]. Being a woman is one of the factors associated with vulnerability to HIV, due to both biological and non-biological factors [25].

A biological disadvantage for women is pregnancy itself, as it can enhance the susceptibility of the female genital tract's susceptibility to HIV [35]. The increased susceptibility could be due to the physiological, biological and behavioural differences that occur during pregnancy compared to non-pregnant women. Hormonal changes that occur during pregnancy may make the pregnant woman more susceptible to HIV [12, 36, 37]. The high infectivity of the female genital tract with HIV might be due to elevated levels of primary endocervical receptors for HIV-1, CCR5, which are associated with a high progesterone state during pregnancy [38]. Previous studies have shown that there is increased HIV incidence during pregnancy [8, 30]. One study by Gray *et al* [5] showed that pregnant women were two times more likely to acquire HIV when compared to breast feeding women, non-breastfeeding women and non-pregnant women. However, the risk of getting HIV by pregnant women cannot be explained by biological factors alone.

Studies have shown that there are certain sexual behaviours during pregnancy that increase the risk of getting HIV among pregnant women. Serocoinfection in pregnancy has been associated with mothers who have unprotected sex during pregnancy [39]. Peltzer *et al.* [40] argue that behavioural factors such as unprotected sex and having multiple sexual partners during pregnancy contribute greatly to the risk of HIV acquisition. Being in a discordant relationship can also increase the risk of vertical transmission. If the woman is negative and the partner is positive this might increase the risk of getting HIV during pregnancy. In a study of HIV serodiscordant couples, Mugo *et al.* [14] observe that the risk of getting HIV was greatest during pregnancy. In Africa most discordant couples are pressured socially to conceive and they engage in unprotected sex, which is usually continued during pregnancy. Mugo *et al.* [14] argue that this may also increase the risk of HIV infection among pregnant women. There seems to be a need for male involvement and partner testing as shown by a study in Rwanda [41]. This is because male partners often use the woman's test result as a proxy for their own status, and can increase the risk of the negative pregnant woman acquiring HIV. It appears that there is need to prevent primary HIV acquisition especially in couples who are discordant relationships using drugs for pre-exposure to HIV such as tenofovir disoproxil fumarate (TDF) or TDF plus emtricitabine (TDF/FTC) as shown by current studies [42-44].

HIV seroconversion among pregnant women has been observed in women with history of sexually transmitted infections (STIs). STIs like Herpes Simplex Virus (HSV 2) have been shown to increase HIV acquisition. In one study, three out of five pregnant women who had seroconverted had chronic HSV2 infection [17].

Changes in sexual activity can also put the pregnant women at risk of getting HIV. A recent cross-sectional study by Keating *et al.* [16] showed that sexual activity among pregnant women decreased as the gestational age increased. The researchers in this study used both questionnaire and in-depth-interviews, which allowed greater comparison of sexual behaviours in early pregnancy, late pregnancy, during lactation and in non-pregnant women to learn more about the beliefs about abstinence during pregnancy. Different authors explain that the decrease in sex acts during pregnancy may be associated with increased partner risky behaviours [6, 15]. However, the recent study by Keating *et al.* [16] reports no difference in spouse's risky behaviours, although some women in the study were afraid that the decrease in sexual activity during pregnancy could result in partner infidelity and HIV infection. Cultural beliefs seem to play a big role in abstinence during pregnancy and most women continue to follow this belief. However, some studies have shown that married women who reported that they practice abstinence were 3.2 times at risk of getting HIV compared to those who did not abstain [45]. Other researchers argue that these women acquire HIV despite them reporting abstinence because they are likely to continue having irregular unprotected sex [16].

A study in Zimbabwe showed that the incidence of HIV among pregnant women was associated with the age of the woman, educational level and time of seroconversion [46]. Women, who are young, especially those less than 19 years, were more likely to seroconvert during pregnancy [21, 46, 47]. Marital status is another factor that previous studies have highlighted; being married does not protect the women from acquiring new HIV infection. Polygamous relationships generally increase the risk of acquiring new infections especially among married women [48]. This could be due to the fact that there are many parties involved in the relationship, and if one partner gets infected everyone becomes at risk.

2.5 Mother-to-child transmission of HIV

2.5.1 Timing of HIV infection and MTCT

The timing of HIV acquisition plays a critical role in MTCT rates. Studies have been done to compare MTCT among women who were infected at onset of pregnancy and those that seroconvert during pregnancy. Gay *et al.* [17] argue that women who are infected in the third trimester have the highest risk of MTCT compared to women who acquired HIV before pregnancy or in the early trimesters. The high levels of viral load during acute HIV infection have been associated with increased MTCT.

Various studies have been done to compare the transmission rate of HIV among women with different viral loads. John *et al.* [49] show that transmission rate was at 9% in women with viral loads less than 10 000 copies/mL versus 45% for women with viral loads above 43 000 copies/mL. When comparing the transmission rate of women who booked for their ANC already HIV positive and those that seroconverted during pregnancy, different authors produce varying results. In a study in Malawi, the mean viral load among women who seroconverted during pregnancy was very high: 1,324,766copies/mL compared to 35,537copies/mL that had tested HIV positive at first ANC visit [17]. However, a study by Rodongpisuthipong *et al.* [50] shows no difference in viral loads between women who seroconverted during pregnancy and those who had initial HIV positive results at first ANC visit. In this study there was similarity in mean viral loads among pregnant women who seroconverted and those who tested positive at initial ANC (17,505 and 20,845 copies/mL respectively). Both studies used a prospective cohort design to explore the association between pregnancy and acquisition of HIV and infant seroconversion. Both studies enrolled pregnant women who tested HIV positive and those who seroconverted during pregnancy.

The outcomes of the pregnancies of women who seroconvert during pregnancy have been compared with those of women who had an initial HIV positive result at first ANC booking. A study by Birkhead *et al.* [51] shows that 22% of infants who tested HIV positive were born from mothers who had a negative HIV result at first antenatal visit and seroconverted during pregnancy compared to 1.8% babies whose mothers booked for ANC already living with HIV. Humphrey *et al.* [19], in the ZVITAMBO trial indicated that women who acquire infection during pregnancy are 4.6 times more likely to infect their infants as compared to women who were already living with HIV at the beginning of the trial. The high percentages of infants born from women who seroconvert during pregnancy might be due to the increased viral loads experienced during acute HIV infection when these mothers are not aware that their HIV status has changed.

2.5.2 Prevention of Mother to Child Transmission of HIV (PMTCT)

Vertical transmission of HIV is preventable. The goal of PMTCT programs is to eliminate pediatric infections. The World Health Organization (WHO) [52] recommended a four pronged strategy for PMTCT so as to prevent pediatric HIV infections and ensure the survival of both the mother and infant. Prong one: prevention of HIV infection among young women and pregnant women. Prong two: prevention of unintended pregnancies among HIV positive women.

Prong three: prevention of HIV from HIV positive women to their infants. Prong four: provision of treatment, care and support to HIV infected women, their children and spouses.

Most of the focus of PMTCT programs has been on prong three, that is the women who are living with HIV at onset of pregnancy. These women are given antiretroviral prophylaxis which includes zidovudine and intrapartum drugs and infant nevirapine [30]. Women who seroconvert during pregnancy might not access the PMTCT interventions because it is difficult to diagnose AHI. The tests that are used in developing countries, Swaziland included, cannot easily identify AHI. During the window period, HIV antibodies are at low levels and hence cannot be detected using standard antibody test kits. To diagnose AHI in pregnant women with negative antibodies for HIV, sophisticated tests are required that will be able to detect HIV Ribonucleic Acid (RNA) or p24. Birkhead *et al.* [51] show that women who seroconverted during pregnancy were less likely to receive intrapartum antiretroviral (ARV) drugs (80.5%) compared to women who were already living with HIV prior to pregnancy (10.5%).

Preventing HIV among young women and reducing the incidence of HIV during pregnancy is the key to lowering the vertical transmission rates of HIV [53]. If there is no HIV infection to start with there will be no MTCT. Health education of the women on HIV testing is very important so as to prevent HIV incidence in pregnant women thus reducing vertical transmission of HIV [54]. Mofenson [53] highlights the importance of retesting especially at delivery and postnatal period for the early diagnosis of women with acute HIV infection. Retesting on its own may not be enough. Strategies are needed to address the issue of seroconversion. The PMTCT programs should have strategies to prevent new infections among pregnant women [48].

2.6 Conceptual framework

There are many factors associated with HIV seroconversion during pregnancy, as presented in the previous section. These factors may be organized into demographic, socio-economic, cultural, health and health seeking behaviours. The proximate determinant framework proposed by McCarthy and Maine [55] provides an approach to organizing these factors and the influence they have on each other and on pregnancy, HIV exposure and HIV seroconversion during pregnancy, as shown in Figure 2.1. The original proximate determinant framework proposed by McCarthy and Maine states that it is possible to identify proximate and distant factors which result in either maternal mortality or morbidity. This framework is suitable for adaptation in this study since there are proximate and distant factors that are associated with exposure or non-exposure to HIV, which lead to infection or non-infection and seroconversion during pregnancy.

In this study the distant factors include socio-cultural, economic and demographic factors. Socio-cultural factors include cultural beliefs that influence sexual activity during pregnancy as shown by Keating *et al.*[16].Socio-cultural expectations for children may influence the discordant couples to engage in unprotected sex [14].Economic factors, including poverty, may predispose a woman to HIV because women might engage in risky behaviours such as transactional sex and polygamous relationships [25, 56]. Age, marital status and educational level are some of the predictor demographic factors identified by various authors [21, 46, 48]. These distant factors on their own do not lead to risk taking behaviours but operate through proximate factors that may reduce or increase the risk of HIV infection.

The proximate determinants include health and reproductive factors, access to health facility and utilization of health services by individuals. Reproductive factors such as parity and gravidity have been shown to be one of the factors associated with HIV seroconversion. Health or biological factors in this study include sexually transmitted infections, use of vaginal herbs and male circumcision as shown by other researchers [17, 46]. Behavioural factors, which are considered risky, include multiple partners and nonuse of condoms. The proximate factors interact with each other to create conditions for HIV exposure and infection leading to seroconversion during pregnancy.

