Pregnancy and acquisition of sexually transmitted infections: risk behaviors and incidence

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Abstract

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This dissertation had three primary aims. The first aim was to systematically review evidence documenting incidence of sexually transmitted infections (STI) during pregnancy. Eighteen papers were included in the final review which reported incidence of five STIs: chlamydia, gonorrhea, human papillomavirus (HPV), herpes simplex virus type 2 (HSV-2) and human immunodeficiency virus (HIV). The review found that there are very limited data on incidence of STIs during pregnancy and even fewer data comparing risk between pregnant and non-pregnant women. Although data are limited, studies suggest that women continue to acquire STIs during pregnancy, with incidence varying by type of infection, population of interest and geographic setting. Highest incidence was found for HPV and chlamydia although some studies of chlamydia showed low proportions of pregnant women infected. Studies in which partners were known to be infected with HSV-2 and HIV showed higher rates of acquisition in pregnant women compared to studies where partner status was not known.

The second aim of this dissertation was to describe the impact of pregnancy on behavioral risk factors and vaginal practices that are associated with increased risk of STI acquisition. Data for this and the following aim came from the Methods for Improving Reproductive Health in Africa (MIRA) study, a randomized clinical trial conducted in South Africa and Zimbabwe 2003-2006. The analysis for the second aim included women in the MIRA trial who had a pregnancy during follow-up. Pregnancy was found to decrease sexual activity, particularly in the third trimester, but women were more likely to report sex without condoms while pregnant. There were lower reports by women during pregnancy of other risk factors for STI acquisition, including anal sex, concurrent sexual relationships and new sex partners. Vaginal wiping and insertion of material into the vagina, potentially important mechanisms for

STI acquisition, were also less common during pregnancy. The data from this aim present a complicated picture of risk for STIs during pregnancy as a result of increased unprotected sex but decreased frequency of other known behavioral risk factors.

The third and final aim of the dissertation was to measure incidence of four STIs in pregnant and non-pregnant women and to evaluate whether women are at greater risk during pregnancy for acquiring four STIs: chlamydia, gonorrhea, trichomoniasis and HIV. This analysis included 4,549 women 18-50 years of age, 17% (N=766) of whom had a pregnancy during follow-up. In general, women continued to be sexually active but reported less overall sex than non-pregnant women. Report of condom use was lower during pregnancy as were other types of high risk sexual behaviors, such as multiple sexual partners, sex in exchange for drugs or money and anal sex, as well vaginal practices. STI incidence was measured during pregnancy and it was found that women continued to acquire STIs when pregnant. In addition, during periods when women became pregnant, they appeared to be a high risk for acquiring chlamydia, trichomoniasis and HIV. Finally, in examining the association between pregnancy status and STI risk, we found that in multivariable models adjusted for demographic and time-varying self-reported behavioral risk factors and vaginal practices, pregnancy was not associated with increased STI risk. However in visit intervals when women became pregnant, they appeared to be at higher risk for contracting chlamydia compared to non-pregnant periods.

Table of Contents

Charts, Graphs and Illustrations

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Dedication

This dissertation is dedicated to my family who have supported me throughout this process, and to my husband, Peter Mustalish, for his encouragement and love.

Chapter 1

Introduction

The World Health Organization (WHO) estimates that every year there are almost 500 million new cases globally of curable sexually transmitted infections (STI) including syphilis, gonorrhea, chlamydia and trichomoniasis.[1] In addition, there are more than 536 million people living with herpes simplex virus type 2 (HSV-2) and an estimated 35 million people living with human immunodeficiency virus (HIV) worldwide.[2,3] Women in sub-Saharan Africa (SSA) have particularly high rates of STIs, 60% of those living with HIV in SSA are female.[3] Untreated STIs can lead to infertility and in pregnant women can cause stillbirth, neonatal death, and low birth weight.[4] It is also possible for infected pregnant women to transmit STIs to their children. Transmitted in utero or during delivery, syphilis, chlamydia and gonorrhea can cause blindness and developmental disabilities.[5-7] Mother-to-child transmission of HSV-2 and HIV causes lifelong infection and can result in mortality in infected children.[8-11]

STIs pose a risk to pregnant women in any setting, however in those with high STI prevalence these infections contribute to significant morbidity and mortality in women and children. Despite the evidence of continued high fertility rates in settings that also have high STI prevalence, there are few data on incidence of STIs during pregnancy. There is also little known about women's sexual behavior and vaginal practices during pregnancy, both of which may impact STI acquisition. In light of the significant consequences of STIs for the health of women and children, it is critical to more fully characterize factors that influence risk for these infections during pregnancy, assess whether pregnancy is a time of increased vulnerability and understand the biological and behavioral pathways through which pregnancy may increase the risk of STI acquisition in women. This information could lead to more effective efforts to prevent STIs in women of reproductive age which would have a beneficial impact on women's health and the health of their children.

The overall goal of this dissertation is to evaluate whether pregnancy is a period of increased STI risk and to investigate the factors that may increase STI incidence during pregnancy. Chapter 2 presents results from a systematic literature review of the existing data on incidence of STIs among pregnant

women and the evidence for whether pregnancy is a time of increased risk of acquisition. This chapter provides and overview and assessment of the available data, synthesizing findings from all existing English-language studies published from 1980 to 2012 which measured incidence and/or compared incidence among pregnant and non-pregnant women.

Chapter 3, the first of two analytic analyses, explores the impact of pregnancy on women's sexual risk behaviors which are thought to contribute to STI acquisition. In addition to sexual risk behaviors, changes in vaginal practices, which are also thought to be important pathways of STI acquisition, are examined. Using data from a randomized clinical trial for HIV prevention, women's behaviors were compared during pregnant and non-pregnant periods. For the analysis, only women with lab confirmed pregnancies were included, with follow-up time prior to and during pregnancies examined to describe the impact of pregnancy on women's sexual risk behaviors and vaginal practices. Behavior change was measured using several different analytic approaches in order to fully characterize changes resulting from pregnancy. This analysis is unique in using prospective follow-up data to characterize sexual behavior change during pregnancy with data from the same women before and during pregnancies. In addition, the restriction of the analysis to only women who had a pregnancy helps to reduce unmeasured confounding between women who did and did not have pregnancies during the trial, adding strength to the findings on behavior change resulting from pregnancy.

Chapter 4 uses data from the same randomized clinical trial to examine incidence of four STIs: chlamydia, gonorrhea, trichomoniasis and HIV and to compare incidence by pregnancy status. All women from the trial 18-50 years of age at with a follow-up visit within 6 months of enrollment were included in the analysis, including 766 (17%) women who had a pregnancy during follow-up. All follow-up visits were classified according to whether women were pregnant or non-pregnant at the visit, as well as the type of hormonal contraception (HC) reported at non-pregnant visits. The analysis then examined differences in reported sexual risk behaviors and vaginal practices based on pregnancy status.

The analysis of differences in risk behaviors between pregnant and non-pregnant women took into account different numbers of visits attended by participants and adjusted for multiple observations per participant. Incidence of each of the four STIs was calculated for the pregnancy exposure groups using person time during follow-up. Cox proportional hazards models adjusted for time-varying exposure and time-varying and time-fixed covariates were used to compare STI incidence among pregnant and non-pregnant women. In addition to these main analyses, several sensitivity analyses were also conducted to examine the potential impact of using different definitions of pregnancy (including women who were newly pregnant compared women in ongoing pregnancies), as well as to assess the impact of excluding a small number of participants who did not have adequate follow-up time for inclusion in the analysis.

This dissertation represents one of the first thorough examinations of the question of sexual risk behavior change and incidence of four common STIs during pregnancy. The analyses utilize robust statistical methods with data from a randomized clinical trial with good follow-up, both of which are important strengths. In addition, the sensitivity analyses that were conducted help to address any questions regarding biases from exclusion of subjects or misclassification of pregnancy status. There are few previous analyses with which to compare the findings and this dissertation presents important new information about STI risk among pregnant women which may contribute to efforts to reduce risk and protect both women and children from potential morbidity and mortality associated with these infections.

Chapter 2

Incidence of sexually transmitted infections during pregnancy: systematic review of incidence and evidence for increased risk among pregnant women

Abstract

Background: Sexually transmitted infections (STIs) are common among pregnant women and pose significant threats to their health and the health of their children. Although high STI prevalence is reported among pregnant women in many parts of the world, STI incidence during pregnancy has not been well documented.

Methods: A systematic literature search of English language peer review journal articles published from 1980 to 2012 was conducted to describe STI incidence during pregnancy and to assess whether pregnant women are at greater risk for STI acquisition compared to non-pregnant women. Prospective follow-up studies with outcome data during pregnancy and studies with repeat screening in pregnant women from all geographic regions were included.

Results: Eighteen papers were included in the final review with incidence of five STIs: chlamydia, gonorrhea, human papillomavirus (HPV), herpes simplex virus type 2 (HSV-2) and human immunodeficiency virus (HIV). Four studies reporting new chlamydia infections found incidence of 4-9%. Recurrence of chlamydia during pregnancy (reinfection after treatment during the same pregnancy) reported in five studies was 9-32%. Only one study reported incidence of gonorrhea and found that 4% of women acquired infection during pregnancy. Two studies reported HPV incidence among pregnant women with 31% of pregnant women in Uganda and 12% in Holland acquiring a new high risk HPV type, respectively. In three studies of HSV-2, pregnant women with unknown partner status ranged from no new infections to incidence of 0.9 per 100 person years. In a study of women HSV-2-infected male partners, 14% of pregnant women acquired infection. Seven studies reported HIV incidence among pregnant women ranging from 1.64 (in Uganda and Zimbabwe) to 10.7 per 100 person years (in South Africa). Very few studies compared incidence among pregnant and non-pregnant women. Only one study of HPV compared incidence and did not find a higher incidence during pregnancy. In four studies

comparing HIV risk by pregnancy status, one study found that pregnant women had two-fold increased risk while three other studies did not show any difference. In nine studies that examined risk factors for STI acquisition during pregnancy younger age was found to be associated with increased incidence.

Conclusions: There are very limited data on incidence of STIs during pregnancy but existing studies show that STI incidence does occur during pregnancy, possibly at high rates for some infections. Younger pregnant women and those with partners known to be infected with an STI appear to be at higher risk for acquisition.

Background

Sexually transmitted infections (STI) are highly prevalent around the globe and are very common among pregnant women. The World Health Organization (WHO) estimates that every year worldwide there are 448 million new cases of curable STIs including syphilis, gonorrhea, chlamydia and trichomoniasis.[12] In addition, non-curable STIs, including HIV, herpes simplex virus type 2 (HSV-2) and human papillomavirus (HPV) are also highly prevalent. There are now over 34 million people living with HIV worldwide and the epidemic continues to disproportionately impact women, particularly in sub-Saharan Africa (SSA) where 60% of those living with HIV are female.[13] The majority of the 536 million people estimated to be infected with HSV-2 worldwide are women.[2] HPV is the most prevalent STI globally and is found in 11.7% of women around the world.[14]

STIs are responsible for significant morbidity in women particularly in relation to their reproductive health. Left untreated, chlamydia and gonorrhea can lead to infertility, while trichomoniasis and syphilis are associated with premature delivery.[4,10,15] HPV causes cervical cancer which is the third most common cancer in women and the fourth most deadly cancer in women around the world.[16] HIV is a leading cause of death for women of reproductive age[17] and has been shown to increase maternal mortality by up to six fold.[18] STIs also impact the health of children. Premature delivery resulting from infections can cause developmental delays[19] and STIs that are transmitted from mother-to-child including chlamydia, gonorrhea and syphilis can cause blindness and other cognitive impairments,[5-7] as well as, lifelong infection and death, in the case of HSV-2 and HIV.[8,10,11,20,21] Mother-to-child transmission (MTCT) of HIV is the most important driver of the HIV epidemic in children – over 90% of infections in children result from MTCT.[13]

Most previous studies examining STIs in pregnant women have described prevalence which has been shown to be high in many parts of the world, particularly among young women. Data from

Mozambique showed that 75% of pregnant women had at least one STI[22] and a 2010 study of pregnant women from Zimbabwe found that half were infected with HSV-2, 25% with HIV and 12% with trichomoniasis.[23] While pregnant women in high resource settings generally have lower STI prevalence than those in sub-Saharan Africa, up to 8% of pregnant women in the United States (US) have been found to have chlamydia.[24,25] Among pregnant adolescents in the US, rates of STIs are much higher; one study found that 31% of pregnant 15-19 year olds were infected with either (or both) chlamydia and gonorrhea.[26]

The question of whether pregnancy increases STI acquisition is important given the large numbers of pregnant women who are found to have these infections. STI prevalence data in pregnant women provides evidence of the significant health threat these infections pose and highlight the importance of routine screening during pregnancy to identify and treat infections in order to protect women's health and to ensure optimal birth outcomes. Prevalence data however do not indicate when pregnant women are acquiring infections, whether they are infected prior to or during their pregnancy, so do not distinguish whether pregnancy is a time when women may be more likely to acquire STIs. Pregnancy has been hypothesized as a time of potentially increased vulnerability to STIs as a result of changes in behavioral and biological risk factors.