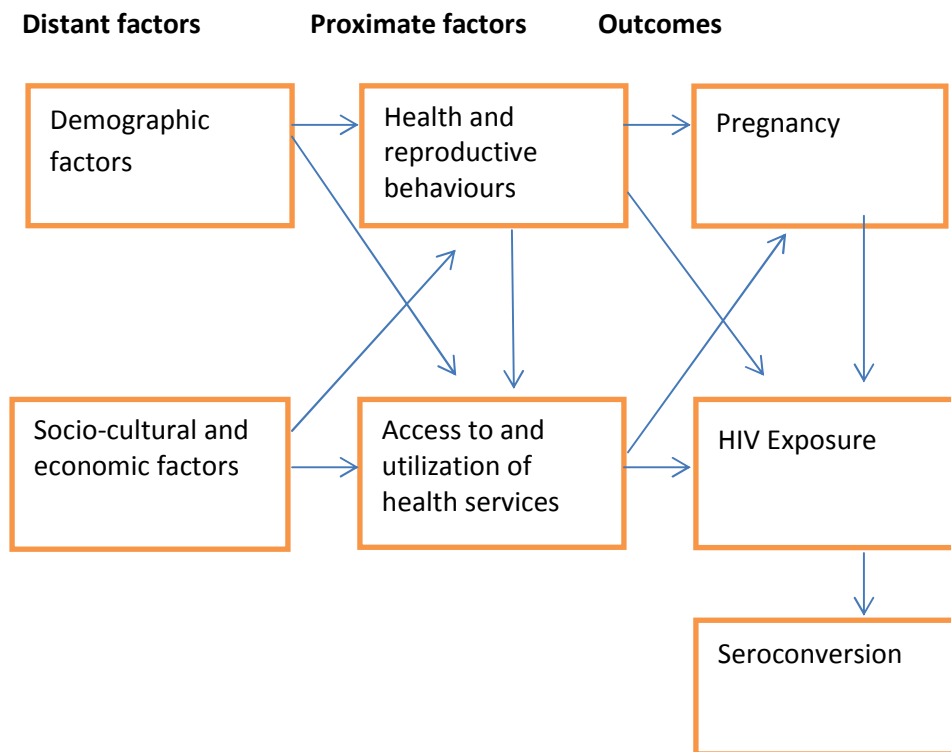


Figure 2.1 Proximate and distant factors conceptual framework for HIV seroconversion in pregnancy

2.7 Summary

The literature review has shown the role of acute HIV infection in MTCT rates. High viral load during the acute HIV infection period is the strongest risk factor for MTCT. Various factors such as socio-cultural, economic, demographic and health behaviours have been shown to be associated with HIV seroconversion. A conceptual framework of distant and proximate factors associated with HIV seroconversion during pregnancy was used to better understand how these factors interact leading in HIV seroconversion.

CHAPTER 3: METHODOLOGY

3.1 Introduction

This chapter describes in detail the methods used to collect and analyze data so as to achieve the aim and objectives of the study. The aim of this research was to determine the factors associated with HIV seroconversion during pregnancy among women delivering at Raleigh Fitkin Memorial Hospital in 2012. The specific objectives were to: determine the proportion of HIV negative pregnant women who are retested for HIV during pregnancy; determine the gestational age at which pregnant women are retested for HIV; establish the proportion of women who were HIV negative and seroconverted during pregnancy; and establish the factors associated with seroconversion during pregnancy. This chapter will explain the study setting, study design, target population, sampling framework, the data collection, storage, and analysis. The ethical considerations in conducting the study will also be presented.

3.2 Study location

The study was conducted in Manzini, at the Raleigh Fitkin Memorial (RFM) Hospital maternity ward. The major reason why RFM was chosen as a study location for this study is because of its central location, which makes the hospital accessible to pregnant women from all the four regions of the country. In Swaziland approximately 33 000 deliveries occur annually and 74% of women are reported to deliver in health facilities. Of the 33 000 deliveries that occur annually in Swaziland approximately 23% occur at Raleigh Fitkin Memorial Hospital.

3.3 Study period

The study participants were recruited from the maternity ward at the RFM Hospital, post-delivery, over a period of three months from April to July 2012.

3.4 Study design

The study design used was observational with descriptive and analytic cross-sectional components. The selected study design enabled the examination of the relationship between exposure and outcome variables. A cross-sectional study design was chosen for this study because of its advantages of being easy and cheap to conduct compared to cohort study designs that were used in similar studies [50, 57]. However, when compared to cohort study designs which were undertaken by other researchers, a cross-sectional study design provides weaker evidence when it comes to determining causation of disease [58].

3.5 Study population

3.5.1 Target Population

The target population was all women who initiate ANC HIV negative in Swaziland.

3.5.2. Study population

The study population was all women delivering at RFM Hospital, who had a record of testing negative for HIV at initiation of ANC. The reason for using women who had tested negative for HIV at first ANC was to help differentiate women who seroconverted from women who were previously sero-positive prior to antenatal care. The first HIV negative test during pregnancy was also used in previous studies as the baseline [17, 19]. Using this unit of analysis allowed comparison of the results of this research with previous studies that have been conducted.

3.5.2.1 Inclusion criteria

All pregnant women who delivered at RFM Hospital and who had a record of an HIV negative result at initiation of antenatal care were included in the study.

3.5.2.2 Exclusion criteria

The following pregnant women were excluded from the study:

- All pregnant women who were living with HIV before initiation of ANC;
- All pregnant women who tested HIV positive at initiation of ANC; and
- All pregnant women who were never tested for HIV during ANC.

3.6 Sampling size and strategy

3.6.1 Sampling size

The sample size comprised a representative proportion calculated from the number of women who deliver at RFM Hospital. The sample size was calculated using Stata version 11 based on the following assumptions:

α -0.05

β - 0.80

Minimal clinical difference- 10%

Seroconversion rate- 5%

The total number of annual deliveries at RFM Hospital in 2010 was 7751. Thus, a sample size of 381 pregnant women was deemed sufficient for fulfilling the objectives of the study.

3.6.2 Sampling strategy

The average monthly deliveries conducted at RFM hospital maternity in 2010 was 645, hence the sampling strategy used in this study was systematic random sampling. The advantage of systematic random sampling is that one does not need to know the complete population in order to do sampling[58]. The first pregnant woman was selected randomly from the delivery register. Each third woman after the starting point was selected into the study until the required sample size was reached. The pregnant women were recruited post-delivery from the maternity ward instead of ANC because most pregnant women initiate ANC late in pregnancy. According to the SDHS [7] the median number of weeks at first booking is twenty weeks. The SDHS states that, “A large proportion of the women continue to delay the initiation of antenatal care.” This makes it difficult for all women to be retested during the antenatal period. Furthermore, recruiting from the maternity ward makes it easier to identify the HIV status of the women post-delivery and permits a complete picture of what happened to the woman during ANC, intrapartum and postpartum periods.

3.7 Data collection

3.7.1 Variables

The following variables in Table 3.1 below were collected, as guided by the conceptual framework.

Table 3.1 List of variables for the study

Dependent variables	Independent variables	Source of data
-Seroconversion	<p><u>Demographic variables:</u> Age marital status, educational level</p> <p><u>Social, economic and cultural variables:</u> Income, history of sexual abuse, use of vaginal herbs</p> <p><u>Health and reproductive variables:</u> Parity, history of multiple partners, age at sexual debut, condom use during pregnancy, presence of syphilis in the pregnant woman, knowledge on HIV,</p> <p><u>Access to and utilization of health services variables:</u> Number of antenatal care visits, gestational age at first booking, gestational age at retest</p>	Interviews with study participants and review of participants’ antenatal care cards and labour records.

3.7.2 Data collection techniques

This study used two techniques to collect data: (i) by conducting structured interviews with women post-delivery and (ii) by undertaking a review of study participants' labour records, which also comprised the ANC card. The following section will describe each technique in detail, highlighting the major strengths and weaknesses of each method.

3.7.2.1 Structured interviews

The structured interviews with the pregnant women were a very valuable data source for this study. During the interviews, the interviewer used a structured questionnaire to avoid misinterpretation of the questions and to minimize variability. The guiding principle behind the development of the questionnaire was the conceptual framework. The variables that were collected during the interview were based on the study objectives using a combination of questions. The questionnaire was interviewer administered as opposed to self-administered because face-to-face interview with the respondent helps to facilitate response and yields quality data. However, interviews are time consuming and costly to conduct [58].

3.7.2.2 Review of records

Review of records was undertaken to complement the information gathered from the interview. The study participants' ANC cards and labor records were reviewed to extract additional data. The data that was collected from reviewing the records include HIV status of participants, reproductive factors such as gestational age, parity, gravidity, history of STIs and retest for HIV. The data extraction tool was developed in a way that data could be collected so as to achieve the objectives. Reviewing records has its own strengths, which include the fact that the information obtained from the records cannot be influenced by the study. Incompleteness of the records is one limitation of this method of data collection [58]. However, most of the variables collected for this study are also key in the management of the pregnant woman hence they were available in the participant records.

3.7.3 Data instruments

The instruments that were used to collect data included an interviewer-administered questionnaire and a data extraction tool to extract data from the participant's ANC card (Appendices A and B respectively).

3.7.4 Data collection process

A pilot study was conducted at Mbabane Government Hospital prior to the commencement of the study to test the data collection instruments. A sample of 34 women was systematically recruited and interviewed. The first pregnant woman was randomly selected and every third woman who fitted the

inclusion criteria was recruited into the pilot. The principal investigator conducted the pilot study. Conducting the pilot study assisted the principal investigator on how to phrase some of the questions for them to be better understood by the participants. The pilot also assisted in editing the questionnaire especially in the flow of the questions. Conducting the pilot helped in establishing the best suitable times to collect the data.

The principal investigator and nurses attending deliveries at the RFM hospital conducted the main study. The first step for the principal investigator was to train the nurses who were going to collect data. The nurses were trained on the purpose of the study, data collection processes, the data collection tools, how to ensure reliability of data, and how to obtain informed consent. The principal investigator and nurses attending to clients delivering at the hospital administered the interviews. The interviews were conducted eight hours after the woman had delivered. The interviews and the data review lasted between 30-45 minutes. The interviews were conducted in the language the participant understood. The majority of the interviews were conducted in SiSwati.

3.7.5 Ensuring validity and reliability

The study was vulnerable to selection bias, as women who deliver at home might have different characteristics than those who deliver in hospital. Women who choose not to deliver at RFM hospital might also be different from those that do.

In this study using structured interviews, whereby the interviewers asked the questions in a standard manner, with similar probes and elaborations for each respondent, ensured the reliability of the data. The responses that the respondents gave were recorded in the questionnaire by the interviewers in a similar way by means of offering options to select from. By administering a standardized questionnaire, reliability of the data was increased.

Validity of data especially information bias, was limited by asking questions exploring similar issues differently. The principal investigator conducted an intensive training of the interviewers and this reduced interviewer variation during the interviews. Periodic checks were done also by the principal investigator on the questionnaires completed by the interviewers and showed them how to deal with errors seen.

3.7.6 Data management and storage

- i. Data quality - Double entry of data was done to ensure that there were no errors in data entry. Data was entered into two different excel spreadsheets and compared for discrepancies. Differences in the entries were resolved by checking the questionnaires.
- ii. Data safety - Electronic databases were password protected and the raw data was kept in a lockable cabinet at Elizabeth Glaser Pediatric AIDS Foundation offices, which are protected by a pin-coded alarm. The principal investigator kept the cabinet keys.
- iii. Data storage- Backup of data in hard drives and compact discs was done regularly.

3.7.7 Data analysis

- i. Data was analyzed using SPSS software version 20 (SPSS Inc).
- ii. Data was summarized using descriptive statistics. Categorical data was summarized using frequency distributions. Exploratory analyses were done for ordinal categorical variables so as to know which summary statistics to use. Symmetrical data was summarized using mean, mode and standard deviation. Asymmetrical data was summarized using median and inter-quartile range.
- iii. Bivariate and multivariate analysis was done. Variables were manipulated in order to perform the appropriate regression analysis. The reference points used in the bivariate analysis were guided by literature.

3.8 Ethical considerations

The basic principles of ethics: autonomy, beneficence, non-maleficence and justice were supported in this study. Ethics approval was provided by the University of KwaZulu-Natal Biomedical Research Ethics Committee, BREC reference number BE 120/11 and the Swaziland Ethics Committee, reference number MH/599C). Permission was sought from the RFM hospital administration to conduct the study at the health facility. The study participants were granted the opportunity to refuse or withdraw from the study. Prior to participation in the study, the participants were requested to provide written informed consent (refer to Appendices F and G). To ensure confidentiality no names of participants were written on the questionnaire; a study number was assigned to each participant.

CHAPTER 4: RESULTS

4.1 Introduction

The aim of this study was to investigate the factors associated with HIV seroconversion during pregnancy in women recruited at RFM hospital, in Manzini. This chapter presents the data collected from women post-delivery in the maternity department at RFM hospital. Three hundred and eighty one women were recruited into the study between April and July 2012. Descriptive results with regard to the demographic characteristics, sexual behaviours, partner factors, reproductive factors and knowledge on HIV will be presented in this chapter. Statistical analysis was used to determine which factors, were associated with HIV seroconversion during pregnancy.

4.2 Sample realization

Three hundred and eighty one women were interviewed for the study, of which three had inconclusive results at second HIV test (n=3) and hence were excluded from the analysis. The final sample for analysis comprised three hundred and seventy eight (N=378) women. Of the 378 women included in the analysis 9.7% seroconverted (n=37) and 90.2% (n=341) remained HIV uninfected.

4.3 Demographics characteristics

Table 4.1 shows the demographic characteristics of study participants, comparing seroconvertors and non-seroconvertors. The mean age for the women was the same between the two groups (22 years $p=0.85$). A greater percentage of the participants were below the age of 30 years in both groups as shown in Table 4.1. With regard to marital status, the women fell into three main groups: married (30%), never married (58%) and cohabiting (11.1%). However, among those who were married a small percentage (7%) was in a polygamous relationship. The majority of women had attained an education level greater than primary schooling. More than half (63%) of the women reported that they were unemployed. Fewer women reported family income greater than 3000 Emalangeni per month (equivalent to 333 USD).

Compared to women 30 years or older, women in the 20 to 23 years category and those in the 24 to 29 years were 2.4 times and 1.78 times respectively at risk of seroconversion during pregnancy though not statistically significant. Individuals who were in polygamous relationships were 2.88 times more likely to seroconvert than those who were in monogamous relationships. However, this was not statistically significant ($p=0.26$).

Table 4.1 Clinical characteristics of study participants by seroconversion status (N=378)

Variables	Categories	HIV seroconvertors n=37 (%)	HIV non-seroconvertors n=341(%)	Odds ratio for seroconversion (95% CI)	p-value
Age	16-19	4 (10.8)	89 (26.1)	0.66 (0.14-3.08)	0.60
	20-23	18 (48.6)	110 (32.3)	2.40 (0.67-8.56)	0.18
	24-29	12 (32.4)	98 (28.7)	1.78 (0.48-6.68)	0.38
	≥30	3 (8.1)	44 (12.9)	REF	
	Mean	22	22		0.85
Marital status	Married	13 (35.1)	101 (29.6)	REF	
	Never married	20 (54.1)	200 (58.7)	0.78 (0.37-1.63)	0.50
	Separated	0	2 (0.6)	0	1
	Divorced	0	0		
	Widowed	0	0		
	Cohabiting	4 (10.8)	38 (11.1)	0.82 (0.25-2.66)	0.74
Polygamous marriages	Yes	2 (15.4)	6 (5.9)	2.88 (0.52-16.04)	0.23
	No	11 (84.6)	95 (94.1)	REF	
Level of education	None to Some primary	8 (21.6)	18 (5.3)	7.85 (1.88-32.83)	0.005*
	Completed primary	8 (21.6)	33 (9.7)	4.28 (1.06-17.3)	0.041*
	Some secondary	11 (29.7)	123 (36.1)	1.58 (0.42-5.89)	0.50
	Completed secondary	7 (18.9)	114 (33.4)	1.09 (0.27-4.36)	0.91
	Tertiary	3 (8.1)	53 (15.5)	REF	
Source of Income	Unemployed	26 (70.3)	211 (61.9)	1.29 (0.29-5.84)	0.74
	Student	0	22 (6.5)	0.000	1
	Formal skilled	2 (5.4)	21 (6.2)	REF	
	Formal unskilled	2 (5.4)	34 (10.0)	0.62 (0.81-4.72)	0.64
	Self employed	7 (18.9)	53 (15.5)	1.39 (0.27-7.23)	0.70
Income level	None	11 (29.7)	114 (33.4)	2.48 (0.67-9.17)	0.17
	Less than E1000	13 (35.1)	84 (24.6)	3.97 (1.10-14.47)	0.037*
	E1000-E3000	10 (27.0)	66 (19.4)	3.89 (1.03-14.73)	0.046*
	≥ E3000	3 (8.1)	77 (22.6)	REF	

*Statistically significant at p-value of <0.05.

Bivariate analysis showed statistically significant associations in two demographic characteristics as shown in Table 4.1: level of education (p=0.002) and income levels (p=0.046). The risk of seroconversion was higher in those with a lower level of education, and there was a trend towards decreasing risk of seroconversion with rising level of education (p <0.001).

In the multivariate logistic regression analysis, level of education retained statistical significance as shown in Table 4.2. Age was not statistically significant in the bivariate analysis and assumed borderline significance ($p= 0.067$) in the logistic regression.

Table 4.2 Logistic regression on the demographic characteristics

Variable	LR Test (X^2 Test)
Age	0.067
Marital status	0.84
Polygamous relationship	0.23
Level of education*	0.002
Source of income	0.20
Income level	0.119

*Statistically significant in the bivariate analysis

4.4 Partner factors

This study assessed several partner factors, as shown in Table 4.3 below, including: level of education, circumcision status, reported faithfulness, reported HIV status, frequency of travelling, and having discussed HIV issues in the relationship.

A high percentage of seroconvertors, 62.2% versus 23.5% non-seroconverters, reported that their partners were frequent travelers. It is evident from Table 4.3 that most partners who travelled did so once a month in both groups. Women with partners who were frequent travelers were associated with increased risk of seroconversion during pregnancy ($p<0.001$). There is a trend towards an increase in risk of seroconversion as the frequency of travel increases. Reviewing the time spent away by partner, a trend is again seen towards an increase in risk of seroconversion during pregnancy as the partner's length of travel increases. Women who reported that their partners were away for several months at a time were 11.5 times at risk of getting HIV during pregnancy ($p= 0.026$) compared to those who reported that their partners travelled for a few days.

Almost all (94%) of the women in the study reported that their partners had completed secondary and tertiary education. Low partner education was associated with increased risk of seroconversion during pregnancy. Women whose partners had no education or some primary school education were 11.1 times more likely to seroconvert versus those with higher education ($p=<0.001$). There is a trend towards increase in risk of seroconversion of mothers during pregnancy as the partner level of education decreases.

More than two thirds of the women in both groups had partners who were not circumcised. Partner circumcision status, as shown in Table 4.3 is a protective factor. However, statistical significance was not established in this study ($p=0.20$).

When it came to discussing HIV issues in the relationship, a high percentage (87%) of the women reported that they did talk about HIV with their partners. In relationships where the women and their partners did not talk about HIV issues, they were 2.48 times more likely to seroconvert during pregnancy. Also the association between discussion of HIV issues in the relationships and seroconversion was statistically significant ($p=0.03$).

Despite the high proportion of women reporting that they have discussed HIV issues with their partners, amongst seroconvertors 73% reported that their partners had not tested or they did not know if their partners had tested. The risk of seroconversion during pregnancy was greatest among women who reported not knowing the HIV status of their partners ($p < 0.001$).

Interestingly, more than 50% of the women who seroconverted perceived that their partner was either not faithful or they doubted the faithfulness. There was a statistically significant association between the perception of partner faithfulness and HIV seroconversion during pregnancy.