STI acquisition may be higher during pregnancy as a result of changes in sexual risk behaviors, primarily lower rates of condom use which may result from the absence of contraceptive concerns. If pregnant women engage in more frequent unprotected sex, this could put them at higher risk for becoming infected with an STI. While existing studies on sexual behaviors of pregnant women have found that coital frequency tends to decline over the course of pregnancy,[27-29] some data show that reported condom use is lower among pregnant women compared to non-pregnant women.[30-33] Pregnant adolescents have also been shown to be less likely to report use of condoms than non-pregnant teens.[34] Male partner sexual risk behavior may also explain high rates of STI incidence during

pregnancy. If some men are more likely to acquire new sexual partners during a woman's pregnancy (higher rates of sexual partner concurrency) this could increase men's risk of incident infection and make them more likely to transmit STIs to their pregnant partners. There are limited data on the sexual behaviors of male partners during pregnancy. A study in Malawi found that pregnant women reported fear of partner sexual concurrency during pregnancy[30] however data from the Rakai community cohort did not find that men with pregnant partners were more likely to report concurrent sexual relationships.[35]

STI acquisition during pregnancy could also be facilitated by biological changes that occur as a result of pregnancy, specifically cervical and immunologic changes. The lower female reproductive tract (FRT) and the mucosal tissue of the cervix are considered the main sites of STI acquisition in women.[36-39] The tissue of the cervix is a critical entryway for STIs, in particular HIV, because of the presence of receptor cells which promote infection.[36,40] Changes to the cellular composition of the cervix during pregnancy, including cervical ectopy, could make women more vulnerable to infection. Cervical ectopy is a condition in which the more fragile epithelium cells of the endocervix extend to the outer areas of the ectocervix potentially increasing vulnerability to pathogens.[36,41] Cervical ectopy is a common condition in the developing cervices of adolescents[42] and the disproportionately high rates of HIV and other STIs among young women are thought to be associated with this condition.[36,43,44] Cervical ectopy is also commonly found in pregnant women[41,45,46] and women using oral contraceptives.[43] In cross sectional data from a study in Kenya, cervical ectopy was present in half of the pregnant women studied.[45] Cervical ectopy has previously been shown to be associated with increased STI acquisition[43] and with prevalent HIV infection in pregnant women.[45]

In addition to cervical ectopy, there are other cervical changes that occur during pregnancy that may increase STI risk. Mouse models have shown that tissue "remodeling" of the cervix begins within days of pregnancy as part of the process of cervical ripening necessary for delivery of the fetus.[47] In a

study of baboons, pregnancy was associated with a thinning of the squamous epithelial tissue and higher leukocyte counts in cervical tissues samples which could increase cervical tissue pathogen susceptibility.[48] Although these studies were conducted in animals and did not specifically aim to examine cervical changes in relation to infection risk in humans, the data suggest that there may be significant changes occurring during pregnancy, possibly from the early stages of pregnancy, that alter the composition of the cervix making it more permeable and potentially more vulnerable to infectious agents.

In addition to histological changes occurring in the cervix, hormonally-regulated immune function during pregnancy could also play a role in STI risk. Pregnancy is associated with up to 11-fold increases in progesterone concentrations and up to 4.8-fold increases in estrogen levels[49] which are both known to alter innate and acquired immune function, including cell production, distribution of immune cells in the FRT and activation of specific immune cells.[50-54] Hormonally-induced immune adaptation experienced during pregnancy has been observed in a number of ways including the alleviation of symptoms of autoimmune disorders,[55] increased vulnerability to and severity of disease from pathogens such as influenza,[54,56] and evidence of diminished immune response to vaccination in pregnant women.[54] Pregnancy has thus been described as an immune-compromised state, an evolutionary adaptation thought to be necessary for sustaining a "semi-allogeneic" fetus.[44,54,56]

Diminished immune capacity during pregnancy may put women at greater risk for STIs. However the precise mechanism for this has not been well characterized. Studies have linked hormonal fluctuations to alterations in antibody levels in vaginal secretions in women.[52] Progesterone in particular has been a focus of attention with regard to HIV risk. Increased progesterone levels have been shown to increase acquisition of simian immunodeficiency virus (SIV) in primates (SIV is a virus related to HIV that is found in primates).[57] In vitro and animal studies have also shown that increased progesterone levels during pregnancy can reduce the number of natural killer immune cells which are part of the body's

defense against infection.[58,59] Progesterone levels may also alter recruitment of immune cells causing inflammation of the genital tract which has been associated with risk of STI and HIV infection.[60] Finally, data are mixed on the impact of progesterone levels on concentrations of CCR5 cells in the cervix.[61,62] CCR5 is considered the most important co-receptor (facilitator) of HIV infection in the FRT and in a small study, pregnant women in the second and third trimesters were found to have higher percentages of tissue cells in the FRT with CCR5 co-receptors compared to non-pregnant women.[62] High progesterone levels in pregnant women may also cause the epithelium thinning described above that has been found in primates to increase vulnerability to pathogens.[57] In addition to viral and bacterial infections, there may also be greater susceptibility to parasitic infections during pregnancy as a result of depressed immune function and/or increased inflammation.[50] Studies in mice have shown greater susceptibility to certain parasites during pregnancy.[63,64]

Studies showing direct evidence for an effect of immune depression or inflammation on infection risk during pregnancy are limited, particularly in humans. A study in mice found that pregnancy increased vulnerability to infection with several STIs including gonorrhea, chlamydia and HSV-2.[52] Anderson et al. investigated differences in the immunologic properties of the cervico-vaginal secretions of pregnant and non-pregnant women to estimate their anti-HIV capacity.[39] Overall, they did not observe differences between samples from pregnant and non-pregnant women in terms of the cellular response to *in vitro* HIV challenge. However significantly higher protein concentrations were found among pregnant women (it is not yet clear whether or how elevated protein concentrations might increase susceptibility to infection).[39] Morrison et al. identified two markers of inflammation and immune activation (RANTES and SLPI) that were elevated prior to HIV sero-conversion among pregnant women but were not found to be associated with HIV infection in non-pregnant women.[65] These data suggest that pregnancy may increase risk of HIV infection through inflammation, or that inflammation is a marker for risk of HIV acquisition among pregnant women.[65]

The evidence above suggests that there may be behavioral and/or biological changes that occur during pregnancy that could lead to increased STI acquisition among pregnant women compared to non-pregnant women. Incidence data are therefore essential for understanding whether and how pregnancy may be a time of increased STI susceptibility in women. In addition to its etiologic importance, this information would have bearing on prevention and screening efforts for pregnant women. It is critical to understand whether screening offered only once during pregnancy is adequate to identify infections and to prevent negative health outcomes for women and children. There is evidence that when women acquire infections during pregnancy there is increased risk of MTCT for both HIV[66] and HSV-2[67] highlighting the importance of prevention of STIs during pregnancy. This paper will describe the findings from a systematic review of studies documenting incidence of STIs during pregnancy and comparisons of STI incidence among pregnant and non-pregnant women. All prospective studies examining incidence of bacterial, viral and parasitic STIs were considered.

Methods

This systematic review to examine incidence of STIs during pregnancy was conducted adhering to standard practices as outlined in the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines.[68] Existing peer-reviewed English language papers from any geographic region published between 1980 and 2012 were identified through electronic web-based searches of three online databases: Ovid/MEDLINE, PubMed and Embase (European database).

The population of interest was pregnant women and therefore only studies that measured STI incidence during pregnancy were included. Incident infection during pregnancy was defined as a positive test following an initially negative test during the same pregnancy or incidence of infection after treatment for an STI found during the same pregnancy (i.e. reinfection or recurrence after treatment). Only studies measuring incidence were considered; this included prospective studies with active enrollment and follow-up (cohort studies and secondary analyses of randomized clinical trials (RCT)), as well as analyses of repeat STI screening in pregnant women collected through routine health care services or as part of a follow-up study. Only studies that measured incidence during pregnancy were included; studies were also included if they measured incidence in non-pregnant women as well as pregnant women, as were studies which reported incidence of multiple STIs. Studies were excluded if they did not include pregnant women, if they did not present data on repeat testing (incidence) during the same pregnancy (studies with testing in the postpartum period were excluded) and if they did not report findings for individual STIs by type. Cross sectional studies, those otherwise limited to reporting prevalence of STIs among pregnant women, and papers that modeled incidence rather than directly observing incidence were excluded.

Searches were conducted using both medical subject headings (MeSH) and keywords in the aforementioned online databases. The search strategy was based on identifying the population of

interest (pregnant women), the outcomes of interest (STIs) and the study designs (follow-up or prospective studies and those with repeat testing). Following this search strategy, the MeSH and keyword searches included the following terms: (1) population of interest: “pregnancy/pregnant”, “pregnancy complications”; (2) outcomes of interest: “chlamydia”, “chlamydia trachomatis”, “gonorrhea”, “gonorrhoe”, “trichomonas” “trichomonas”, “trichomonas vaginitis”, “trichomonas vaginalis”, “HIV”, “HIV seropositivity”, “HIV-1”, “syphilis”, “chancre”, “neurosyphilis”, “human papillomavirus”, “HPV”, “condylomata acuminate”, “genital warts”, “herpes simplex”, “herpesvirus 2”, “HSV 2”, “sexually transmitted diseases”, “sexually transmitted”, “STD”; (3) study design/measure of association: “incidence”, “acquisition”, “recurrence”, “reinfection”. The search results were further refined to exclude papers published prior to 1980 and after 2012, those that described results of studies not conducted in humans, those for which the full manuscript was not available in English and papers that were a format other than articles or reviews.

A total of 3,036 unduplicated publications were identified through the search combining results from the online databases (Figure 2.1). Of the 3,036 titles found, 454 abstracts were reviewed for relevance; the remaining 2,582 titles were excluded based non-relevance, including studies of prevalence with no follow-up, studies of infections other than those of interest for this analysis, *in vitro* studies and non-human studies, modeling analyses, clinical guidelines and case series. Of the 454 abstracts reviewed, 55 full manuscripts met the inclusion criteria and were selected for detailed review by the author and an independent additional reviewer. Studies were divided into disease-specific groups and then categorized into tiers according to the study design and procedures for follow-up. Tier 1 consists of RCTs and prospective studies with active and systematic follow-up. Tier 2 contains studies considered to have “passive” follow-up and/or studies with repeat testing during pregnancy that were not conducted with systematic follow-up (i.e. no fixed interval of time between testing) and those that relied on existing data, such as health facility data or patient held records for testing results. Tier 2 also

includes retrospective cohorts and cohorts for which recruitment procedures and exclusions of subjects were not fully reported as this may have introduced selection bias. Tier 3 consists of studies that only reported reinfection or recurrence among pregnant women who tested positive for curable STIs at enrollment and were followed after treatment to measure reinfection with the same STI.

For the purposes of presenting results from the studies included in the review, a table was designed to present information on each study. Table 2.1 is organized by infection type (studies that included more than one STI are included in the sections for each individual STI) and by tier. Table 2.1 includes information on the study location, population and incidence, risk factors for STI incidence and comparisons of incidence between pregnant and non-pregnant women for those studies that included non-pregnant women. Many of the studies included prevalence data at baseline for the full sample and follow-up incidence data from repeat testing on a smaller number of women included in the study; the total sample size reported in the study and sample size relevant to the findings on incidence are noted in Table 2.1. In studies using multivariable modeling, the factors that were adjusted for are also included.

For all studies of curable STIs, results were examined to distinguish incidence among two groups of women: (1) those testing negative for STIs at the first test during pregnancy and (2) women who had a positive test for an STI at their first test during pregnancy, received treatment and were followed to measure repeat infection with the same STI (recurrence). It is important to differentiate incidence in these two groups as they represent different populations of pregnant women who may have very different risk factors and incidence of infection during pregnancy. Women who are positive at the first test during pregnancy may be at higher risk of acquiring the same infection during their pregnancy as a result of sexual risk behaviors, particularly if they have partners who are positive and do not receive treatment. For studies measuring recurrence, it is critical to understand whether the study included test of cure (TOC) after treatment for curable STIs – without TOC documentation, recurrence may be overestimated and reflect failure of treatment rather than acquisition of new infections. Table 2.1

included information on whether the studies reporting recurrence among women who were positive for an STI at the first test during pregnancy included TOC procedures.

For the presentation of findings on incidence of STIs, separate estimates are shown in Table 2.1, where possible, for women who were negative at the first test and for recurrence in women who were positive at the first test. For some studies of curable STIs the estimated incidence proportions (including recurrence) reported by the authors were considered underestimates with regard to the two types of incidence of interest noted above (i.e. among women who tested negative at their first test during pregnancy and those who were positive at their first test). This occurred in studies in which the total number of women with repeat testing was used as the denominator for calculating incidence proportions rather than using the subsets of women with negative and positive first test results as separate denominators. For studies that calculated incidence proportions using women with repeat testing, results as reported by the authors are shown in Table 2.1. In addition, we re-calculated incidence proportions (where possible) using the available data in the paper using as the denominator women with negative first test results during pregnancy and those with positive first test results in order to distinguish new incident infections among women not infected at study enrollment from reinfection (recurrence) among women with infections at study enrollment.

Results

A total of 18 studies were included: five studies reporting incidence of chlamydia, one of which also included gonorrhea, two studies reporting HPV, four studies reporting HSV-2, and seven studies reporting HIV incidence among pregnant women. All of the studies reported incident infections (or reinfections) among women during the same pregnancy. Overall, eight studies were conducted in the US, two in Europe (one each in the Netherlands and Finland) and eight in sub-Saharan Africa (Botswana, Kenya, Malawi, Rwanda, South Africa, Tanzania, Zambia and Zimbabwe). The ages of participants enrolled in the studies was not reported uniformly across all studies, two were exclusively in young adults (under 25 years) and adolescents (12-18 years of age), while the others included both young and adult women (up to age 45). The earliest study data came from 1978 and the most recent from 2009.

Of the studies reporting chlamydia, four of five were considered tier 2 as a result of reliance on health facility records for some or all testing outcome data or because they did not define study selection exclusions and/or follow-up procedures.[26,69-71] One additional study of chlamydia was considered tier 3 because it only reported recurrence among women who were infected and then treated after the first test during pregnancy.[72] Only one study was found reporting outcomes for gonorrhea[26] (which also reported chlamydia) and was classified as tier 2 as the selection factors were not explained. The two studies of HPV were considered tier 1 as both included active systematic follow-up and fully described participant exclusions.[73,74] For the studies of HSV-2, one was considered tier 1[75] and three were considered tier 2 studies.[76-78] Among the studies of HIV, four were tier 1[31,33,35,79] and three were tier 2 as a result of relying on routinely collected health data.[30,32,80] One study of HIV did not report the individual figures for incidence (of HIV) among pregnant and non-pregnant women but only report the hazard ratio for the comparison of incidence among pregnant and non-pregnant women.[32]

Among the studies reporting outcomes for chlamydia, all five reported incident chlamydia infections as proportions. Four studies of chlamydia, all from the US, reported incidence of infection among women who were initially negative at the first test during pregnancy, these studies also reported recurrence in women who were positive at the first test during pregnancy and then tested subsequent to treatment.[26,69-71] One additional study of chlamydia only included women who were infected with chlamydia at the first test during pregnancy.[72] For the four studies reporting new infections among women who were negative at the first test during pregnancy, the reported incidence proportions ranged from 4% in women with unspecified ages to 9% in women 14-39 years of age.[26,69-71] As noted, results from studies that used all women with repeat testing as the denominator for incidence proportions (women who tested negative and those positive for chlamydia at the first test) were recalculated to estimate separate incidence proportions for women with negative first tests and for women who had positive first tests. Incidence proportions were recalculated for two studies[70,71] while results from one study could not be recalculated with the available data in the paper.[26] Using the recalculated estimates, incidence of acquiring chlamydia among pregnant women who initially tested negative in pregnancy ranged from 4% to 11% (the original estimate of 9% for infections in this study was calculated using the number of new infections (numerator) divided by the total women at enrollment (including those who were positive for chlamydia); the recalculated figure for new infections is higher because the denominator is smaller (only women initially negative) but the numerator remained unchanged).

All five studies of chlamydia also reported incidence of recurrence or repeat infection. Only one of the studies[72] had complete documentation of test of cure after the positive initial test while the remaining four either had no test of cure or incomplete data on test of cure. Reported incidence proportions for recurrence of chlamydia during pregnancy ranged from 9% in adolescents (12-18 years) to 32% in women of unspecified ages. Recurrence estimates were recalculated for one study,[70] increasing the upper range of estimates to 65% in women 14-39 years of age; estimates from a fourth

study could not be recalculated using the available data in the paper.[26] Only one study of incident chlamydia during pregnancy reported risk factors and found that black race, age <25 years, being single and not completing high school were associated with acquiring chlamydia during pregnancy in the US .[69] No studies of chlamydia compared incidence among pregnant and non-pregnancy women.

Only one study reported outcomes for incidence of gonorrhea during pregnancy which was conducted in US adolescents (12-18 years of age).[26] This study reported incidence as a proportion. As noted in Table 2.1 and explained above for chlamydia outcomes, the incidence proportion of gonorrhea among pregnant women in this paper was calculated using all women with two tests during the same pregnancy as the denominator, rather than calculating separate incidence for women with initially negative tests and for those positive at the first test during pregnancy. The authors reported that out of all pregnant women with repeat testing, 4% had a negative test followed by a positive test result whereas 3% had two positive test results (there was no test of cure in this study).[26] The authors did not present separate incidence proportions for women with negative and positive first tests and it was not possible to recalculate the incidence in this way using the data provided in the paper.

Two studies reported HPV incidence among pregnant women, one in women under 25 years of age and the other in women 18-29 years of age. One study measured acquisition of both low and high risk HPV types[73] while the other reported only high risk types[74] (both studies defined high risk genotypes of HPV as 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 and 73 as these types have been linked to cervical cancer). The two studies of HPV incidence included women with and without HPV at enrollment and measured acquisition of new HPV types during pregnancy (one study of HPV also included a comparison group of non-pregnant women[74]). In the study from Uganda, 42.9% of pregnant women acquired a new HPV infection, 15.2% acquired new low risk HPV types and 30.5% acquired new high risk HPV types.[73] This study also found that younger age was associated with acquiring new HPV infections during pregnancy. In the study from Holland, 11.8% of pregnant women acquired a new high risk HPV

infection.[74] This study also examined HPV acquisition among non-pregnant women and found that 15.7% acquired new high risk HPV infections during follow-up. The authors reported that there was no significant difference in the proportions of pregnant and non-pregnant women who acquired HPV in nonlinear mixed modeling adjusted for age, education, smoking, history of STI and condom use (p=0.57).[74]

There were four studies reporting HSV-2 incidence among pregnant women. One study conducted among women with known HSV-2-infected partners found that 14% of pregnant women acquired HSV-2. The authors reported that after adjusting incidence estimates for duration of pregnancy the HSV-2 incidence would have been 20%.[75] This study did not include a comparison of non-pregnant women. Incidence of HSV-2 in three studies among pregnant women with unknown partner status was much lower – one study of 558 women in xxx found that no pregnant women acquired HSV-2.[76] Boucher et al. reported that 0.25% of 1,580 women who were negative for HSV-2 at the first test during pregnancy became infected[77] and Brown et al. found incidence of 0.9 per 100 person years in 7,046 pregnant women in Seattle, Washington.[78] No studies of HSV-2 incidence compared pregnant and non-pregnant women. Two studies examined risk factors for incidence of HSV-2 during pregnancy; one found that shorter duration of partnership was associated with higher HSV-2 risk [75] while the other found no significant predictors.[78]

The largest number of studies reporting STI incidence among pregnant women were found for HIV. All seven studies were conducted in sub-Saharan Africa; incidence of HIV during pregnancy was reported in six of the seven studies and four studies compared HIV incidence among pregnant and non-pregnant women. HIV incidence in pregnant women ranged from 1.64 (in Uganda and Zimbabwe) to 10.7 per 100 person years (in South Africa).[30,31,33,35,79,80] Among the three studies that reported HIV incidence among non-pregnant women, the range of estimates was 1.1 (Uganda) to 3.0 per 100 person years.[31,33,35] Only one of the four studies comparing incidence among pregnant and non-pregnant

women found a higher risk among pregnant women; after adjusting for behavioral risk factors, including male partner risk behavior, pregnant women in Rakai, Uganda were found to have two fold increased risk of HIV infection compared to non-pregnant women (risk ratio: 2.0, 95%CI 1.3-3.1).[35] Three other prospective cohort studies failed to find increased HIV risk that was statistically significant among pregnant women after adjusting for potential confounding.[31-33] However, in the study of HIV sero-discordant couples, pregnant women with HIV-infected male partners did appear to have higher risk (hazard ratio: 1.7) but the p-value was 0.08; it should be noted that the study only included 1,085 couples with an HIV-uninfected woman.[31] Risk factors for HIV infection were reported in five studies and included both demographic characteristics (younger age, never married and lower parity), behavioral risk factors (multiple, new and high sex partners, and alcohol use) and clinical characteristics (recent STI, bacterial vaginosis and other medical symptoms).[30,32,33,35,79] One study reported no significant risk factors for HIV incidence among pregnant women.[80]

Discussion

There are very limited data on incidence of STIs during pregnancy and even fewer data that compare risk between pregnant and non-pregnant women. Only 18 studies were found reporting new infections among pregnant women and five studies were identified that compared incidence of STIs in pregnant and non-pregnant women. The data included in this review were limited to only five types of infections; no studies were found on trichomoniasis and syphilis, STIs that can have significant impact on birth outcomes, was well as the health of women and children. The lack of data overall and on specific STIs highlights the need for more studies in order to describe STI incidence during pregnancy and to identify whether pregnancy increases the risk of STI acquisition. Although the data are limited, they suggest that women do acquire STIs during pregnancy although incidence varied within and across infection types, populations and geographic setting.

The studies included in this review also varied in the types of incidence measures reported, from proportions to rates using person time, making comparisons somewhat challenging. Highest incidence was found for HPV[73] and chlamydia[70] although some studies of chlamydia showed low proportions of pregnant women infected.[71] Studies in which partners were known to be infected with HSV-2[75] and HIV[31] showed higher rates of acquisition in pregnant women compared to studies where partner status was not known. Most of the studies measuring repeat infections of curable STIs (chlamydia and gonorrhea) had limited data on test of cure so while the findings are suggestive that women who have an STI at the first test during pregnancy may be at very high risk for a repeat infection during pregnancy, they may also indicate that pregnant women receive inadequate STI treatment.

Overall the quality of evidence on incidence of bacterial STIs in particular is lacking. None of the studies of chlamydia or gonorrhea included full descriptions of follow-up and exclusion procedures and the studies tended to focus on reinfection (without test of cure in most cases) rather than newly

infected pregnant women. In addition, most studies only included characteristics of women who were included in the analyses (i.e. those with complete data at baseline and follow-up) and did not report characteristics of women who did not have repeat testing data and were excluded. The lack of information on women missing testing data is significant as selection bias may have been introduced and without further information on who was excluded, the potential bias is difficult to assess. It is possible that if women with more complete data (repeat testing) were less likely to have infections compared to those who only had one test or no testing data during pregnancy, the reported incidence proportions may have been underestimated and the STI incidence among pregnant women may in fact be higher. Conversely if women who were deemed to be at higher risk for STI acquisition were more likely to have repeat testing through targeted screening or study enrollment, the incidence reported may be an overestimate.

There are also additional data quality concerns with regard to the evidence presented in this review. In relation to data on recurrence or repeat infections among pregnant women, only one of five studies of chlamydia and no studies of gonorrhea included test of cure to establish that women were free of infection before being retested. This may have led to overestimates of the incidence of recurrence of infections among pregnant women. In addition, only two studies tested male partners for STIs, some others included information collected from women about their partners' status and still others had no information on partner STI status or efforts to provide treatment for infected partners. Information on partner STI infections, while difficult to collect, is important for understanding the estimates of risk as women with infected partners are likely to have high rates of infection. Many of the studies that reported incidence as proportions had very small sample sizes, some with less than 100 women. Given the small sample sizes, data quality issues, and geographic diversity of the studies, the evidence for incidence of STIs in women is challenging to interpret and to generalize to any specific population.

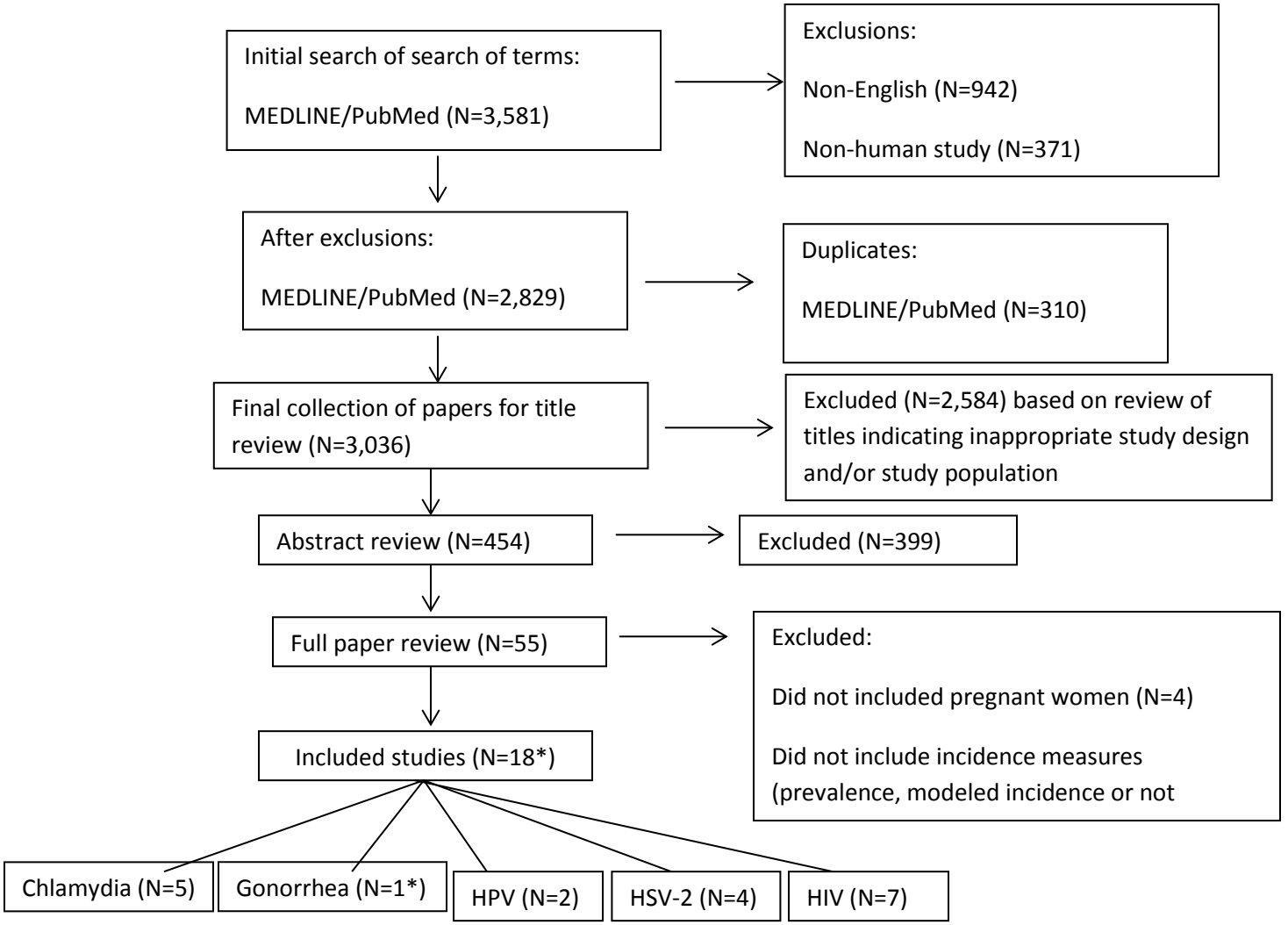
Despite the limited evidence and limitations of many of the studies, there does appear to be continued incidence of STIs during pregnancy which suggests that repeat screening would benefit women, particularly those at highest risk for acquiring STIs during pregnancy. Unfortunately few studies examined risk factors for STIs during pregnancy so that it is difficult to know how repeat screening efforts could be targeted towards high risk women. Younger age (less than 25 years) does appear to increase STI risk among pregnant women[30,32,33,35,69,71-73,79] and may result from biological factors, such as cervical ectopy,[36,43,44] or from higher rates of behavioral risk factors, including lower reported rates of condom use.[34] Further studies of pregnant adolescents could help to understand the potential interaction between pregnancy and younger age. The data also showed that women with infected partners may have higher risk of acquiring STIs.[31,75] This finding underscores the need for expanded efforts to test partners and to provide treatment for curable STIs, as well as the need to enhance counseling for all pregnant women around safer sex practices. Although the number of studies was limited, it appeared that the risk of acquiring a new high risk HPV type was higher in sub-Saharan Africa compared to the Netherlands among young pregnant women.[73,74] This finding is in keeping with data showing higher prevalence of STIs in sub-Saharan Africa and other low resource settings.[1,14]

There is very little direct evidence addressing the question of whether pregnant women are at higher risk of acquiring STIs compared to non-pregnant women. Only five studies were found that directly compared incidence among pregnant and non-pregnant women, one on HPV and four on HIV (no studies were found reporting comparisons by pregnancy for bacterial or parasitic STIs). Among the studies comparing HIV incidence in pregnant and non-pregnant women, only one found that pregnant women were at increased risk.[35] A closer reading of the studies on HIV highlights the complexity of evaluating whether and how pregnancy may increase HIV risk. In three of the studies of HIV, pregnancy was associated with decreases in many of the risk factors associated with HIV acquisition such as multiple sexual partners and high risk sex for drugs or money.[32,33,35] Pregnant women did however

report more sex acts without the use of condoms which may have led to increased acquisition. More data are needed to evaluate the question of whether and how pregnancy may increase STI risk, not only for HIV but also for common curable STIs.