Table 4.3 Partner characteristics

Categorical predictor Variables	Categories	HIV seroconvertors n=37 (%)	HIV non-seroconvertors n=341(%)	Odds ratio for seroconversion (95% CI)	p-value
Frequent traveler	Yes	23(62.2)	80(23.5)	5.33(2.63-10.86)	<0.001*
	No	14(37.8)	260(76.5)	REF	
Frequency of travel	Once a week	3(13.0)	28(34.6)	REF	
	Once a month	14(60.9)	40(49.4)	3.27 (0.86-12.44)	0.08
	Every three months	5(21.7)	12(14.8)	3.89(0.80-18.94)	0.09
	Every six months	1(4.3)	1(1.2)	9.33(0.46-190.63)	0.14
	Once a year	0	0		
Length of travel	A few days	3(13)	23(28.4)	REF	
	A week	7(30.4)	27(33.3)	1.98(0.46-8.58)	0.36
	A month	10(43.5)	29(35.8)	2.64(0.65-10.74)	0.17
	Some months	3(13)	2(2.5)	11.5(1.33-99.33)	0.026
Level of education	None to Some primary	4(10.8)	7(2.1)	11.10(2.62-47.06)	<0.001*
	Completed primary	4(10.8)	9(2.6)	8.64(2.13-35.07)	0.003*
	Some secondary	12(32.4)	70(20.5)	3.33(1.25-8.84)	0.016*
	Completed secondary	10(27)	119(34.9)	1.63(0.63-4.42)	0.34
Circumcision	Tertiary	7(18.9)	136(39.9)	REF	
	Yes	7(18.9)	30(81.1)	REF	
Discuss HIV issues	No	96(28.4)	242(71.6)	0.59(0.25-1.38)	0.22
	Yes	28(75.7)	301(88.5)	REF	
Partner HIV status as reported by women	No	9(24.3)	39(11.5)	2.48 (1.09-5.64)	0.03
	Infected	1(2.7)	5(1.5)	5.2(0.55-49.23)	0.15
	Uninfected	9(24.3)	234(68.6)	REF	
Faithfulness as reported by women	Unknown	27(73)	102(29.9)	6.88 (3.12-15.16)	<0.001*
	Yes	16(43.2)	235(69.1)	REF	
	No	11(29.7)	22(6.5)	0.14(0.056-0.33)	<0.001*
	Unknown	10(27)	83(24.4)	0.24(0.091-0.64)	0.004*

*Statistically significant at p-value of 0.05.

In the logistic regression, several partner factors retained statistical significance, except for partner length of travel as shown in Table 4.4. The factors that retain significance include: partner being a frequent traveler, partner level of education, discussing HIV issues with partner, reported partner HIV status and perceived partner faithfulness.

Table 4.4 Logistic regression on partner

Variable	LR (X² Test)
Frequent traveler*	<0.001
Frequency of travel	0.17
Length of travel*	0.13
Level of education*	<0.001
Circumcision	0.20
Discuss HIV issues*	0.04
Partner HIV status as reported by women*	<0.001
Partner faithfulness as reported by women*	<0.001

*Statistically significant in the bivariate analysis

4.5 Sexual behaviour factors

Table 4.5 shows the reported sexual behaviours of study participants. More than 50% of the participants reported having one sexual partner in the past 12 months. A majority of women in the study reported having two lifetime partners. A greater percentage (88%) of women who participated in the study had their sexual debut between 17 and 20 years. The mean age for sexual debut was 17 years among seroconvertors versus 18 years in the non-seroconvertors. As revealed by Table 4.5, there was a trend towards decrease in risk of HIV seroconversion with increase in age of sexual debut.

Condom usage at sexual debut was very low (27%) among seroconvertors and these women were 4.38 times more likely to seroconvert during pregnancy than those who used condoms on sexual debut (<0.001). High numbers of women reported ever having used condoms in their sexual history. However, during pregnancy less than 25% of seroconvertors used condoms and notably none of them used condoms consistently. Worth noting is the number of women who reported that they abstained from sex during pregnancy, 2.7% and 7.3% among seroconvertors and non-seroconvertors respectively. Among seroconvertors 35.7% reported that their children were from the same father, vs. 78% among non-seroconvertors.

A few participants reported using vaginal herbs during pregnancy in both groups. There was no significant difference of sexual abuse between seroconvertors and non-seroconvertors. The women diagnosed with syphilis were 12 times more likely to seroconvert when compared to those who did not seroconvert ($p < 0.001$). More than 25% seroconvertors reported history of STI, compared to less than 10% non-seroconvertors.

In Table 4.5 the results of the bivariate analysis show that a number of sexual behaviours were associated with HIV seroconversion during pregnancy. There was a statistically significant association between having multiple partners in the past 12 months and HIV seroconversion during pregnancy ($p = 0.002$). There was also a statistically significant association between number of lifetime partners and risk of HIV seroconversion during pregnancy ($p = 0.016$). Sexual debut between 14-16 years was statistically associated with increased risk of HIV seroconversion during pregnancy ($p = 0.035$). In addition, women whose sexual debut was without condoms and those who had unprotected sex during pregnancy experienced increased risk of seroconversion during pregnancy ($p < 0.001$). Furthermore, a reactive RPR test for syphilis and a history of STI were associated with HIV seroconversion during pregnancy ($p < 0.001$).

Table 4.5 Sexual behaviours of participants

Categorical predictor Variables	Categories	HIV seroconvertors n=37 (%)	HIV non-seroconvertors n=341(%)	Odds ratio for seroconversion (95% CI)	p-value
No of sexual partners in the last 12 months	1	28(75.7)	315(92.4)	REF	0.002*
	≥2	9(24.3)	26(7.6)	3.89(1.67-9.12)	
Number of lifetime partners	1	6(16.2)	126(37)	REF	0.016*
	2	18(48.6)	116(34)	3.26(1.25-8.49)	
	≥ 3	13(35.1)	99(29)	2.76(1.01-7.52)	
Age of sexual debut	14-16	16(43.2)	54(15.8)	4.05(1.11-14.83)	0.035*
	17-20	18(48.6)	246(72.1)	1(0.28-3.55)	
	≥ 20	3(8.1)	41(12)	REF	
	Mean	17	18		
Condom use sexual debut	Yes	10(27)	211(61.9)	REF	<0.001*
	No	27(73)	130(38.1)	4.38(2.05-9.35)	
Ever used condoms in their lifetime	Yes	34(91.9)	318(93.3)	REF	0.76
	No	3(8.1)	23(6.7)	1.22(0.35-4.28)	
Condom use during pregnancy	Yes	9(24.3)	185(54.3)	REF	<0.001*
	No	27(73)	131(38.4)	4.24(1.93-9.31)	
	Abstinence	1(2.7)	25(7.3)	0.82(0.1-6.77)	
Frequency of condom use during pregnancy	Always	0	82(43.6)	REF	1
	Sometimes	10(100)	106(56.4)	152403257	
Different biological fathers of children	Yes	10(35.7)	138(78)	6.37 (2.72-14.91)	<0.001*
	No	18(64.3)	39(22)	REF	
Use of vaginal herbs	Yes	5(13.5)	41(12)	1.14(0.42-3.1)	0.79
	No	32(86.5)	300(88)	REF	
History of sexual abuse	Yes	1 (2.7)	11 (3.2)	0.83 (0.11-6.64)	0.86
	No	36 (97.3)	330 (96.8)	REF	
Syphilis status	Reactive	6(16.2)	5(1.5)	12.9(3.74-45.18)	<0.001*
	Non-reactive	29(78.4)	314(92.1)	REF	
	Unknown	2(5.4)	22(6.5)	0.98(0.22-4.39)	
History of STI	Yes	13(35.1)	25(7.3)	6.85(3.11-15.06)	<0.001*
	No	24(64.9)	316(92.7)	REF	

*Statistically significant at p-value of 0.05.

With logistic regression all the sexual behaviours retained their significance as shown in Table 4.6 below.

Table 4.6 Logistic regression on sexual behaviours of participants

Variable	LR Test (χ^2 Test)
Number of sexual partners in the last 12 months*	0.004
Number of lifetime partners *	0.027
Age at sexual debut*	0.001
Condom use at sexual debut*	<0.001
Condom use during pregnancy*	<0.001
Frequency of condom use during pregnancy	0.006
Different biological fathers of children*	<0.001
Syphilis status*	<0.001
History of STIs*	<0.001

*Statistically significant in the bivariate analysis

4.6 Risk of HIV infection

Most women perceived themselves as not at risk of acquiring HIV during pregnancy after receiving the initial HIV result as shown in Table 4.7. Women selected different reasons for thinking they were risk-free, as shown by Figure 4.1 below. The women could select more than one reason which made them perceive themselves as not at risk of acquiring HIV after the initial HIV negative result. The most common reason given by the women was having one sexual partner (63.2% seroconvertors and 62.7% non-seroconvertors). Partner faithfulness was the second most common reason selected by the women, with the third reason selected was 'being married'.

In the bivariate analysis, consistent use of condoms during pregnancy was a protective factor and its statistical significance was shown in the logistic regression analysis as shown in Table 4.7 below.