Overall this review found that there are very limited data on incidence of STIs during pregnancy. The studies included in this review show that STI incidence does occur during pregnancy, possibly at high rates for some infections. Identifying pregnancy as a time of increased risk for STIs could lead to more effective interventions to reduce overall incidence of STIs among women. Reducing the number of maternal infections during pregnancy would not only decrease the impact of STIs on women's health and improve birth outcomes, it would also decrease vertical transmission, particularly for HIV; incident HIV infections during pregnancy are accounting for a growing proportion of the new HIV infections among children around the world, including in the US.[81-86] While there is no evidence regarding incident infection and MTCT in relation to other STIs, it is possible that, as with HIV, there may be higher risk of congenital infection with gonorrhea and chlamydia when women are infected during pregnancy. More research is needed to more fully describe STI incidence during pregnancy and to compare incidence during pregnancy to non-pregnant periods in order to establish whether and how pregnancy may make women more vulnerable to these infections.

Figure 2.1 Diagram of search strategy and results



**The only paper with data on gonorrhea also reported data on chlamydia*

Table 2.1 Studies with incidence of STIs during pregnancy

Author	Title	Setting	Years	N	Study population	Incidence during pregnancy	Comparison with non-pregnant	Risk factors for incidence	Adjustment
Chlamydia									
Tier 2									
Allaire[69]	Initial and repeat screening for chlamydia trachomatis during pregnancy	US	1993-1994	2484 (total) 2173 for relevant findings	Pregnant women presenting for prenatal care at <20wks (no age range specified)	5.7% of pregnant women negative at first test during pregnancy acquired chlamydia (109 of 1929) Recurrence: 32% of pregnant women with positive first test had positive subsequent test (78 of 244) No test of cure	N/A	race, younger age, not currently married and not completing high school	race, age (<25 or above), marital status, education (high school or no)
Berggren[26]	Prevalence of chlamydia trachomatis and Neisseria gonorrhea and repeat infection among pregnant urban adolescents	US	2003-2005	125 (total) 95 for relevant findings	Pregnant teens 12-18 years enrolled in prospective cohort at one hospital in Washington, DC	4%* of pregnant women negative at first test during pregnancy acquired chlamydia (4 of 95 women) Recurrence: 9%* of pregnant women with positive first test had positive subsequent test (9 of 95) Incomplete test of cure data **Incidence proportion using all women rather than only those with negative/ positive first test (see note below table), data in paper in sufficient to re-calculate*	N/A	Not reported	Not reported

Chlamydia *continued*									
Heggie[70]	Chlamydia trachomatis infection in mothers and infants	US	1978-1980	1,327 (total) 131 for relevant findings	Pregnant women 14-39 years of age attending prenatal care at one hospital in Cleveland, Ohio	9%* of pregnant women negative at first test during pregnancy acquired chlamydia (12 of 131) Recurrence: 13%* of pregnant women with positive first test had positive subsequent test (17 of 131) **Incidence proportion using all women rather than only those with negative/ positive first test; recalculated incidence using figures from paper:* *12 of 105 (11%) had negative first test and subsequent positive test* *Recurrence: 17 of 26 (65%) had positive first test and subsequent positive test* No test of cure	N/A	Not reported	Not reported
Miller[71]	Initial and repeat testing for chlamydia during pregnancy	US	1998-2000	752	Pregnant women in community based prenatal program in New Orleans, LA (no age range specified; included women over and under 19)	3.9%* of pregnant women negative at first test during pregnancy acquired chlamydia (29 of 752) Recurrence: 13.3% of pregnant women with positive first test had positive subsequent test (14 of 105) **Incidence proportion using all women rather than only those with positive first test; recalculated incidence using figures from paper:* *29 of 647 (4.5%) had negative first test and subsequent positive test* No test of cure	N/A	younger age, lower parity, attending fewer prenatal visits	Not reported

Chlamydia *continued*									
Tier 3 (Repeat testing)									
Miller[72]	Recurrent chlamydial colonization during pregnancy	US	1992-1996	149	Pregnant women in New Orleans, LA with positive test for chlamydia (no age range specified; included women ≤20)	16.8% of pregnant with a positive first test and test of cure had second chlamydia infection during pregnancy (25 of 149)	N/A	younger age	Not reported

Author	**Title**	**Setting**	**Years**	**N**	**Study population**	**Incidence during pregnancy**	**Comparison with non-pregnant**	**Risk factors for incidence**	**Adjustment**
Gonorrhea									
Tier 2									
Berggren[26]	Prevalence of chlamydia trachomatis and Neisseria gonorrhea and repeat infection among pregnant urban adolescents	US	2003-2005	125 (total) 95 for relevant findings	Pregnant teens 12-18 years enrolled in prospective cohort at one Washington, DC hospital	4%* of pregnant women negative at first test during pregnancy acquired gonorrhea (4 of 95) Recurrence: 3%* of pregnant women with positive first test had positive subsequent test (3 of 95) Incomplete test of cure data **Incidence proportion using all women rather than only those with negative/ positive first test (see note below table), data in paper in sufficient to re-calculate*	N/A	Not reported	Not reported

Author	Title	Setting	Years	N	Study population	Incidence during pregnancy	Comparison with non-pregnant	Risk factors for incidence	Adjustment
Human papilloma virus (HPV)									
Tier 1									
Banura[73]	Prevalence, incidence and clearance of human papillomavirus infection among young primiparous pregnant women in Kampala, Uganda	Uganda	2004	987 (total) 105 for relevant findings	Primiparous women 14-24 years of age living within 20km and coming for antenatal care at one hospital in Kampala, Uganda	42.9% (95%CI: 33.2-52.9) of pregnant women acquired new HPV type (45 of 105 women acquired new HPV type not found at first test); 15.2% (95%CI: 9.0-23.6) acquired new low risk HPV type (16 of 105); 30.5% (95%CI: 21.9-40.2) of pregnant women acquired new high risk HPV type (32 of 105 women)	N/A	younger age	Not specified
Schmeink[74, 87]	HPV detection in pregnant women	Holland	2007	102	Women 18-29 years of age not previously screened for HPV, recruited from the community in 3 cities in Holland (no women were pregnant at enrollment; for analysis matched non-pregnant comparison	11.8% of pregnant women acquired a new high risk type of HPV (6 of 51 women found to have a new high risk HPV type not found at first test); among non-pregnant women 15.7% were found to have a new high risk HPV type not found at first test (8 of 51)	Incidence of high risk HPV types pregnant vs. non-pregnant women: Adjusted odds ratio (aOR) 1.09 (95%CI 0.20-5.82)	Not reported	matched stratum

Author	Title	Setting	Years	N	Study population	Incidence during pregnancy	Comparison with non-pregnant	Risk factors for incidence	Adjustment
Herpes simplex virus type 2 (HSV-2)									
Tier 1									
Gardella [75]	Risk factors for herpes simplex virus transmission to pregnant women: a couples study	US	1992-2000	3192 (total) 125 for relevant findings	Pregnant women and male partners attending care at two health facilities in Seattle, Washington (no age range specified)	14% of pregnant women acquired HSV2 (17 of 125 found to have HSV-2 antibodies at second test); estimate after adjustment for length of gestation 20% (range: 12-29%)	N/A	shorter duration of partnership	length of gestation and duration of sexual relationship with partner
Tier 2									
Alanen[76]	Sero-prevalence, incidence of prenatal infections and reliability of maternal history of varicella zoster virus, cytomegalovirus, herpes simplex virus and parvovirus B19 infection in South-Western Finland	Finland	2000	558 (total) 506 for relevant findings	Pregnant women 16-45 years attending antenatal care during 3 month period at one hospital in Finland	No women acquired HSV-2 during pregnancy	N/A	None (no infections)	Not reported

HSV-2 *continued*									
Boucher[77]	A prospective evaluation of primary genital herpes simplex virus type 2 infections acquired during pregnancy	US	1987-1989	1891 (total) 1580 for relevant findings	Pregnant women 10-44 years of age attending one health facility in California	0.25% of pregnant women acquired HSV-2 (4 of 1580 women found to have HSV-2 antibodies at second test)	N/A	not reported	no adjustment
Brown[78]	The acquisition of herpes simplex virus during pregnancy	US	1989-1993	8538 (total) 7046 for relevant findings	Pregnant women receiving prenatal care at two hospitals in Seattle, Washington (no age range specified)	HSV-2 incidence in pregnant women: 0.9 per 100 person years (19 of 2033 found to have HSV-2 antibodies at second test); adjusted rate sero-conversion risk over all during pregnancy 1.4 +/- 0.3	N/A	no significant predictors	age, race, marital status, parity

Author	Title	Setting	Years	N	Study population	Incidence during pregnancy	Comparison with non-pregnant	Risk factors for incidence	Adjustment
Human immunodeficiency virus (HIV)									
Tier 1									
Gray[35]	Increased risk of HIV during pregnancy in Rakai, Uganda: a prospective study	Uganda	1994-1999	2188	Women 15-49 years enrolled in the Rakai, Uganda Community Cohort	HIV incidence in pregnant women: 2.3 per 100 person years (23 pregnant women with 997 py of follow-up seroconverted during pregnancy); incidence in non-pregnant (non-lactating women): 1.1 p/py (275 women contributing 24,161 py)	HIV incidence in pregnant vs. non-pregnant women: Risk ratio 2.03 (95%CI: 1.33-3.11)	younger age, previous marriage or never married, multiple sex partners, symptoms of genital ulcer disease	age, marital status, education, multiple sex partners, genital ulcer disease, condom use (reported by both female and male partner)
Mbizvo[79]	HIV-1 sero-conversion incidence following pregnancy and delivery among women seronegative at recruitment in Harare, Zimbabwe	Zimbab-we	1991-1995	372	Pregnant women attending antenatal care at four health clinics in Harare, Zimbabwe (age range not specified, included women under 17 and up to and over 40)	HIV incidence in pregnant women: 4.32 per 100 per person years (16 of 372 women seroconverted during pregnancy)	N/A	younger age, no reported condom use, lower education in male partners, recent medical conditions reported (urinary tract infection, recent upper respiratory infection, history of unexplained diarrhea)	not clearly specified: age, education, occupation, partner occupation, recent medical conditions

HIV *continued*									
Morrison[33]	Pregnancy and the risk of HIV-1 acquisition among women in Uganda and Zimbabwe	Uganda and Zimbab-we	1999-2004	4439	Women 18-35 years seeking general, reproductive health or STI services at health facilities in Uganda and Zimbabwe	HIV incidence in pregnant women: 1.64 p/py (13 women contributing 793 py seroconverted during pregnancy); incidence in non-pregnant (HC use) women: 2.94 p/py (126 women contributing 4288 py); incidence in non-pregnant (no HC use) women: 2.70 p/py (39 women contributing 1447 py)	HIV incidence in pregnant vs. non-pregnant: Hazard ratio (HR) 0.60 (95%CI: 0.31-1.16)	study site, not living with partner, younger age, higher participant behavioral risk, higher male partner behavioral risk, alcohol use	location, age, condom use, socio-demographic characteristics, STI prevalence, time-varying sexual behavior variables
Mugo[31]	Increased risk of HIV-1 transmission in pregnancy: a prospective study among African HIV-1 sero-discordant couples	Bots-wana, Kenya, Rwanda, SA, TZ, Uganda and Zambia	2004-2007	3321 (total) 1085 for relevant findings	HIV sero-discordant couples (both partners >18 years of age) recruited from health centers in seven countries	HIV incidence in pregnant women: 7.35 p/py (12 women contributing347 py seroconverted while pregnant); incidence in non-pregnant women 3.01 p/py (46 women contributing 2905 py)	HIV incidence in pregnant vs. non-pregnant women: HR 1.71 (95%CI: 0.93-3.12)	not reported	randomization arm, age, region, number of children, STI prevalence, HSV-2, circumcision, time-varying sexual behavior variables
Tier 2									
Keating[30]	High HIV incidence and sexual behavior change among pregnant women in Lilongwe, Malawi: implications for the risk of HIV acquisition	Malawi	Jan-Oct 2009	1087	Pregnant women attending prenatal care and delivering at one large hospital in Malawi	HIV incidence in pregnant women: 4.0 per 100 person years (95%CI: 2.2-7.2) (11 of 1087 seroconverted during pregnancy)	N/A	younger women, lower parity	Not reported

HIV *continued*									
Moodley[80]	Cofactors for HIV-1 incidence during pregnancy and postpartum	South Africa	2006-2007	2377	Pregnant women attending antenatal care at three health facilities in South Africa, women testing HIV-negative at first antenatal visit included (no age range specified, included women <20 and >40 years)	HIV incidence in pregnant women: 10.7 per 100 person years (72 of 2377 women seroconverted during pregnancy)	N/A	no significant risk factors	Not reported
Reid[32]	Pregnancy, contraceptive use and HIV acquisition in HPTN 039: relevance for HIV prevention trials among African women	South Africa, Zambia, Zimbabwe	2003-2007	1358	Women 16 years and older recruited from family planning, well-baby and voluntary HIV testing clinics	Not reported	HIV incidence in pregnant vs. non-pregnant women: HR 0.64 (95%CI: 0.23-1.80)	younger age, new partner and bacterial vaginosis	randomization arm, study site, socio-demographic characteristics, STI prevalence and symptoms, time varying sexual behavioral variables, contraceptive use

**Studies where incidence proportion reported in paper was calculated using all women with repeat testing were recalculated to estimate proportions for women with negative first tests and for women with positive first tests (recurrence)*

Chapter 3

Sexual behavior and vaginal practices during pregnancy: a comparison of pregnant and non-pregnant periods among women enrolled in a randomized clinical trial

Abstract

Background: Data suggest that pregnant women, particularly young pregnant women and those in resource limited settings have high prevalence of sexually transmitted infections (STI). We examined changes in sexual risk behaviors and vaginal practices during pregnancy.

Methods: Data for the analysis came from the Methods for Improved Reproductive Health in Africa (MIRA) study which was conducted in South Africa and Zimbabwe 2003-2006. HIV-negative women aged 18-45 years were included in this analysis. We used a cross-over design comparing behaviors during pregnant and non-pregnant periods in women with a lab confirmed pregnancy during follow-up. Modified Poisson regression models were fitted to estimate and compare sexual risk behaviors and vaginal practices during pregnant and non-pregnant periods.