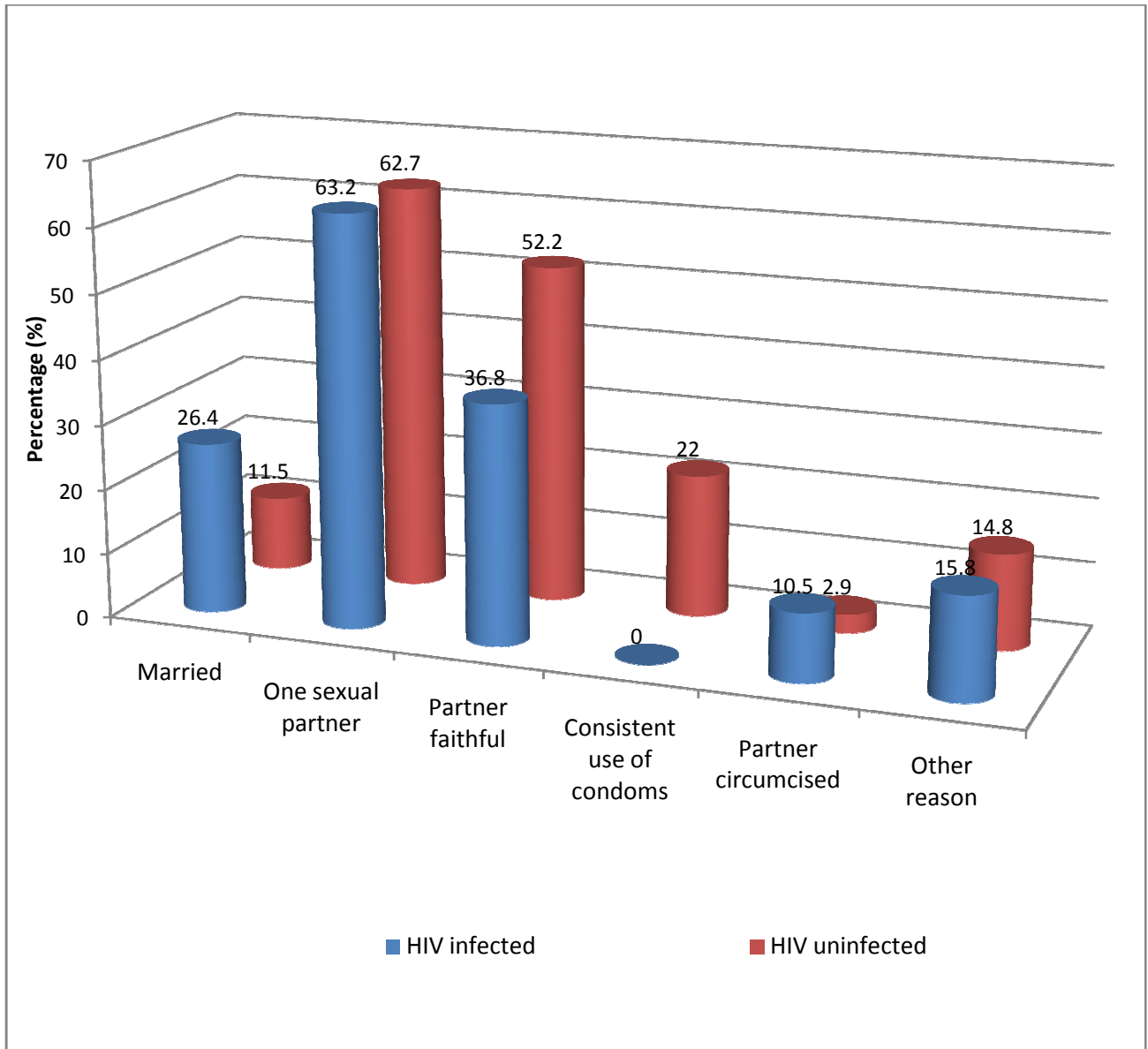


Figure 4.1 Shows the percentage women giving a reason for perceiving not at risk of acquiring HIV after initial HIV test result (More than one reason could be given by one woman).

Table 4.7 Perceived risk of HIV infection

Categorical predictor Variables	Categories	HIV seroconvertors <i>n</i> =37 (%)	HIV non-seroconvertors <i>n</i> =341(%)	LR Test (χ^2 Test)	Odds ratio for seroconversion (95% CI)	p-value
How do you rate your Risk of HIV	No risk	1(33.3)	56(16.5)	0.17	REF	
	Small risk	0	115(33.9)		0.00	1
	Moderate risk	0	67(19.8)		0.00	1
	High Risk	1(33.3)	11(3.2)		5.09(0.29-87.68)	0.26
	Don't know	1(33.3)	90(26.5)		0.62 (0.04-10.15)	0.73
Perception of risk after initial negative HIV test	Yes	18(48.6)	134(39.3)	0.27	1.46(0.74-2.89)	0.27
	No	19(51.4)	207(60.7)			
Reasons for perception of 'no risk						
Being married	Yes	5 (26.4)	24 (11.5)	0.93	2.75 (0.911-8.32)	0.07
	No	14 (73.7)	185 (88.5)			
One sexual partner	Yes	12 (63.2)	131 (62.7)	0.97	1.02 (0.39-2.70)	0.97
	No	7 (36.8)	78 (37.3)			
Partner faithful	Yes	7 (36.8)	109 (52.2)	0.20	0.53 (0.20-1.41)	0.21
	No	12 (63.2)	100 (47.8)			
Consistent use of condoms	Yes	0	46 (22)	0.003	0.00	1
	No	19 (100)	163 (78)			
Partner circumcised	Yes	2 (10.5)	6 (2.9)	0.15	3.98 (0.75-21.25)	0.11
	No	17 (89.5)	203 (97.1)			
Other reasons	Yes	3(15.8)	31(14.8)	0.91	1.07(0.3-23.91)	0.91
	No	16(84.2)	178(85.2)			

*Statistically significant at p-value of 0.05.

4.7 Reproductive factors

The reproductive factors associated with HIV seroconversion are presented in Table 4.8. More than half of all women had four or more antenatal care visits, 62.2% and 76% among HIV seroconvertors and non-seroconvertors respectively. The number of women who were pregnant for the first time was 29.7% among seroconvertors versus 54.8% in the non-seroconvertors group. Women who were pregnant for the second time were more likely to be infected compared with women of higher gravida.

The mean gestational age at ANC booking was 21.5 weeks for HIV seroconvertors whereas that of non-seroconvertors was 20 weeks. A high percentage (98%) of the women who participated in the study had an HIV test at first ANC visit. A large proportion (98%) of women had an HIV repeat test done during ANC. The mean gestational age at retest was 30 weeks among seroconvertors and 29 weeks among non-seroconvertors.

There was a statistically significant association between the number of pregnancies and HIV seroconversion during pregnancy. Compared to primigravida the risk of seroconversion was 3.48 times higher for women with two pregnancies ($p=0.002$). Interestingly, there was a trend towards increase in risk of HIV seroconversion during pregnancy as the gestational number of weeks at first ANC increased. The results show that parity was associated with HIV seroconversion during pregnancy ($p=0.04$).

With logistic regression analysis, the number of pregnancies, gestational age at first ANC and parity retained their statistical significance as shown in Table 4.9.

Table 4.8 Reproductive factors of participants

Categorical predictor Variables	Categories	HIV seroconvertors n=37 (%)	HIV non-seroconvertors n=341(%)	Odds ratio for seroconversion (95% CI)	p-value	
ANC visits	<4	14(37.8)	82(24)	1.92(0.95-3.91)	0.071	
	≥4	23(62.2)	259(76)	REF		
	Mean	4	4			
Number of pregnancies	Primigravida	11(29.7)	187(54.8)	REF		
	2	17(45.9)	83(24.3)	3.48(1.56-7.76)	0.002*	
	≥2	9(24.3)	71(20.8)	2.16(0.86-5.42)	0.1	
Gestational age at first ANC	<16	1(2.7)	67(19.6)	REF		
	16-25	27(73)	226(66.3)	8.00(1.07-60.0)	0.043*	
	≥26	9(24.3)	48(14.1)	12.56(1.54-102.48)	0.018*	
	Mean	21.5	20			
HIV testing at first ANC	Yes	36(97.3)	334(97.9)	REF		
	No	1(2.7)	7(2.1)	1.33(0.16-11.08)	0.80	
Retest at ANC	Yes	37(100)	335(98.2)	REF	1	
	No	0	6(1.8)			
Gestational age at retest	<30	REF	19 (51.4)	138 (41.2)	0.66(0.34-1.31)	0.24
	>30		18(48.8)	197 (58.8)		
	Mean		31	29		
Exit test at 32 to 36 weeks	Yes	20(57.1)	224(65.7)	REF		
	No	15(42.9)	117(34.3)	1.44(0.71-2.91)	0.32	
Retest at Labour	Yes	12(35.3)	89(26.1)	REF		
	No	22(64.7)	252(73.9)	0.65(0.31-1.36)	0.25	
Parity	0	12 (32.4)	193 (56.6)	REF		
	1-2	23 (62.2)	125 (36.7)	2.96 (1.42-6.16)	0.04*	
	≥3	2 (5.4)	23 (6.7)	1.40 (0.29-6.64)	0.67	

*Statistically significant at p-value of 0.05.

Table 4.9 Logistic regression on reproductive factors of participants

Variable	LR Test (X² Test)
ANC visits*	0.078
Number of pregnancies*	0.007
Gestational age at first ANC *	0.02
HIV testing at first ANC	0.80
Retest at ANC	0.26
Gestational age at retest	0.24
Exit test at 32-36 weeks	0.32
Retest at labour	0.26
Parity *	0.011

*Statistically significant in the bivariate analysis

4.8 Knowledge on HIV

Table 4.10 shows the various questions which were used to assess the knowledge on HIV and PMTCT of the study participants. The women were assessed on the signs and symptoms of HIV, MTCT, HIV seroconversion and HIV prevention. Most of the women were knowledgeable on all the areas assessed. There was no association between knowledge on HIV and HIV seroconversion.

Table 4.10 Knowledge of HIV

Categorical predictor Variables	Categories	HIV seroconvertors n=37 (%)	HIV non-seroconvertors n=341(%)	LR Test (X ² Test)	Odds ratio for seroconversion (95% CI)	p-value
Does a person with HIV always show Signs and symptoms	Yes	1 (2.7)	9 (2.6)	0.90	1.01 (0.13-8.53)	0.97
	No	33 (89.2)	311 (91.2)		REF	
	Don't know	3 (8.1)	21 (6.2)		1.35 (0.38-4.76)	
Mother to child HIV transmission	Yes	34 (91.9)	324 (95)	0.74	REF	0.56
	No	2 (5.4)	12 (3.5)		1.59 (0.34-7.39)	
	Don't know	1 (2.7)	5 (1.5)		1.91 (0.22-16.8)	
HIV seroconversion during pregnancy	Yes	34 (91.9)	322 (94.4)	0.84	0.63 (0.07-5.42)	0.68
	No	1 (2.7)	6 (1.8)		REF	
	Don't know	2 (5.4)	13 (3.8)		0.92 (0.07-12.28)	
Healthy looking man HIV transmission	Yes	36 (97.3)	314 (92.1)	0.23	REF	1
	No	0	13 (3.8)		0.00	
	Don't know	1 (2.7)	14 (4.1)		0.62 (0.08-4.88)	
Use of condoms to prevent HIV transmission	At every sexual encounter	36 (97.3)	325 (95.6)	0.48	REF	0.91
	At most encounters	1 (2.7)	8 (2.4)		1.13 (0.14-9.28)	
	At high risk encounters	0	7 (2.1)		0.00	

*Statistically significant at p-value of 0.05.

4.9 Multivariate analysis

Multivariate analysis of all statistically significant variables using a multinomial logistic model showed that women with history of STI, women who reported not using condoms at sexual debut, women who reported having multiple sexual partners in the past 12 months and women who reported having a partner who was a frequent traveler were independently associated with acquiring HIV infection during pregnancy as shown in Table 4.11 below.