Results: Among the 4,948 women who were <45 at enrollment, 483 (10.1%) women who had a lab confirmed and known pregnancy and adequate follow-up time were included in the analysis. Compared to periods when women were not pregnant, pregnancy was associated with less frequent sex (adjusted risk ratio (ARR) 0.9;95%CI 0.8-1.0) but more unprotected sex acts (ARR 1.3;95%CI 1.2-1.5). Pregnancy was also associated with decreased reporting of other sexual risk behaviors including anal sex, multiple sexual partners and sex in exchange for drugs or money (ARR 0.7;95CI 0.6-0.9). Visits that occurred during pregnancy included lower reports of all types of vaginal practices including washing, wiping and insertion of cotton, cloth or paper for non-menstruation related reasons.

Conclusion: We found that pregnancy decreased sexual activity but increased the risk of sex without a condom. The frequency of other high risk sexual behaviors known to be associated with STI acquisition was lower during pregnancy. Vaginal practices were also less common during pregnancy. These data present a complicated picture of risk for STIs during pregnancy as a result of increased unprotected sex but decreased frequency of other important risk factors.

Background

Epidemiologic data suggest that pregnant women, particularly young pregnant women and those in resource limited settings, have high prevalence of sexually transmitted infections (STI). Data from East and Southern Africa have shown that up to 7% of pregnant women are infected with chlamydia, 4% have gonorrhea[88], up to 15% are infected with syphilis[89] and more than a quarter of all pregnant women in East and Southern Africa have been shown to have trichomoniasis.[88] Herpes simplex virus type-2 (HSV-2) and human immunodeficiency virus (HIV) have also been commonly found in many pregnant women in sub-Saharan Africa (SSA). A 2010 study from Zimbabwe found that half of pregnant women were infected with HSV-2 and 25% were HIV-infected.[23] In South Africa, antenatal HIV prevalence in 2010 was over 30%. [90] Pregnant women in the United States (US) have also been found to have high STI prevalence; up to 8% of pregnant women may be infected with chlamydia [24,25] and pregnant adolescents have been shown to have combined prevalence of chlamydia and gonorrhea of up to 31%.[26] These figures are higher than those in a nationally representative survey of youth 18-26 years of age in the US which found 4.7% of adolescent females to be infected with chlamydia and 0.4% to be infected with gonorrhea.[91]

STIs cause considerable morbidity in women, with infections which can lead to infertility and other adverse health outcomes, including mortality in the case of HIV. [10,15] STIs are of particular concern during pregnancy as they cause birth complications including pre-term delivery and premature rupture of membranes which can be life threatening for both pregnant women and infants.[10] In addition, some infections can be transmitted to infants through mother-to-child transmission (MTCT) causing congenital defects such as blindness and developmental disabilities, and in the case of HIV and HSV-2, lifelong infection and mortality for children perinatally infected.[10,11] Evidence on HIV and HSV-2 suggests that incident infection during pregnancy confers significantly higher risk of MTCT. [67,84,92]

Most studies of STIs in pregnant women have reported prevalent infections identified at routine screening during antenatal care. These data are important for assessing the magnitude of the STI epidemic among pregnant women however they do not provide information on etiology and timing of infection. There have been few studies examining when pregnant women acquire STIs – prior to, concurrent with or during their pregnancies. Evidence regarding whether and how pregnancy may be a time of increased risk for STI infection is very limited and questions remain regarding biological and behavioral mechanisms that may increase women's STI susceptibility. In order to understand more about the risk of STI acquisition during pregnancy and the factors that may contribute to incidence during this period, more information is needed on sexual risk behaviors during pregnancy.

Sexual risk behaviors and factors that have been previously associated with STI prevalence include sex without a condom (unprotected sex), frequency of sex, concurrent sexual partnerships, number of lifetime sex partners, transactional or "exchange" sex (for shelter, money or drugs) and engaging in anal sex without a condom have been associated with STI acquisition.[93-97] There have been few examinations of whether these sexual risk behaviors change during pregnancy, particularly in low resource settings where STI prevalence is high. Existing studies on sexual behaviors of pregnant women have found that coital frequency tends to decline over the course of pregnancy with the least amount of intercourse in the third trimester.[27-29] Data from a cross sectional survey of women in Malawi showed a decline in frequency of intercourse over the course of the pregnancy with pregnant women overall reporting fewer sex acts than non-pregnant women [30] whereas a study from South Africa showed relatively high rates of sexual activity in late pregnancy and less frequent sex in the post-partum period. In the South African study, 67% of women 24-30 weeks gestation reported sexual activity in the previous week whereas only 49% reported recent sexual activity at 3 months post-partum.[98]

Sex without a condom has been more frequently reported by pregnant women compared to non-pregnant women in several studies [31-33,35], however other risk behaviors associated with STI

transmission appear to be less common among pregnant women including concurrent sexual partnerships and transactional sex. [32,33,35] There are few data on the sexual risk behaviors of male partners of pregnant women which is also a critical factor in women's STI acquisition. Pregnant women in Malawi reported greater fear of male partner infidelity during pregnancy than non-pregnant women. [30] However, in studies that have data from male partners, men in South Africa and Uganda were not more likely to report additional sex partners during pregnancy.[35,98]

In addition to sexual risk behaviors, vaginal practices have been identified as a potentially important mechanism for STI acquisition. Vaginal practices include a broad array of behaviors such as douching or cleaning the vagina which may be done for hygienic reasons or for pregnancy prevention. Vaginal practices also include behaviors related to altering the vagina, such as inserting absorbent materials to tighten or dry the vagina which is considered sexually desirable in some settings.[99,100] Vaginal practices are commonly reported in studies conducted in sub-Saharan Africa and as a result have been studied as a potential causal factor in women's STI risk [99-101], most notably in regard to HIV. However, limited data have also shown vaginal practices to be associated with increased risk for chlamydia [102] and other STIs.[103] In a meta-analysis including over 20,000 women, use of cloth or paper, inserting materials to tighten or dry the vagina and cleaning with soap were associated with increased HIV infection.[101] It is not fully understood how vaginal practices increase STI and HIV risk; it may be through abrasions to vaginal tissue or through alteration of the vaginal pH level which causes bacterial vaginosis (BV). BV has been estimated to increase women's susceptibility to HIV by 40-70%.[101,104] It has also been documented that some women engage in vaginal practices more often when they have transactional sex [100] which could increase risk if transactional sex partners are more likely to be infected with STIs or the sex involves more risky behaviors.

There are few studies describing vaginal practices among pregnant women. In a cross sectional study of douching practices among pregnant women in Cote d'Ivoire, 97% reported douching before

antenatal medical visits and 98% reported that it was a common practice.[105] Data on other types of vaginal practices have not been widely reported among pregnant women. Given the limited data on vaginal practices during pregnancy and because they are considered an important modifiable behavioral risk factor for HIV infection, and potentially other STIs, it is critical to understand what role they may play in STI risk for pregnant women.

In light of the high rates of STIs found among pregnant women, the risks associated with incident infections during pregnancy and the lack of data on whether pregnancy changes risk factors for acquiring STIs, more information describing the impact of pregnancy on behaviors associated with STI acquisition is needed. Using data from a randomized clinical trial (RCT) which assessed the diaphragm for HIV prevention in women, self-reported data on sexual and vaginal practices were examined to explore the impact of pregnancy on risk factors for STI acquisition. The analysis compares sexual risk behaviors and vaginal practices reported by the same group of women during pregnant and non-pregnant periods among women with lab confirmed pregnancies during two years of follow-up.

Methods

Data source

The data for this analysis come from the Methods for Improved Reproductive Health in Africa (MIRA) study, an open-label RCT of the diaphragm and lubricant gel for prevention of HIV infection in women which has been previously described.[106] Briefly, the trial enrolled HIV-negative women 18 years and older in Zimbabwe and South Africa from 2003 to 2006. The trial compared incident HIV infection in women who were randomized to one of two arms: the intervention arm received a clinician-fitted diaphragm, lubricant gel and condoms; the control arm received condoms only (both arms received counseling about behavioral risk reduction). As previously reported, the trial found low use of the diaphragm and did not find a benefit from the diaphragm for HIV prevention; the relative hazard in the intent to treat analysis between the diaphragm and condom vs. condom-only arm was 1.05 (95%CI 0.84-1.32).[106]

Study procedures

During 12-24 months of follow-up (depending on when women were enrolled in the study), study participants attended quarterly visits with a clinician-administered interview on recent medical history, pregnancy and routine testing for chlamydia, gonorrhea, trichomoniasis and HIV at all visits and other STI testing based on symptoms (with treatment as needed). Study visits also included physical examinations when clinically indicated. Diagnosis of chlamydia, gonorrhea and trichomoniasis was assessed using urine samples and confirmed with DNA polymerase chain reaction (PCR) testing (Roche Pharmaceuticals, Branchburg, NJ, USA). HIV testing was conducted with two rapid tests on whole bloods samples from finger prick or venipuncture; Determine HIV-1/2 (Abbot Laboratories, Tokyo, Japan) and Oraquick (Orasure Technologies, Bethlehem, PA, USA) with discordant rapid tests confirmed with ELISA. Assays were performed at labs in Zimbabwe and South Africa which were monitored for quality control

during the study. All women testing positive for HIV were referred for care and treatment services and continued follow-up in the study. Women with positive tests for curable STIs were given treatment.

At each visit, participants reported sexual activity, use of contraception and condoms, vaginal practices and diaphragm use (for those in the intervention arm) through clinician administered interviews and audio computer-assisted self-interviewing (ACASI). At all study visits, participants received risk reduction counseling and unlimited supplies of male condoms. Women who became pregnant during study follow-up continued in the study during and after pregnancy; those in the intervention arm were free to choose whether to discontinue use of diaphragm and gel during pregnancy. Participants who became HIV infected were referred to HIV care and treatment services. The MIRA trial was approved by institutional review boards (IRB) at the University of California at San Francisco, Research Council of Zimbabwe, the Medicines Control Authority of Zimbabwe, Biomedical Research Ethics Committee of the University of KwaZulu-Natal, Human Research Ethics Committee of the University of the Witwatersrand, and Western Institutional Review Board. The study was externally monitored and audited by Quintiles Corporation. The study is registered with ClinicalTrials.gov, number NCT00121459.

We examined the association between pregnancy status and self-reported sexual behaviors for women in the MIRA trial with a documented pregnancy using a cross-over design restricting the analysis sample to only those women with a pregnancy during follow-up. The current secondary analysis of de-identified data was deemed non-human subjects research by the Columbia University IRB.

Participant inclusion criteria for analysis

Only women in the MIRA trial who were HIV-negative and between the ages of 18 and 45 years of age at enrollment were included in this analysis of pregnancy (women older than 45 were excluded based on the unlikeliness of pregnancy). In order to examine changes to sexual risk behaviors and

vaginal practices associated with pregnancy, follow-up visit data were examined only for women who had a lab confirmed and known pregnancy. Women who reported a pregnancy but did not have lab confirmation and those women who had a lab confirmed pregnancy but did not know about their pregnancies were excluded from further statistical analyses. In addition, women were only included in the analysis of follow-up data if they had at least one visit during a pregnancy and had a visit when they were not pregnant within 60 days prior to the pregnant visit. Women who only had non-pregnant visits after their pregnancy visit or who had had a non-pregnant visit greater than 60 days prior to the pregnancy visit were excluded.

Follow-up visit inclusion criteria

For the analyses of pregnant and non-pregnant visits, only scheduled quarterly follow-up visits with both laboratory and self-reported behavior data were examined; additional, off-schedule visits were excluded. Visits after HIV diagnosis for women who seroconverted were excluded as sexual and vaginal practices may have been influenced by the HIV diagnosis. In addition, visits that were more than 6 months apart were excluded (women's follow-up time was truncated at the last visit before a 6 month gap) in order to ensure that the comparison between pregnant and non-pregnant visits were in close proximity. For women with multiple pregnancies, only visits prior to and during the first pregnancy were included.

Pregnancy status

Pregnancy status was measured in two ways in the MIRA study: laboratory testing and self-report. At all quarterly study visits a urine pregnancy test was administered and women were asked during clinician interview whether they had been pregnant at any time since the last study visit, as well

as the date when they first knew they were pregnant. Women who became pregnant also reported resolution of pregnancies, including deliveries, miscarriages and terminations.

For the analysis, data from pregnancy tests and self-report of pregnancies were used to classify pregnancy status for the interval preceding each visit (pregnancy status at the visit was assigned to the interval prior to the study visit). Because this analysis is interested in changes in behaviors resulting from pregnancy, determination of pregnancy status was contingent on women having knowledge of pregnancy prior to the study visit based on the assumption that in order for women to change behavior in response to pregnancy, they must be aware of the pregnancy. Specific information on how pregnancy exposure was assigned to visits/time intervals is below.

- Visits classified as pregnant:
    - Visits with a positive lab pregnancy test *and* report of knowledge of pregnancy at least two weeks prior to visit
    - Visits occurring within first six weeks after a reported delivery date
- Visits classified as non-pregnant:
    - Any visit with a negative lab pregnancy test, including visits where women were lab negative and did not report a pregnancy and also visits where women were lab negative but did report a pregnancy between visits (including reports of miscarriages and terminations in the period between the prior and current visit)
    - Visits with positive lab pregnancy tests but no report of knowledge of pregnancy before visit (including visits with report of knowing on same day as visit or less than two weeks prior to visit); the next scheduled visit after pregnancy was coded as a pregnant visit

Visits that occurred between 6 weeks and 12 months after a reported delivery were considered post-partum and were excluded from the analysis as data suggest that sexual behaviors may change in

the period after delivery and the focus of this analysis is on comparing non-pregnant periods prior to a pregnancy and the changes that occur during pregnancy.[98,107]

Gestational age

Visits that were identified as occurring during pregnancy were further classified according to the estimated gestational age at the visit. The date of last menstrual period was not collected for the study and therefore the first date women reported knowing of their pregnancy or the first positive pregnancy test were used to estimate gestational age. Delivery dates were also used to estimate gestational age (assuming a 40 week pregnancy) for women who reported the date of delivery. Gestational age was estimated in weeks and then visits grouped by trimester with visits in the first 28 weeks of pregnancy considered to be in the first/second trimester ("early pregnancy") and visits after 28 weeks and up to 6 weeks postpartum in the third trimester ("late pregnancy") (behavior reported at visits in the first 6 weeks postpartum were considered to have occurred mainly during pregnancy for the visit interval).

Hormonal contraceptive use status

Non-pregnant visits were classified according to hormonal contraceptive use as reported by women at all quarterly study visits. Hormonal contraceptive use was categorized into three groups: oral contraception (pills) (including combined and progesterone only pills), injectables (including Depo-Provera and Net-N) and no hormonal contraceptive use which included visits where women only reported condom use, natural contraceptive methods or no contraceptive use.

Sexual risk behaviors and intra-vaginal practices

The aim of the analysis was to examine sexual risk behaviors and vaginal practices during pregnant and non-pregnant periods. Sexual behavior data was collected at enrollment and at each follow-up visit through ACASI. The information collected at each quarterly visits included the frequency of sex per week, sex without a condom, sex with regular and other partners, sex in exchange for money

or drugs, anal intercourse (with or without a condom), and new and multiple sexual partners. This information was reported by women for the interval between visits (3 months); most questions related to the entire visit interval, for instance how often they used condoms during the entire visit interval (every time, more than half the time, less than half the time, never), while some questions gave specific time frames such as condom use at their last sex act.

Reported sexual risk behaviors were examined individually as dichotomous variables. In addition, a dichotomous variable was created to identify "high risk sex" indicated if women reported any of the following: vaginal sex without a condom, exchange sex, any anal intercourse, having two or more partners and having a new sex partner. Women also reported on male sexual partner behaviors at enrollment, including having a known HIV-positive partner, a partner who was away from home for >1 month, suspecting or knowing that a partner had other sex partners, partners use of alcohol or drugs before sex and partners who forced sex. At follow-up visits, women only reported whether male partners were known or suspected to have other partners and abuse by partners. A indicator variable was created at enrollment to identify women with male partners who were "high risk", those who were HIV+, away for more than 1 month, had known or suspected other partners and those who used drugs.

Self-reported data on vaginal practices was also collected at all quarterly follow-up visits. Vaginal practices were classified into three categories: vaginal washing, wiping or insertion of products not related to menstruation. Frequency of the three types of vaginal practices as reported by women was assessed during categories for reported daily, weekly, monthly or none, and a summary variable indicating any (or no) engagement in each of the three vaginal practice behaviors.

Statistical analysis

Enrollment characteristics

Descriptive statistics were used to compare demographic characteristics and frequency of reported sexual behaviors and vaginal practices at enrollment for all women 18-45 years of age in the MIRA trial. Women with lab confirmed and known pregnancies were compared to women with no reported or lab confirmed pregnancies. Differences in characteristic and behaviors reported at enrollment were compared between women with and without pregnancies using chi-square tests for categorical variables and Wilcoxon tests for continuous variables.

Association between pregnancy status and reported behaviors at follow-up visits

Descriptive statistics were used to compare reported sexual behaviors and vaginal practices at all quarterly study follow-up visits prior to and during first pregnancies. Visits were classified as either pregnant or non-pregnant. To compare frequency of reported behaviors and in order to adjust for an unequal number of visits completed by individual study participants, logistic regression models fitted with general estimating equations (GEE) were used to measure the association between pregnancy status at a visit in order to estimate robust standard errors (for continuous variables linear regression with GEE was used). Unadjusted (univariable) logistic models were fitted with pregnancy status modeled as the independent variable and sexual risk behavior and vaginal practice as the dependent variables. Odds ratios with 95% confidence intervals are reported for the individual models run for each sexual risk behavior and vaginal practice. For modeling categorical dependent variables with more than two levels, individual logistic regression models was with GEE were fitted using a common referent group.

Association between gestational age and HC use with reported behaviors at follow-up visits

In addition to examining pregnant and non-pregnant visits, we also examined the association between sexual and vaginal practices with gestational age and HC use. For the gestational age comparison first/second trimester (early pregnancy) visits were compared to third trimester visits (late pregnancy). For the HC analysis, pregnant visits were compared to non-pregnant visits categorized

based on the type of HC reported: oral, injectable and no contraceptive use. These analyses were conducted using the same statistical approach described above.

Pre/post pregnancy analysis

A further analysis measured change across two visits for each woman, the visit just prior to her pregnancy and the first pregnant visit. For this analysis, McNemar's test for paired binomial categorical data was used to compare reported behaviors at the two visits.

Modeling risk of behaviors during pregnancy

Finally, modified Poisson regression models were fitted to estimate risk ratio of sexual risk behaviors and vaginal practices in pregnant and non-pregnant periods (modified Poisson regression was used to estimate risk ratio using a dichotomous outcome variable and to correct variance estimates for repeated measures per participant).[108] Pregnancy status was modeled as the dependent variable and sexual behaviors and vaginal practices were included as independent predictor variables in bivariate and multivariable models using an exchangeable correlation matrix. Multivariable models were adjusted for age, study randomization arm and study location (city). All statistical procedures were conducted using SAS 9.3 (SAS Institute, Cary, NC, USA).

Results

Among the 4,948 women in the final analysis of the MIRA trial, 4,801 were 18-45 years of age at enrollment and had visit data eligible for this analysis (Figure 3.1). Among women 18-45 years, 3,830 (79.8%) had no lab confirmed or reported pregnancies during follow-up, 26 (0.5%) women had only self-reported pregnancies that were never lab confirmed and 283 (5.9%) women had a lab confirmed pregnancy but no visits where they had prior knowledge of the pregnancy. Among the 662 (13.8%) women who had lab confirmed and known pregnancies, there were a total of 676 pregnancies (14 women had 2 pregnancies during follow-up) which resulted in 343 deliveries, 34 miscarriages, 5 terminations, 38 pregnancies that ended with no information about the outcome, and 206 pregnancies that were ongoing at the woman's last study visit. Among all women with lab confirmed and known pregnancies, 483 (73.0%) were included in the analysis. Of the 662, 179 (27.0%) women were excluded from the analysis for the following reasons: 56 women had no follow-up visits when they were not pregnant which was required for the crossover design; 121 women had a non-pregnant visits that were more than 6 months prior to their pregnant visit; and 2 women had a non-pregnant visits only after pregnancy (but no visits before).

Enrollment: demographic characteristics, reported sexual risk behaviors and vaginal practices for women who never got pregnant compared to those included in the analysis

Compared to the 3,829 (88.8%) of women with no pregnancies during follow-up, the 483 (10.1%) women with lab confirmed/known pregnancies included in the analysis were more likely to come from the Harare, Zimbabwe sites and were more likely to be younger (Table 3.1). The median age of women with pregnancies included in the analysis was 24 years (range 18-44 years) compared to 27 years (range 18-45) for women with no pregnancies ($p<0.0001$). Women with pregnancies were more likely to have been employed at study enrollment and less likely to have had a previous pregnancy.

Pregnant and non-pregnant women did not differ by marital status or cohabitation with male partners. (Table 3.1)

At enrollment, more women who did not have pregnancies during follow-up reported using some form of contraception. Among women with no pregnancies, 27.9% reported current use of injectable hormonal contraceptives compare to only 13.3% of women no pregnancies. Women who went on to have pregnancies were more likely to report use of combined oral contraceptive pills at enrollment. (Table 3.1) More than 70% of women in both groups reported use of a condom at the last sex act but more than 70% of women in both groups also reported some sex in the last three months without a condom. (Table 3.1) Reported male partner characteristics did not differ by group; just over 40% of women in both groups had a male partner who was at least five years older and roughly 30% of women in both groups reported knowing or suspecting male partners of having other sexual partners. Over 30% of women with and without pregnancies during follow-up reported verbal and/or physical abuse by male partners and over 36% in both groups reported sex when partners were under the influence of drugs or alcohol.

At enrollment, similar proportions of women with and without pregnancies during follow-up reported all three types of vaginal practices (Table 3.1). Over 81% of women in both groups reported any vaginal washing, including 61% who reported daily washing. Just over half of women in both groups reported vaginal wiping, with roughly 40% reporting wiping as a daily activity. About half of women in both groups reported any vaginal insertion of cotton or other fabric and paper products not for menstruation but few reported daily insertion.

At enrollment low prevalence of gonorrhea (<1% in both groups) was observed but almost 4% of women in both groups were infected with trichomoniasis at enrollment. Slightly more women who had pregnancies during follow-up were infected with chlamydia at enrollment, 5.8% (vs. 4.2% of women

with no pregnancies), however this did not reach the level of significance (p=0.09). Overall 57.7% of women had positive serological tests for HSV-2 at enrollment; significantly more women with no pregnancies during follow-up were HSV-2 positive at enrollment (59.0% vs. 48.7%; p<0.0001).

In an additional analysis shown in Appendix 3.1, enrollment characteristics of women who were excluded from the analysis were compared to those who did not have pregnancies and those women who did have pregnancies during follow-up. The group of excluded women is a mixture of women who reported pregnancies and/or pregnancy outcomes between visits but never had a lab confirmed pregnancy, those with lab confirmed pregnancies who never had a visit with knowledge of the pregnancy and those who had large gaps between visits or no non-pregnant visits prior to a pregnancy. The excluded women were generally more similar to the women with pregnancies with regard to age and parity but were the least likely to be married of the three groups. (There were no differences in reported sexual behaviors or vaginal practices between those included and those excluded from the analysis (Appendix 3.1)).

Association between pregnancy status and behaviors reported at follow-up visits

The analysis comparing non-pregnant and pregnant visits attended by 483 women with lab confirmed/known pregnancies and at least one non-pregnant visit included 2,540 follow-up visits: 904 (35.6%) during a pregnancy; 1.636 (64.4%) visits when the woman was not pregnant (Table 3.2). The median time between visits was 91 days [interquartile range (IQR): 90-95]; the median follow-up time for women (using visits included in the analysis) was 15 months [IQR: 12-21]. The median number of visits per woman (among visit included in the analysis) was 5 (range 2-8); and the median number of visits women had while pregnant was 2 (range 1-4) and while not pregnant was 3 (range 1-7).

At almost all quarterly study visits, regardless of pregnancy-status, women reported some sexual activity, however women were less likely to report more than 3 sex acts per week when they

were pregnant (odds ratio (OR) 0.8; 95%CI 0.7-0.9). During pregnancy, women were more likely to report last sex without a condom (OR 0.8; 95%CI 0.7-0.9) and any unprotected sex (without a condom) in the past three months (OR 1.4, 95%CI 1.2-1.7). There were lower reports of other risk factors during pregnancy, including anal sex, having two or more sexual partners and having a new sexual partner in the past three months (Table 3.2). Overall, during pregnancy women were less likely to report high risk sex (indicated by any of the following: ≥2 sex partners, a sex partner, exchange sex or sex under the influence of drugs or alcohol) (OR 0.6, 95%CI 0.5-0.7) (Table 3.2). Differences in vaginal practices were observed during pregnant and non-pregnant periods; women were significantly less likely to report any vaginal washing, wiping and insertion of products during non-pregnant periods. In particular, vaginal wiping on a daily, weekly and monthly basis were lower during pregnancy; compared to non-pregnant periods, the odds of daily wiping during pregnancy was 30% lower (OR 0.7, 95%CI 0.6-0.8).

Association between gestational age with behaviors reported at follow-up visits

In an additional analysis, pregnant visits were categorized according to gestational age and differences in reported sexual behaviors and vaginal practices reported in the first and second trimester (early pregnancy) were compared to those reported in the third trimester (late pregnancy). Of the 904 pregnant visits included in the analysis, 492 (54.4%) were estimated to have occurred during the first/second trimester and 412 (45.6%) occurred during the third trimester (Table 3.3). 228 (47.2%) women had both an early and late pregnancy visit, 174 (36.0%) had only a first/second trimester visits and 81 (46.8%) had only a third trimester visit.

Compared to visits in early pregnancy, during the third trimester women reported less sex at visits, including report of any sex between visits (OR=0.39; 95%CI 0.22-0.72) and fewer reported ≥3 sex acts per week (OR=0.6; 95%CI 0.5-0.7). During the third trimester women were also more likely to report sex without a condom between visits compared to visits earlier in pregnancy (Table 3.3). There were no

other differences in sexual risk behaviors between early and late pregnancy, nor were any differences observed in vaginal practices.

Association between hormonal contraceptive use and behaviors reported at follow-up visits

In another additional analysis, non-pregnant visits were categorized according to women's reported hormonal contraceptive (HC) use and were compared to pregnant visits. Of the 2,540 visits included in the analysis, 1636 (64.4%) occurred when women were not pregnant; at 504 of the non-pregnant visits (33.2%) women reported using oral contraceptives (including both combined and progesterone only pills), at 165 visits (10.1%) women reported that they were using injectable HC and at 928 (56.7%) of visits women reported using no HC (these visits were compared to the 904 visits that occurred during a pregnancy).

Compared to visits during pregnancy, women were more likely to report any sex and $\geq$3 sex acts per week at non-pregnant visits when they were using oral contraceptives (OR 2.1; 95%CI 1.3-3.4) (Table 3.4). This differed from visits with report of injectable HC use where women were less likely to report any sex between visits compared to pregnant visits (OR=0.6; 95%CI 0.3-1.0). No difference was observed in any sex or sexual frequency between pregnant visits and non-pregnant visits where women were not using HC. Visits when women were using oral contraceptives and those where no HC was used included fewer reports of any sex without a condom in the previous three months compared to pregnant visits (OR for oral contraceptive visits=0.7; 95%CI 0.6-0.9). Visits with injectable HC use did not differ from pregnant visits with regard to report of unprotected sex. Compared to pregnant visits, reports of other sexual risk behaviors, including anal sex, having had more than one sex partner between visits and indication of any high risk sexual behaviors was significantly more common at all non-pregnant visits, particularly those with reported use of injectable HC (OR 2.2;95%CI 1.4-3.4) (Table 3.4).

Vaginal washing appeared to be more common at visits when women were using oral contraceptives compared to pregnant visits overall and on a daily and monthly basis, however, visits with injectable HC and no HC use did not differ from pregnant visits with regard to report of vaginal washing (Table 3.4). At visits when women were using oral or injectable HC, there were more reports of vaginal wiping (OR=1.8;95%CI 1.3-2.4) and they were much more likely to report insertion of materials (OR=1.7;95CI 1.0-2.7). At visits with women were not using any HC, there were only differences observed in vaginal wiping compared with pregnant visits (Table 3.4).

Pre/post pregnancy analysis

As a further examination of the changes to sexual risk behaviors and vaginal practices during pregnancy, we conducted a matched analysis of paired data for each woman comparing the last non-pregnant visit just prior to the first pregnant visit (Figure 3.2). There were significant changes observed in reporting of any sex without a condom; 15.7% of women who had not reported sex without a condom before pregnancy reported sex without a condom at the first visit during pregnancy ($p<0.05$). During pregnancy there appeared to be decreased anal sex and in having more than one sexual partner between visits, meaning that significantly more women changed from reporting these behaviors prior to pregnancy and not reporting them during pregnancy (compared to women who stayed the same in reporting or increased reporting of behaviors during pregnancy). More women also changed from reporting some type of high risk sex when not pregnant to reporting no high risk sex when pregnant ($p<0.01$). Pregnancy did not appear to change vaginal washing or insertion but did appear to decrease vaginal wiping ($p=0.05$).