4.10 Summary

The results of the study show that demographic factors such as age ($p=0.067$) and educational level ($p=0.002$) are associated with HIV seroconversion during pregnancy in women who initially tested negative for HIV at initiation of ANC. The findings also highlight how the partner factors play a role in HIV seroconversion. The study findings show the following partner factors to be associated with HIV seroconversion among pregnant women: frequency of partner travel ($p<0.001$), level of partner education ($p<0.001$), discussing HIV issues in the relationship ($p=0.04$), reported partner HIV status ($p<0.001$), and perceived partner faithfulness ($p<0.001$). The results of the study indicate that sexual behaviours of the women contribute greatly to HIV seroconversion and these include; multiple partners in the past 12 months ($p=0.004$), multiple partners in lifetime ($p=0.027$), early sexual debut ($p=0.001$), condom use at sexual debut ($p<0.001$), condom use during pregnancy ($p<0.001$), different biological fathers of children ($p<0.001$), syphilis status ($p<0.001$), and history of STI ($p<0.001$). Women who had gravidity of two ($p=0.002$), booked for ANC at 16-25 weeks ($p=0.043$) and above 25 weeks ($p=0.018$) and had one or two children ($p=0.04$) were more likely to seroconvert during pregnancy.

Table 4.11 Adjusted odds ratio for factors associated with HIV seroconversion

Variables	Categories	All data	p-value
		Odds Ratio (95% CI)	
Level of education	None to Some primary	0.058 (0- 10.65)	0.30
	Completed primary	0.051 (0- 7.61)	0.26
	Some secondary	0.024 (0- 4.72)	0.17
	Completed secondary	0.014 (0- 3.30)	0.12
	Tertiary	REF	
Discuss HIV issues	Yes	REF	
	No	1.54 (0.14- 16.89)	0.73
Number of sexual partners in the last 12 months	1	REF	
	≥2	73.34 (3.05- 1764.2)	0.008*
Number of life time partners	1	REF	
	2	0.18 (0.019- 1.63)	0.13
	≥ 3	0.57 (0.033- 9.89)	0.70
Age of sexual debut	14-16	3.26 (0.15- 66.96)	0.44
	17-20	2.99 (0.19- 47.32)	0.44
	≥ 20	REF	
Condom use at sexual debut	Yes	REF	
	No	7.80 (1.36- 44.27)	0.021
Condom use during pregnancy	Yes	REF	
	No	1.23E-9 (1.23E-9 to 1.3E-8)	1
	Abstinence	2.35E-25 (4.84E-10 to 1.14E-8)	<0.001*
Different biological fathers of children	Yes	4.41 (0.52- 37.83)	0.18
	No	REF	
Syphilis status	Reactive	2.33E-25 (0 - 3.12E-23)	1
	Non-reactive	REF	
	Unknown	0.65 (0.024- 17.28)	0.8
History of STI	Yes	12.55 (1.72- 91.66)	0.013*
	No	REF	
Number of pregnancies	Primigravida	REF	
	2	0.36 (0.058- 2.21)	0.27
	≥2	1.62E-6 (0 - 3.56E-5)	1
Gestational age at first ANC	<16	REF	
	16-25	0.22 (0.019- 2.64)	0.18
	≥26	1.63E29 (0- 8.33E30)	1
Parity	0	REF	
	1-2	0.036 (0 - 5.76)	0.20
	≥3	71961.15 (0.001- 182856)	1
Partner frequent traveller	Yes	13.08 (2.0- 87.45)	0.008*
	No	REF	
Partner Level of education	None to Some primary	0.001 (2.08E-5 - 0.10)	0.003*
	Completed primary	0.044 (0.001 - 3.87)	0.17
	Some secondary	0.025 (0.001 - 0.44)	0.011
	Completed secondary	0.22 (0.026 - 1.96)	0.18
	Tertiary	REF	

*Statistically significant at p-value of 0.05 level

CHAPTER 5: DISCUSSION AND CONCLUSION

In this chapter the results are discussed in relation to each other and in light of previous studies reported in the literature. The aim of this study was to determine factors associated with seroconversion during pregnancy among women delivering at Raleigh Fitkin Memorial Hospital in 2012. The results of this study show that there is seroconversion rate of 37/378 (9.7%) among pregnant women who had tested HIV negative at initial ANC booking. The seroconversion rate is higher than the 5% found by Kieffer *et al.* [59] in Swaziland, the 3.9% found in Nigeria [60], and the 1% in Lilongwe, Malawi [16]. This shows that HIV seroconversion rate among pregnant women varies between regions and countries [1].

The risk of HIV infection in pregnant women in Manzini region, Swaziland, was associated with distant and proximate factors. The significant distant factors included demographic determinants such as age, level of education, income and partner factors. The significant proximate factors comprised mainly reproductive and sexual behaviours. HIV seroconversion risk during pregnancy was independently associated with history of STI, not using condoms at sexual debut, multiple sexual partners in the past 12 months and having a partner who was a frequent traveler.

Demographic determinants are important components in the conceptual framework of factors associated with exposure or non-exposure to HIV, which lead to seroconversion during pregnancy. In regard to demographical factors, this study shows that women who had few years of education, were below the age of 24 years and had low income were at higher risk of acquiring HIV during pregnancy. Studies done in other African countries like Zimbabwe, Malawi and Tanzania also highlight that women with less education are more likely to seroconvert during pregnancy [17, 46, 61]. In contrast a study in Uganda reported high HIV incidence in pregnant women with higher levels of education and there was no interaction between education and HIV acquisition [5]. In the general population there are conflicting results on the effect of the level of education on HIV acquisition. Most researchers have noted a shift in HIV acquisition from more educated to least educated in the past decade [62-64]. De Walque *et al.* [63] concluded that the higher the level of education, the more likely is a change in sexual behavior.

Hargreaves *et al.* [65] in South Africa reported that women and men with higher levels of education were less likely to acquire HIV because of higher reported condom usage. Contrary to these studies Msisha *et al.* [66] found no association between increasing education and HIV acquisition in both men and women.

Women with few years of education have a greater chance of acquiring HIV during pregnancy because they are more vulnerable economically compared to educated women [66]. It is well documented that low levels of education amongst women are associated with low income and financial dependence on men [68-70]. In this study low income was indeed associated with increased risk of HIV seroconversion. The results of this study differ to previous studies reporting higher HIV prevalence among wealthy women compared to poor women [71]. However, the results of this study concur with those of a study done in Zimbabwe, where HIV prevalence was low among wealthy men and women compared to the poor [72]. This study underscores yet again that knowledge of HIV prevention alone is not protective against acquiring HIV, even during pregnancy. As in the general population (Dunkle *et al.* [insert reference]), it may be difficult for pregnant women financially dependent on their partners to apply their knowledge, exercise control over their sex life and negotiate for safe sex, thus placing themselves at greater risk of acquiring HIV [62].

To help prevent new infections in all women, including the pregnant, there is need to empower girls by ensuring that their education goes beyond primary level. According to UNFPA [73], "Investing in girls' education is one of the most effective ways to reduce poverty". Achieving universal primary education for all is one of the millennium development goals (MDGs) and attaining this goal will have an impact in all MDGs including reduction of new HIV infections [74]. In Swaziland the proportion of women who obtained education higher than secondary school level is five percent which is very low [7]. There is need for the Government of Swaziland to ensure that education is available and accessible to girls at secondary and tertiary levels. Retaining a girl child at school for longer duration may contribute significantly to the reduction of HIV acquisition because each year a girl child stays in schools she gains greater independence and becomes empowered to make decisions regarding her sexual life [63, 75-78].

Another demographic risk factor seen to be associated with seroconversion among pregnant women in this study was age. The majority of seroconvertors were below the age of 24 years. Similar findings among pregnant women have been reported in previous studies done in Zimbabwe [46, 79]. In these studies most seroconvertors were below 25 years of age.

The results deviate with findings on HIV acquisition in the general population. According to the SDHS [7] the prevalence of HIV is highest among women aged 25-29 years at 49% and 45% in men aged 35-39 years. The Swaziland antenatal seroprevalence survey of 2010 showed that HIV prevalence was highest in women aged 30-34 years [29]. In a cohort study of women in Durban, South Africa, high risk of HIV acquisition was reported among the 25-29 year age group [80]. According to the UNAIDS report [81] in

sub-Saharan Africa prevalence of HIV among women is highest in the 30-34 age group whilst in men it peaks in the late 30s and early 40s. Gregson *et al.* [82] in Zimbabwe observed a decline in HIV prevalence by 49% in women aged 15-24 years. Comparing with general population, this present study indicates that pregnant women are at high risk of seroconverting during their pregnancy at a younger age.

Given the seroconversion during pregnancy at a younger age, it is important for programs to target young adolescents and intensify preventative messages before they become sexually active. Also at this stage values and life decisions begin to be made. According to Gallant *et al.* [83] targeting women before they engage in sexual activities is the key to keeping them HIV negative because protective behaviours can be established at this stage rather than having programs later in life that attempts to change behaviour. This means the protective behaviours the girls acquire when they are young will benefit them later in life, whether pregnant or not. Previous studies [84, 85] have shown that life skills orientation programmes at schools are also key to behaviour change and can be implemented in all schools, including primary level.

Partner factors highlighted in this study associated with HIV seroconversion among pregnant women include: partner being a frequent traveler; level of education; reported partner HIV status and discussing HIV issues with partner. Partners who travel frequently can place the women at risk of getting HIV as suggested by the results of this study, especially if they are away from home for several months. Previous studies have shown that frequent travelers who are away for longer periods, especially migrant workers, are more likely to engage in risky sexual behaviours because they establish other sexual networks [68, 79]. It is important that more focus is placed on educating men to practice safe sex and use condoms when they travel. The women who reported that their male partners were less educated were more likely to seroconvert, because the level of understanding the risk could be low and they might have limited access to knowledge on HIV [21].

Women who reported that their partners were not tested for HIV were at higher risk of acquiring infection. Not knowing the partner's HIV status could result in couples not knowing they are in a serodiscordant relationship. It has been reported in several studies [86-88] that men generally are not keen to test for HIV because they see themselves as healthy, others are afraid of a positive result and others are afraid of stigma. Furthermore, men are reported to use the women's HIV results as proxy for their own status, thus assuming they are negative if their partner tests negative at initiation of ANC. It is

important to ensure men get tested by bringing HIV services closer to them, especially in their workplace and recreational places [89, 90]. A study by Yeganeh *et al.* [91] demonstrated that women who had tested for HIV reported that their partners had tested too. However, in this study it was noted that all the women knew their status but only two thirds knew the partner's HIV status.

Despite the women having access to knowledge on the importance of testing for HIV especially during pregnancy, partner testing is still a challenge. There is need to intensify male involvement efforts especially during ANC visits, so as to increase the number of partners who know their status [92-94]. ANC provides an opportunity to reach out to the men when a woman is pregnant compared to the non-pregnant women. Pre exposure prophylaxis for HIV should be made available in resource-limited areas with high HIV prevalence for pregnant women whose partners refuse HIV testing or who have an unknown HIV-status, so as to protect the pregnant women from acquiring new infections unknowingly and seroconverting during the pregnancy.