Modeling risk of behaviors during pregnancy

In regression models adjusted for age, diaphragm randomization arm, city, it appeared that being pregnant was not associated with report of any sex in last 3 months but was associated with less

frequent sex (adjusted risk ratio (ARR) sex ≥3 times per week 0.9;95%CI 0.8-0.9) (Table 3.5). Pregnancy was associated with less reported condom use at last sex (ARR=0.8, 95%CI 0.7-0.9) but more reports of any unprotected sex (ARR 1.3; 95%CI 1.2-1.5). Pregnancy was also associated with less reporting of anal sex (ARR 0.7, 95%CI 0.5-0.9) and lower risk of any indication of high risk sex (ARR 0.7;95%CI 0.6-0.9). Pregnancy was not associated with lower risk of vaginal washing or insertion of materials but was found to decrease the risk of vaginal wiping on a daily and weekly basis; pregnancy was associated with 26% lower risk of any vaginal wiping (ARR 0.8; 95%CI 0.8-0.9).

Discussion

This analysis examined changes in self-reported sexual behaviors and vaginal practices during pregnancy in order to understand pregnancy's impact on potential risk factors for STI acquisition. We found that pregnancy tended to decrease sexual activity, particularly in the third trimester. We also observed that compared to non-pregnant periods, there was more vaginal sex without condoms reported during pregnancy but lower frequency of other risk factors for STI acquisition, including anal sex, concurrent sexual relationships and new sex partners. Vaginal wiping and insertion of material into the vagina, potentially important mechanisms for STI acquisition, were also less common during pregnancy. In a further examination of non-pregnant periods using pregnancy as a common reference group for comparisons, we found that, although the number of visits with injectable HC use was low, high risk sexual behaviors (anal sex, exchange sex, concurrency, new partners and drugs and alcohol use during sex) were reported most often when women were using injectable hormonal contraception, compared to oral contraceptives and no HC use. The association between vaginal practices differed depending on the type of HC use. At visits when women were using oral contraceptives, they were significantly more likely to report all types of vaginal practices compared to pregnant visits. For visits with injectable HC, vaginal wiping and insertion were more common than during pregnancy and only vaginal wiping was associated with non-pregnant visits where women were using no HC. There are few previous examinations of changes to sexual risk behaviors for STIs during pregnancy and no previous analyses of the impact of pregnancy on vaginal practices. These data are therefore an important contribution to developing better understanding of women's risk for STIs during pregnancy.

At enrollment in the randomized clinical trial from which woman in our analysis were selected, there were few demographic and behavioral differences distinguishing women who went on to have pregnancies from those who did not have pregnancies during study follow-up. Women who had a pregnancy were somewhat younger at enrollment and less likely to have children, but equally likely to

be married and to live with male partners. There were few differences in reported sexual behaviors and vaginal practices with similarly high proportions of women in both groups reporting unprotected sex, sex in exchange for drugs or money, having older partners and having high risk partners. While 70% of all women reported using a condom at last sex act, 70% of women also reported unprotected sex in the previous three months. Women who did not have a pregnancy during follow-up were significantly more likely to be infected with HSV-2 which was expected based on their older age which is associated with higher HSV-2 prevalence [2]; there were no differences in other STIs at enrollment. A high proportion of all women (33%) reported physical or verbal abuse by male partners, including being threatened with a weapon. This finding is in keeping with previous reports of high levels of intimate partner violence from South Africa and Zimbabwe.[109-112]

In the analysis of behavior change during pregnancy, our findings suggest that sexual activity decreases, particularly in the third trimester, and that sex not protected by a condom is more commonly reported during pregnancy. Both findings are consistent with previous studies from resource limited settings.[30-33,35,98] We also found that while unprotected sex may be more common during pregnancy compared to non-pregnant periods, other risk behaviors, such as anal sex and having multiple concurrent sexual partners occur less during pregnancy. These findings are also in keeping with previous studies examining HIV risk during pregnancy [31-33,35] and highlight the complexities of estimating STI risk factors during pregnancy. Lower frequency of sexual activity and fewer additional sexual partners would suggest that women might have lower risk of acquiring STIs during pregnancy. In addition, women who become pregnant may be in more stable partnerships (we did not examine changes in relationship stability over time but this has been reported previously)[113]. The lower rates of condom use among pregnant women could however increase exposure to STIs, and risk could be further increased if male partners have concurrent relationships during pregnancy. Changes to male partner sexual behaviors

during pregnancy have only been measured in a small number of studies which have not shown an increase in partner concurrency during pregnancy.[35,98]

Similar to other studies, at more than 60% of all follow-up visits women reported using a condom at the last sex act, including during pregnancy, however sex without a condom at any time between the visits was also reported frequently (over 68% of follow-up visits) regardless of pregnancy status. These findings indicate inconsistency in condom use which is one of the most important risk factors for STI acquisition. The women in this analysis were enrolled in an HIV prevention trial and were counseled at all study visits, including those during pregnancy, to use condoms for HIV and STI prevention. Despite counseling, most women reported some unprotected sex which increased significantly during pregnancy. There has been little study of condom use during pregnancy, including qualitative studies on women's understanding of continued risk for STIs in the absence of contraceptive concerns, and few evaluations of efforts to improve condom use during pregnancy. Greater efforts are needed to support women's consistent condom use for those in relationships with positive men or men with unknown STI status and to ensure they understand the continued risk of STI acquisition during pregnancy.

Potential bias in reporting of sexual behaviors is an important consideration when examining evidence from analyses such as this. Self-report of sexual behaviors, particularly with regard to use of condoms, has been shown to be an unreliable marker of risk.[114,115] A study from Zimbabwe showed no association between reported condom use by women over two years of follow-up and HIV incidence.[116] In an analysis of 910 participants from MIRA trial sites in Zimbabwe, prostate specific antigen (PSA) in vaginal secretions was used as a biomarker of recent unprotected sex to validate self-reported condom use. The analysis found that almost half of the women with measureable PSA reported that they had not had any unprotected sex in the previous two days.[117] A recent review of the studies using biological validation of self-reported HIV risk behaviors estimated that on average about 38% of participants over

report condom use.[118] Different reasons are put forward to explain the discrepancies found between self-report and biological markers of risk behaviors, including poor recall, misunderstanding of questions and deliberate misreport of behaviors as a result of social desirability bias.[115]

The potential misclassification resulting from over reporting of condom use is concerning. This type of bias would generally be presumed to be *non-differential*, i.e. not systematically different based on pregnancy status, and would lead to an estimated effect that would be biased towards the null, in other words, the misclassification would mask any impact of pregnancy on risk behaviors. It is also possible that the misclassification could be *differential* based on pregnancy status, for instance if pregnant women were more accurate in reporting condom use while non-pregnant women over reported condom use, this would lead to a more concerning bias as it would suggest that pregnant women have more unprotected sex whereas they may in fact report accurately while non-pregnant women under report unprotected sex creating an effect of pregnancy where no, or potentially the opposite, effect exists. This potential bias could explain the effect of pregnancy that has been observed in several studies showing increased unprotected sex during pregnancy (including ours). Alternatively, pregnant women could be less accurate in reporting other risk behaviors, including anal sex, multiple partners and exchange sex which could be an explanation for the lower report of these behaviors during pregnancy. Unfortunately there are no existing studies that have tried to measure this type of potential bias by validating self-reported behaviors among pregnant women.

Our analysis also found some differences in sexual behaviors based on the type of hormonal contraceptives that women were using. The comparison of reported behaviors across HC types to pregnancy status was conducted as a result of conflicting evidence regarding the impact of HC use on STI risk. Some studies have shown progesterone-only HCs to be associated with increased risk of infections including chlamydia [51], gonorrhea [119] and HIV [120] however evidence, specifically in regard to HIV, is controversial and as yet inconclusive.[121-124] In this analysis, we observed that women tended to report

high risk sex more frequently when they were using injectable HC compared to periods when they were pregnant or using oral contraceptives, however the number of visits when women were using injectable HC was low (in part because we excluded postpartum visits when women may have been more likely to be using this form of contraceptive)[125]. These results suggest that women's behavior during non-pregnant periods may vary depending on what type of HC type they are using. This has bearing for examinations of STI risk during pregnancy as comparisons to non-pregnant periods must take HC use into account in order to properly examine and adjust for risk behaviors.

Vaginal practices were commonly reported by women in this analysis which is in keeping with other studies from sub-Saharan Africa. Vaginal washing was the most commonly reported practice with daily washing reported at over half of all visits, regardless of pregnancy status and any vaginal washing reported at over three quarters (77%) of all follow-up visits. Vaginal wiping and insertion of cloth, cotton or paper for non-menstruation related reasons were less common but any engagement in these practices was reported at 42% and 12% of visits, respectively. A strong association was observed for decreased vaginal practices, all three types, during pregnancy. This is the first report comparing vaginal practices during pregnant and non-pregnant periods in the same women and as such there are no other data with which to compare it. The decrease in vaginal practices could be a result of less sexual activity during pregnancy or as a result of reduced frequency of exchange sex which has been found to be associated with intra-vaginal practices.[100] It is also possible that the reduction in vaginal practices during pregnancy was in part caused by the general decline in these behaviors over study follow-up as reported by van der Straten et al.[126] Pregnancies that occurred later in the study may have had lower report of vaginal practices not as a result of pregnancy but potentially as a result of this general trend. The reasons for changes in vaginal practices observed during pregnancy warrants further study as they are an important risk factor for STI acquisition and may also impact pregnancy outcomes. While douching has been found to be associated with increased risk of ectopic pregnancy and other adverse

reproductive health outcomes,[127] the effect of other vaginal practices (wiping, insertion of other substances and materials) have not been studied.

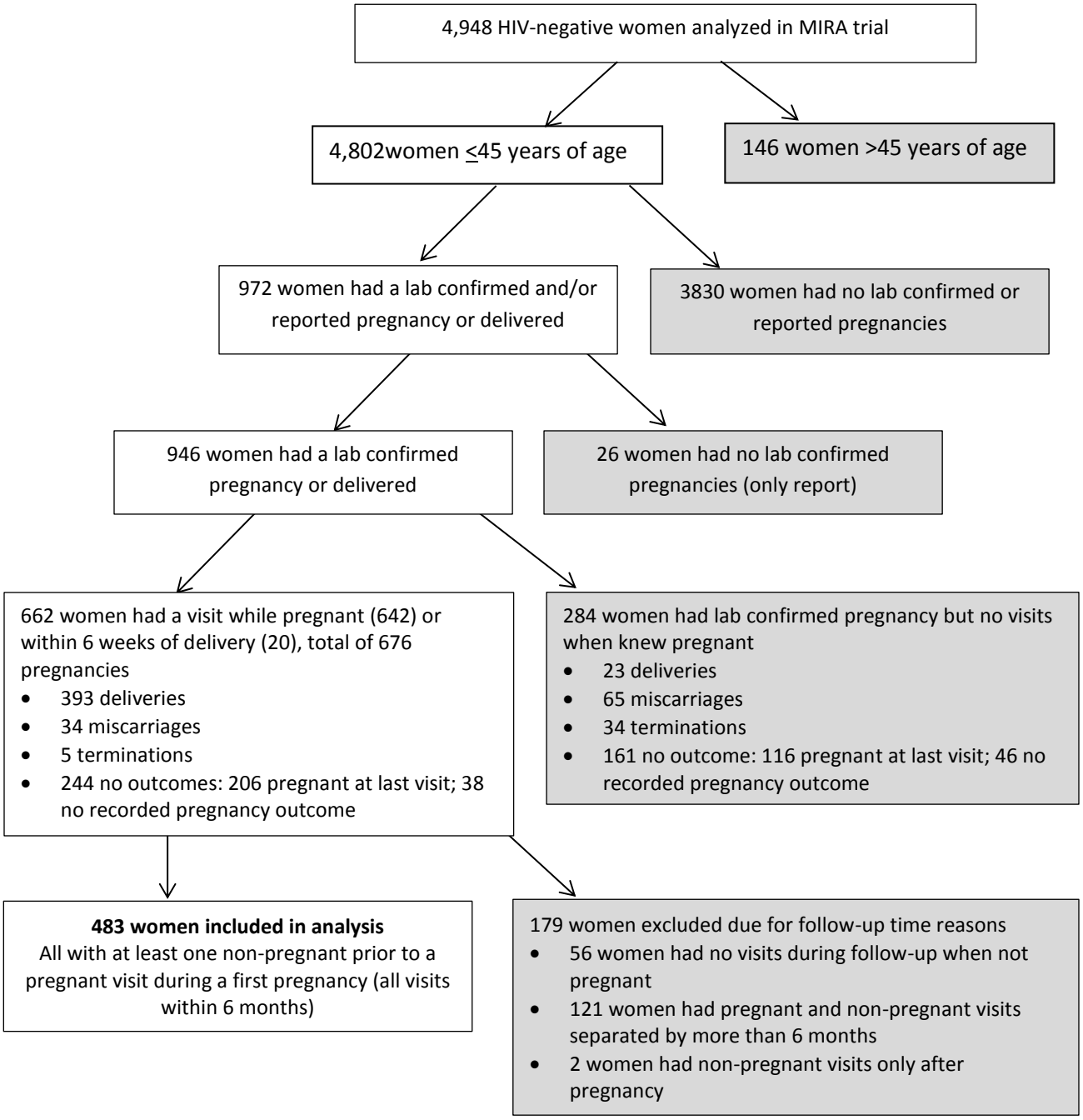
This analysis included more than 480 women with both pregnant and non-pregnant follow-up time. The data came from a randomized clinical trial with rigorous follow-up and data collection procedures. Data on sexual behaviors and vaginal practices were collected primarily through ACASI which may have contributed to more valid responses.[117] This is one of the only studies to examine sexual behaviors during pregnancy and one of the few to compare data from pregnant and non-pregnant periods in the same women. The methods used were also a strength of the analysis, including a cross over design with women serving as their own controls which adjusted for factors and characteristics of women which may be associated with having a pregnancy and could not be easily adjusted for had we compared women with pregnancies to those without pregnancies. We examined changes in behavior using several different analytic approaches in order to accurately capture change at all visits and in specific time periods.