In this study women who reported that their partners were not faithful were more likely to seroconvert during pregnancy. A study conducted in Zimbabwe [45,46] yielded similar results: pregnant women who reported partner infidelity were four times more likely to seroconvert during pregnancy or postnatally. In a recent study in Malawi on sexual behaviours during pregnancy, the researchers reported that the women verbalized that they were concerned that their partners are not faithful when they are pregnant and this could increase their chances of acquiring HIV [16]. According to Smith and Watkins women and men in general worry about HIV infection from their partners if there is infidelity [95].

More than 75% of the women who participated in this study reported that they discussed HIV issues with their partners. Studies have shown that when women discuss HIV issues in their relationships, it becomes easier for them to bring their partners for testing [91]. Another study showed that women who were in concurrent multiple relationships had difficulty in discussing HIV issues especially around condom usage [96]. In the general population, researchers reported that in a relationship where there is no communication on HIV issues, condom usage was less frequent [97].

This shows that being in a relationship where HIV issues are not discussed puts all women, including those pregnant, at risk of acquiring HIV. This could be due to the fact that risk reduction strategies cannot be discussed freely in that relationship. If HIV issues are not discussed in the relationship, this makes it difficult for pregnant women to put the HIV prevention knowledge acquired during counseling into practice.

Culture might be a hindrance, preventing women from discussing sexual and HIV issues with their partners. Men may feel undermined if women are the ones starting conversations on sexual issues. However, women can be empowered to initiate the discussions in the relationship. The ANC setting provides health care workers more opportunity at each visit to equip the pregnant women with communication skills that will improve their ability to negotiate condom usage compared to the non-pregnant counterparts. Sexual communication is very important for PMTCT to be effective and it may be the key to men changing their sexual behaviours [94, 98-100]. According to Peltzer *et al.* [101] male involvement may influence and reduce risk of HIV acquisition among HIV negative women. Sexual communication especially relating to HIV issues might be a challenge. Women might be afraid to talk about HIV with their partner for fear of physical abuse because the partner might suspect the woman is suspicious of their infidelity. One study highlights that it is easier to discuss HIV issues in stable relationships, especially among those who are married and those living with partners [91]. Being married however does not mean the women are safe from acquiring HIV, as has been shown in different studies. In this study, despite the majority of women not being married or living with partners, women reported discussing HIV issues in their relationship. This could mean that women perceived their relationships to be stable and solid.

Sexual behaviours and associated factors were assessed in this study. This study demonstrated that sexual risk behaviours were observed among pregnant women and they include: multiple partners; concurrent and lifetime, unsafe sex, early sexual debut and history of STI. Other studies have also shown that pregnant women continue to engage in risky sexual behaviours [5, 13, 16, 40, 91]. The results of the study concur with findings on HIV acquisition in general population as shown by different researchers [65, 80]. Women who reported more than one partner in the past twelve months were at greater risk of acquiring HIV. The pregnant women might engage in concurrent relationships so that they are able to survive by having a partner who will buy food, another for paying rent and another for luxurious gifts. Karim *et al.* [102] argue that poorer women enter into a series of monogamous relationships for their own survival and that of their offspring.

Women might get pregnant to ensure financial support from the father of the child, even after a separation and as a result some of them have children from different fathers. A study by Jewkes *et al.* [103] showed that to get financial support a woman can continue to have sex with the father of one or more of her children even when they have separated. This study yielded similar results as previous studies and showed that women whose children were from different fathers had increased risk of

acquiring HIV[46]. According to Dunkle *et al.* [56] pregnant women who reported they had transactional sex were more likely to be HIV seropositive. The women may have greater chances of acquiring HIV because they are concerned with getting money and will be ignorant about protecting themselves in the process, as long as they survive.

Having children with different fathers may also be a strong indication that women had more than one lifetime partner as shown by the results of the study with whom they engaged in risky sexual activities thus exposing themselves to HIV. Multiple serial partners may relate to the aforementioned financial dependence on men, which may pose a barrier to women's capability of practicing the HIV prevention knowledge they acquired during counseling at the health facilities [68]. There is need to have socioeconomic development programs in place especially at community level to ensure women are empowered to fend for themselves.

Early sexual debut is one of the sexual factors shown by this study to be associated with HIV seroconversion. The findings of this study concur with other studies that reported association between early sexual debut and high HIV prevalence among the youth because they may not be biologically or mentally mature for sex [104, 105]. According to Wand and Ramjee [106] early sexual debut was associated with increased HIV prevalence and incidence in young people. Researchers argue that women who engage in sex early at a young age are more likely to also engage in other risky behaviours such as having multiple partners and not using condoms [80, 107, 108].

Young women especially adolescents have been reported to be more at risk of acquiring HIV because their genitals are immature and are more likely to be injured during sex compared to older women [109]. In a study done in Zimbabwe researchers reported that girls who were less than 19 years became pregnant and acquired HIV at their sexual debut[21]. Delaying sexual debut is very important in reduction of HIV incidence especially among young women. Yotebieng *et al.* [110] showed that in boys when sex is delayed there is 1.44 times likelihood that a condom will be used at debut and this also increases the chances it will be used subsequently.

Unprotected sex is one of the risks for acquiring HIV and this study showed that women who reported unprotected sex during pregnancy were at higher risk of HIV seroconversion. Pregnancy usually occurs in a relationship where love and trust have been established and most women see condoms as not acceptable in such stable relationships. Wingood and DiClemente [67] state that women who are in steady relationships are three to four times likely to never use condoms compared to those with casual

partners. The reason why women may not use condoms when they are pregnant is that they view condoms as a family planning method and do not see the need to use them because they are already pregnant [111]. Condoms generally in most societies are associated with being promiscuous [102], hence all women including those that are pregnant will not see themselves at risk.

Despite most pregnant women undergoing counseling and knowing the risks, condom usage remains a major challenge as highlighted by the results of this study. This study shows that the majority of women had four ANC visits, which can be seen as an opportunity for intensifying counseling on safe sex throughout pregnancy. Also the regular contact with the health care setting helps to increase access to condoms for pregnant women compared to general population. There is need for healthcare workers to offer condoms to all pregnant women as a part of the package of care so that prevention opportunities are not missed. There is need to intensify prevention campaigns and target pregnant women. A recent study in South Africa highlights the importance of condom use especially during pregnancy, in a context of increased sexual risk behaviours [40].

History of STIs was another factor associated with HIV seroconversion. Literature has shown that HIV infection is more likely to occur when there is an STI present, especially of the ulcerative or inflammatory type [21, 112]. Presence of syphilis or other STI during pregnancy makes the women susceptible to HIV because their genital mucosa is damaged and increases their chances of HIV infection therefore seroconversion as shown by this study and previous researchers [17]. There is need for pregnant women to be taught to identify STI early and seek treatment promptly. During ANC, syphilis screening is one of the tests done and this allows for early detection compared to non-pregnant counterparts who only seek symptomatic treatment.

Women in this study who attended their first ANC at a late period of gestation of more than 16 weeks were more likely to seroconvert. The results concur with the findings of the SDHS [7] that shows that on average women book for their initial ANC at 20 weeks or more.

There is need to work with communities to encourage women to initiate ANC in the first trimester. Earlier initiation will provide greater opportunities to emphasize HIV prevention strategies throughout pregnancy.

Importance of early initiation of ANC can be part of health education for all pregnant women so that on subsequent pregnancies they can come early for ANC. Women who had one to two children were at high risk of acquiring HIV because they might be in stable relationship where issues of trust and love

makes it difficult to practice safe sex as they see the condom fit for people who are not in serious relationship [102].

One of the limitations of this study is that it included sensitive questions on sexual practices to which the participants might have not wanted to divulge full information, as the questionnaire was interviewer administered. However, the interview was conducted in a private place so as to facilitate the participants to answer openly and freely the questions. The study was conducted in a low-income mission hospital and this might limit generalization of the findings of this study to other settings like private hospitals, as the women might have different characteristics compared to those delivering at RFM. However, the central location of RFM makes it accessible to all the four regions of the country making possible that a fair representative sample of the general population of pregnant women in Swaziland.

HIV seroconversion during pregnancy remains a major challenge for PMTCT programs especially in countries of high prevalence. This means that PMTCT programs require interventions that are able to keep HIV negative women free of HIV throughout pregnancy and lactation periods. Most PMTCT interventions for a long time have focused on women already living with HIV and little attention has been given to women who are HIV negative. Recent studies have shown an increase in incidence of HIV among pregnant women. This study focused on HIV seroconversion with the particular aim of determining the factors associated with HIV seroconversion during pregnancy. In the absence of any studies on HIV seroconversion in Swaziland, a cross-sectional study design facilitated the collection of data, which provided the factors associated with HIV seroconversion.

The results of the study show that despite the women having adequate knowledge on HIV issues, they continue to engage in unprotected sex. Counseling on preventative measures especially condom use should be strengthened throughout the ANC visits. There is need for PMTCT programs especially in high prevalence settings like Swaziland to explore the possibility of providing pre exposure ARVs prophylaxis during pregnancy so as to reduce new HIV infections among pregnant women. Understanding how these factors interact to lead to HIV seroconversion is very important; as this will help programs provide interventions that target all the factors holistically.

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APPENDICES

Appendix A: Administered questionnaire

Study Number__ __

Section A: Demographics

I'd like to start the interview with some questions about you and your family.

1. What is your age: __ __ (years)
2. What is your date of birth: __ __ / __ __ / __ __ __ __ (dd-mm-yyyy)
3. What is your marital status?

(1) Married

(2) Never married / not living with a partner

(3) Separated

(4) Widowed

(5) Divorced

(6) Living with partner

4. Is your marriage a polygamous one? (1) Yes (2) No

5. What is the highest level of education you completed at school?

(1) Some primary

(4) Completed secondary

(2) Completed primary

(5) Higher education

(3) Some secondary

(6) Never attended school

6. What do you do earn money?.....
7. What is your monthly income?

- (1) none
- (2) Less than E1000
- (3) E1000-3000
- (4) 3000-5000
- (5) 5000 or more

8. How many times have you been pregnant?

9. How many children are alive?

Section B: Relationship History

10. What is the level of education of your partner?

- (1) Some primary
- (2) Completed primary
- (3) Some secondary
- (4) Completed secondary
- (5) Higher education
- (6) Never attended school

11. Is your partner a frequent traveler?

- (1) Yes (if yes, go to question 12)
- (2) No (if no, go to question 14)

12. How often is your partner away from home?

- (1) a week
- (2) a month
-

- (3) Every three months
- (4) Every six months
- (5) Once a year

13. How long is your partner away from home?

(1) a days

(2) a week at a time

(3) a month

(4) several months

14. Has your partner tested for HIV?

(1) Yes

(2) No

(3) Don't know

15. What is the HIV status of your partner?

(1) Positive

(2) Negative

(3) Don't know

16. Is your partner circumcised?

(1) Yes

(2) No

17. Do you think your partner is faithful to you?

(1) Yes

(2) No

(3) Do not know

18. Have you ever been sexually abused? (1) Yes (2) No

Section C: Sexual behavior

19. Did you use intravaginal herbs during pregnancy?

Yes

No

20. At what age did you have your first sex act?

21. Did you use a condom at your first sexual encounter? (1) Yes (2) No

22. Have you ever used condoms?

(1) Yes

(2) No

23. Did you use condoms during pregnancy?

(1) Yes (If yes, go to question 24)

(2) No (if no, go to question 25)

(3) Abstained from sex (go to question 25)

24. How often did you use condoms?

(1) Always

(2) Sometimes

25. How many partners have you had in the past year?

26. How many sexual partners have you had in your lifetime?

27. Do your children have the same father? (1) Yes (2) No

Section D: Knowledge factors associated with acquisition of HIV

28. How did you rate your chances of acquiring HIV

- (1) No risk
- (2) Small risk
- (3) Moderate risk
- (4) High risk
- (5) Don't know

29. Did you think you were at risk at the time of, your first HIV test?

- (1) Yes (if answer is yes, go to question 31)
- (2) No (if answer is no, go to question 30)

30. If you do not think you were not at risk, why did you think you were not at risk?

- (1) I married
- (2) I have one sexual partner
- (3) My partner is faithful
- (4) I use condoms consistently
- (5) My partner is circumcised
- (6) Other
- (7) Not applicable

31. To prevent STI/HIV, condoms should be used when?

- (1) every sexual encounter
- (2) At sexual encounter
- (3) At high risk encounters

32. Can you always tell if someone is living with HIV?

(1) Yes (always show signs)

(2) No (can look healthy)

(3) Do not know

33. Can a healthy looking man transmit HIV?

(1) Yes

(2) No

(3) Do not know

34. Can a woman living with HIV transmit HIV to her baby?

(1) Yes

(2) No

(3) Do not know

35. Can a HIV non-infected woman become HIV positive during pregnancy?

(1) Yes

(2) No

(3) Do not know

36. Do you talk about HIV with your partner?

(1)Yes

(2)No

Thank you for participating in the study.

Appendix B: Checklist/ Data extraction Tool

1. Parity of the woman.....
2. Gravida of the woman.....
3. Number of ANC visits during pregnancy.....
4. Gestational age at first ANC.....
5. Was HIV testing done at first ANC visit?
 (1) Yes (if answer is yes, go to number 7)
 (2) No (if answer is no, go to number 6)
6. At which visit was test eventually offered?
 (1) Second visit
 (2) Third or fourth visit
7. What was the gestational age at first HIV test?.....
8. Was HIV result given same day of testing?
 (1) Yes (2) No
9. Was retesting done at ANC?
 (1) Yes, if yes proceed to number 9
 (2) No, proceed to number 11
10. Gestational age at retest.....
11. Was HIV exit test done between 32- 36 weeks?
 (1) Yes
 (2) No

12. Was retesting done at delivery?

(1) Yes

(2) No

13. Second HIV test result

(1) Reactive (2) Non-reactive (3) Inconclusive

14. RPR result

(1) Reactive (2) Non-reactive

15. History of sexually transmitted diseases during this pregnancy

(1) Yes

(2) No

16. Partner HIV status

(1) Reactive (2) Non-reactive (3) Not tested

Appendix C: University of KwaZulu-Natal Biomedical Research Ethics Committee (BREC) Approval Letter



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29 March 2012

Dr. S Wusumani
Department of Public Health
Nelson R Mandela School of Medicine
University of KwaZulu-Natal

Dear Dr Wusumani

PROTOCOL: HIV Seroconversion during pregnancy in Swaziland, in 2011. REF: BE120/11

Further to our letter to you dated 23 October 2011, this letter serves to notify you that a sub-committee of the Biomedical Research Ethics Committee provisionally approved the study pending appropriate responses to queries raised. Your responses dated 14 March 2012 have been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given full ethics approval and may begin as from 29 March 2012.

The following related study document has been reviewed and approved:

- FHI Ethics Training Certificate
- Site Permission letter from Raleigh Fitkin Memorial Hospital

This approval is valid for one year from 29 March 2012. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2004), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at <http://research.ukzn.ac.za/ResearchEthics11415.aspx>.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

A handwritten signature in black ink, appearing to read 'D R Wassenaar', written in a cursive style.

PROFESSOR D R WASSENAAR
Chair: Biomedical Research Ethics Committee

Appendix D: Swaziland Ethics Committee Approval Letter

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MINISTRY OF HEALTH
P. O. BOX 5
MBABANE
SWAZILAND

THE KINGDOM OF SWAZILAND

FROM: The Chairman
Scientific and Ethics Committee
P. O. Box 5
Mbabane

TO: Sibongile Wusumani
Principal Investigator
P.O Box
Mbabane

DATE: 11th October 2011

REF: MH/599C

RE: Factors Associated with HIV Seroconversion during pregnancy in Swaziland, 2011

The committee thanks you for addressing the issues raised by the committee and the clarity on responses to the protocol amendment.

In view of the responses submitted after concerns raised and the fact that the study is in accordance with ethical and scientific standards, the committee therefore grants you authority to conduct the study. You are requested to adhere to the specific topic and inform the committee through the chairperson of any changes that might occur in the duration of the study which are not in this present arrangement.

The committee wishes you the best and is eagerly awaiting findings of the study to inform proper planning and programming to use for analysis.


Yours Sincerely,

A handwritten signature in black ink, appearing to read 'Dr. S.V. Magagula'.

Dr. S.V. Magagula
Chairperson Scientific and Ethics Committee
cc: Sec Members



Appendix E: RFM Hospital Permission Letter

 **CHURCH OF THE NAZARENE**
**Raleigh Fitkin
Memorial Hospital**

P.O. Box 14,
Manzini, Swa
Southern Afr
Phone: Mar.....
(09268) 505 2211
Fax: (09268) 505 5077
Telex: 2356 WD

15 November 2011

Sibongile Wusumani
PO BOX 193
Motshane
Swaziland

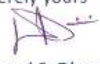
Dear Madam

RE: AUTHORIZATION TO CARRY OUT A RESEARCH IN THE HOSPITAL

Your application on the fore mentioned endeavors has been duly considered and Authorization granted on the following conditions please;

a). That confidentiality is strictly observed
b). That the hospital receives a copy of the report on the proposed research.

Again thank you for considering the Institution for such a task and wishing you all The best.

Sincerely yours


Leonard S. Dlamini (Mr.)
HOSPITAL ADMINISTRATOR

CC: Chief Medical Officer
Matron 1

Appendix F: Information document

Title of study: Factors associated with HIV Seroconversion during pregnancy in Swaziland, in 2011

Greeting: Good morning/afternoon, how are you?

Introduction: I, Sibongile Wusumani, am doing a research on seroconversion among pregnant women who were initially HIV non-infected. Research is a process to answer a question. In this study I want to find out factors that contribute to seroconversion among pregnant women.

Invitation to participate: I am inviting you to participate in a research study.

What is involved in this study: The purpose of this research is to find out factors that make pregnant women who were initially HIV non-infected become HIV positive. We will be interviewing all pregnant women who were initially HIV non-infected. We will sample 344 pregnant women. Information will be collected through an interview which will last about 30-45minutes. We will ask questions about you, your relationship history and sexual behavior issues. Your responses will be written on paper by the interviewer.

What are the possible risks: This is a verbal interview with no physical risks. However some of the questions may be sensitive; this may result in psychologically/ emotionally distress. If this occurs you will be provided with counseling.

Potential benefits: There are no direct benefits to you for taking part in the study. You will have the opportunity to share your views and experiences. The results will be used to help other pregnant women to remain HIV negative.

Participation is entirely voluntary: refusal to participate will not result in penalty and you may discontinue the study at any time, without penalty.

Confidentiality: interviews will take place in a private place. All information given during the interview will be kept confidential. Absolute confidentiality cannot be guaranteed; organizations such as the Biomedical Research Ethics may inspect your research records for quality assurance and data analysis.

Questions: if you have any questions about the research contact the principal investigator on 76269590.

Reporting complaints/problems and questions about your rights as a participant: if you have any questions or concerns regarding your rights as a research participant you may contact the **Biomedical Ethics Research Office**, UKZN, Private Bag X54001, Durban 4000.

Telephone: 0027 (0) 31 260 4769/ 260 1074

Fax: 0027 (0) 31 260 4609

Email: BREC@ukzn.ac.za

Swaziland National Ethics Committee on 2404 7712, contact person Sisi Lukhele.

Appendix G: Informed Consent

Study number _____

Consent to participate in Research

Greetings: Good morning/afternoon. How are you?

Principal investigator: Sibongile Wusumani

Title of Study: Factors associated with HIV Seroconversion during pregnancy in Swaziland, in 2011

You have been asked to participate in a research study to learn more about factors that contribute to seroconversion of HIV among pregnant women.

You have been informed about the study by.....

You may contact Sibongile Wusumani at 7626 9590 anytime if you have any questions about the research.

You may contact the **Biomedical Ethics Research** Office on 0027 (0) 31 260 4769 or 260 1074 or Email: BREC@ukzn.ac.za or Swaziland National Ethics Committee on 2404 7712 if you have any questions about your rights as a participant.

Your participation in this research is not forced. You may refuse or decide to stop participation in this study at any time for any reason. This will not affect your future care at this health facility or with your nurse or doctor.

The details of the research study and the above information have been described to me orally. I have understood this information and I agree to participate in this study in my free will. I have been given time to ask questions that I might have about participation in this research.

Signature/ Thumb of Participant

Date

Signature of witness

Date