There are several important limitations of the analysis. In addition to the issues discussed above relating to validity of self-report, there were also challenges with accurately classifying pregnancy exposure periods and gestational age. For the analysis we considered visits to be during a pregnancy only if the woman knew about the pregnancy prior to the visit in order to measure behavior change from pregnancy. It is possible that some women may not have accurately reported when they knew about their pregnancies which could have led to misclassification of visits. There were also challenges with regard to visits with reported miscarriages and terminations; visits where women were not pregnant according to lab testing but reported these events were considered non-pregnant as the date of the end of the pregnancy was not ascertained in the study. It is possible that women were pregnant during some part of these periods and their behavior may have been different as a result. In both instances noted above, the resulting misclassification would have been non-differential and would have

attenuated the observed effect of pregnancy in changing sexual risk behaviors and vaginal practices. We also did not have data on last menstrual period and thus the estimates of gestation age are not based on when the woman became pregnant but rather when she knew or had a lab positive pregnancy test. This may have led to misclassification of gestational age and most likely results in women who were farther along in pregnancy being considered first or second trimester rather than third. Another limitation of the data was not having any information reported by male partners which is an important risk factor for women. Further study is needed of men's behavior during pregnancy and whether they may engage in more concurrency which could increase women's STI risk during this this period.

These data provide important insights into the sexual risk behaviors and vaginal practices of pregnant women. The inconsistency of condom use found among women during non-pregnant periods and the high frequency of unprotected sex during pregnancy is evidence that greater efforts are needed to improve consistent condom use among women in high STI/HIV prevalence settings. Targeted efforts to increase condom use for STI prevention during pregnancy have not been studied but may be needed to prevent infections that pose threats to the health of pregnant women and their infants. Studies are also needed to understand the impact of vaginal practices that are common in sub-Saharan Africa on women's health and pregnancy outcomes, as well as on STI risk. The analysis highlights some of the conflicting evidence and complexities of understanding and estimating women's risk for STI's during pregnancy.

Figure 3.1 Patient flow diagram



5,045 women were randomized in the trial: 3 were excluded because they were underage, 3 women discontinued on date of randomization, 72 had no HIV results post-enrollment and 19 were found to have been infected with HIV at baseline (after testing positive during follow-up)

Table 3.1 Characteristics at enrollment by pregnancy status during follow-up, N=4,313

Characteristics at enrollment	Total		Women with no pregnancies		Women with lab confirmed & known pregnancies in analysis		p value
	N	%	N	%	N	%	
	4313	100.0%	3830	88.8%	483	11.2%	
Study location							
Harare	2155	50.0%	1881	49.1%	274	56.7%	0.01
Durban	1289	29.9%	1165	30.4%	124	25.7%	
Johannesburg	869	20.2%	784	20.5%	85	17.6%	
Age							
Median age (range)	27	(18-45)	27	(18-45)	24	(18-44)	<0.0001
18-24	1635	37.9%	1388	36.3%	247	51.1%	<0.0001
25-34	1751	40.6%	1552	40.5%	199	41.2%	
≥35	927	21.5%	890	23.2%	37	7.7%	
Completed high school (11 yrs school)	2263	51.5%	1987	51.9%	276	57.1%	0.03
Paid employment	985	22.8%	888	23.2%	97	20.1%	0.13
Married	2546	61.3%	2250	58.8%	296	61.3%	0.29
Lives with male partner	2928	67.9%	2591	67.7%	337	69.8%	0.42
Number of previous pregnancies							
None	357	8.3%	294	7.7%	63	13.0%	<0.0001
1 previous pregnancies	1373	31.8%	1178	30.8%	195	40.4%	
2+	2583	59.9%	2358	61.6%	225	46.6%	
Current birth control (not mutually exclusive)							
Combined pills	940	21.8%	791	20.7%	149	30.9%	<0.0001
Injectable hormones	1132	26.3%	1068	27.9%	64	13.3%	<0.0001
Progesterone only pills	660	15.3%	600	15.7%	60	12.4%	0.06
Intrauterine device	16	0.4%	15	0.4%	1	0.2%	0.77
Condoms (male or female) (only)	1635	37.9%	1453	37.9%	182	37.7%	0.92
None (natural, withdrawal, tradition)	230	5.3%	192	5.0%	38	7.9%	0.01
Regular sexual partner	1763	40.9%	1577	41.2%	186	38.5%	0.37
Age at first sex (mean and range)	18	(10-31)	18	(10-31)	18	(11-26)	0.75
Sex acts per week							
None	1732	40.2%	1522	39.7%	210	43.5%	0.10
1-2 per week	264	6.1%	229	6.0%	35	7.3%	
≥3 per week	2317	53.7%	2079	54.3%	238	49.3%	
Number of sexual partners in past 3 months							
None	250	5.8%	239	6.3%	11	2.3%	0.00
1 partner	3694	85.9%	3268	85.6%	427	88.4%	
2+	357	8.3%	312	8.2%	45	9.3%	
Condom use (male or female) at last sex	3040	70.5%	2701	70.5%	339	70.2%	0.88
Condom use (vaginal sex) in last three months							
Never	1259	29.3%	1123	29.4%	136	28.2%	0.69
Sometimes	1672	38.9%	1487	38.9%	185	38.3%	
Every time	1371	31.9%	1209	31.7%	162	33.5%	
Exchange for money or drugs past 3 months	344	8.0%	306	8.0%	38	7.9%	0.92

Table 3.1 continued

Characteristics at enrollment	Total		Women with no pregnancies		Women with lab confirmed & known pregnancies		p value
	N	%	N	%	N	%	
Anal sex (ever)	629	14.6%	550	14.4%	79	16.4%	0.25
Sex while intoxicated	156	26.9%	136	26.8%	20	27.8%	0.86
High risk (>2 partners, exchange, anal, sex w/ drugs/alcohol)	1002	23.2%	879	23.0%	123	25.5%	0.22
Partner age							
Same age (+/-5 years)	2391	58.0%	2114	57.8%	277	58.9%	0.21
Younger (more than 5 years)	39	1.0%	38	1.0%	1	0.2%	
Older (more than 5 years)	1694	41.1%	1502	41.1%	192	40.9%	
Partner tested HIV positive							
Yes	150	3.5%	136	3.6%	14	2.9%	0.45
No/don't know	4149	96.5%	3680	96.4%	469	97.1%	
Partner away from home >2 months	472	10.9%	425	11.1%	47	9.7%	0.37
Partner circumcised	927	21.6%	821	21.5%	106	22.0%	0.57
Suspects/knows male partner concurrency	1302	30.3%	1164	30.5%	138	28.6%	0.39
Physical or verbal abuse	789	33.3%	715	33.3%	74	33.0%	0.94
Partner used force to get sex	234	9.9%	213	9.9%	21	9.4%	0.80
Sex w/ partner w/ drugs or alcohol	1547	36.1%	1368	36.0%	179	37.1%	0.62
Partner high risk (HIV+, away >1 month, other partners, drugs)	2685	62.2%	2386	62.3%	299	61.9%	0.87
Vaginal washing							
Daily	2632	61.2%	2331	61.1%	301	62.3%	0.54
Weekly	623	14.5%	562	14.7%	61	12.6%	
Monthly or less	299	7.0%	268	7.0%	31	6.4%	
None	747	17.4%	657	17.2%	90	18.6%	
ANY	3566	82.7%	3173	82.9%	393	81.4%	0.41
Vaginal wiping							
Daily	1741	40.5%	1546	40.5%	195	40.4%	0.50
Weekly	418	9.7%	362	9.5%	56	11.6%	
Monthly or less	267	6.2%	237	6.2%	30	6.2%	
None	1875	43.6%	1673	43.8%	202	41.8%	
ANY	2438	56.5%	2157	56.3%	281	58.8%	0.44
Vaginal insertion (non-menstruation related)							
Daily	560	13.0%	502	13.2%	58	12.0%	0.87
Weekly	144	3.4%	126	3.3%	18	373.0%	
Monthly or less	178	4.1%	158	4.1%	20	4.1%	
None	3415	79.5%	3028	79.4%	387	80.1%	
ANY	898	20.8%	802	21.0%	96	19.9%	0.59
Diaphragm arm	2132	49.4%	1901	49.6%	231	47.8%	0.45
Positive for STI (enrollment)							
Chlamydia	187	4.3%	159	4.2%	28	5.8%	0.09
Gonorrhea	33	0.8%	30	0.8%	3	0.6%	0.70
Trichomoniasis	157	3.6%	137	3.6%	20	4.1%	0.53
HSV-2	2492	57.8%	2257	59.0%	235	48.7%	<0.0001

Table 3.2 Risk behaviors reported at 2,540 follow-up visits (unadjusted frequencies) and association (odds ratio) with pregnancy status, N=483 women (odds ratios adjusted for clustering with GEE)

Reported behaviors at follow-up	Frequency of report at all visits		Frequency of report at non-pregnant visits		Frequency of report at pregnant visits		Odds ratio pregnant vs. non-pregnant periods		
	N	%	N	%	N	%	OR	95%CI	p value
	2540	100.0%	1636	64.4%	904	35.6%			
Any vaginal sex since last visit	2409	94.8%	1560	95.4%	849	93.9%	0.9	0.6-1.2	0.40
Vaginal sex frequency per week									
mean (range)	3.6 (0-10)		3.8 (0-10)		3.4 (0-10)		**0.7**	**0.6-0.9**	**<0.0001**
≥3 times per week	1607	69.4%	1022	71.2%	585	66.5%	**0.8**	**0.7-0.9**	**<0.01**
Condom use at last sex	1654	68.7%	1103	70.7%	551	64.9%	**0.8**	**0.7-0.9**	**0.02**
Unprotected sex (any report)	1646	68.3%	1018	65.3%	628	74.0%	**1.4**	**1.2-1.7**	**<0.01**
Anal sex since last visit	136	5.4%	104	6.4%	32	3.5%	**0.5**	**0.4-0.8**	**<0.01**
Sex in exchange for money or drugs	79	3.3%	56	3.6%	23	2.7%	0.8	0.6-1.1	0.18
≥2 male sex partners since last visit	156	6.5%	130	8.3%	26	3.1%	**0.3**	**0.2-0.5**	**<0.0001**
New sex partner	264	10.9%	188	12.0%	76	9.0%	**0.7**	**0.5-0.9**	**<0.01**
Suspects/knows male partner concurrency	573	23.7%	384	24.5%	189	22.3%	0.9	0.8-1.1	0.16
High risk sex (exchange, anal sex, >2 partners, sex with drugs/alcohol)	431	17.0%	316	19.3%	115	12.7%	**0.6**	**0.5-0.7**	**<0.0001**
Vaginal washing									
Daily	1424	56.1%	914	55.9%	510	56.4%	**0.8**	**0.7-0.9**	**0.01**
Weekly	306	12.1%	200	12.2%	106	11.7%	0.9	0.7-1.1	0.17
Monthly or less	224	8.8%	162	9.9%	62	6.9%	**0.7**	**0.6-0.9**	**<0.01**
None (ref)	586	23.1%	360	22.0%	226	25.0%	1.0	-	-
ANY	1954	76.9%	1276	78.0%	678	75.0%	**0.8**	**0.7-0.9**	**<0.01**
Vaginal wiping									
Daily	795	31.3%	543	33.2%	525	27.9%	0.7	**0.6-0.8**	**<0.0001**
Weekly	158	6.2%	113	6.9%	45	5.0%	0.6	**0.5-0.8**	**<0.01**
Monthly or less	117	4.6%	82	5.0%	35	3.9%	0.6	**0.5-0.9**	**<0.01**
None (ref)	1470	57.9%	898	54.9%	572	63.3%	1.0	-	-
ANY	1070	42.1%	738	45.1%	332	36.7%	**0.7**	**0.6-0.8**	**<0.0001**
Vaginal insertion (non-menstruation related)									
Daily	173	6.8%	118	7.2%	55	6.1%	0.8	0.6-1.0	0.06
Weekly	50	2.0%	35	2.1%	15	1.7%	0.7	0.4-1.2	0.22
Monthly or less	75	3.0%	54	3.3%	21	2.3%	0.7	0.5-1.1	0.09
None (ref)	2242	88.3%	1429	87.4%	813	89.9%	1.0	-	-
ANY	298	11.7%	207	12.7%	91	10.1%	**0.7**	**0.6-0.9**	**0.02**

Table 3.3 Risk behaviors reported at 904 pregnant visits (unadjusted frequencies) and association (odds ratio) with gestational age at pregnant visit, N=483 women (odds ratios adjusted for clustering with GEE)

Reported behaviors at follow-up	Total		Frequency of report at 1st/2nd trimester visits		Frequency of report at 3rd trimester visits		Odds ratio 1st/2nd vs. 3rd trimester periods		
	N	%	N	%	N	%	OR	95%CI	p value
	904	100.0%	492	54.4%	412	45.6%			
Any vaginal sex since last visit	849	93.9%	473	96.1%	376	91.3%	**0.4**	**0.2-0.7**	**0.0024**
Vaginal sex frequency per week									
mean (range)	3.4 (0-10)		3.6 (0-10)		3.2 (0-10)		**0.6**	**0.5-0.8**	**<0.0001**
≥3 times per week	585	66.6%	341	71.6%	244	60.6%	**0.6**	**0.5-0.7**	**<0.0001**
Condom use at last sex	551	64.9%	298	63.0%	253	67.3%	1.2	1.0-1.6	0.09
Unprotected sex (any report)	628	74.0%	364	77.0%	264	70.2%	**0.7**	**0.5-0.9**	**0.02**
Anal sex since last visit	872	96.5%	476	96.8%	396	96.1%	1.2	0.6-2.3	0.58
Sex in exchange for money or drugs	23	2.7%	9	1.9%	14	3.7%	1.7	0.9-3.4	0.11
≥2 male sex partners since last visit	26	3.1%	15	3.2%	11	2.9%	0.8	0.5-1.2	0.23
New sex partner	76	9.0%	42	8.8%	34	9.0%	0.9	0.6-1.4	0.63
Suspects/knows male concurrency	189	22.3%	113	23.9%	76	20.2%	0.8	0.6-1.0	0.06
High risk sex (exchange, anal sex, >2 partners, sex with drugs/alcohol)	115	12.7%	59	12.0%	56	13.6%	1.1	0.8-1.6	0.64
Vaginal washing									
Daily	510	56.4%	275	55.9%	235	57.0%	1.0	0.8-1.2	0.90
Weekly	106	11.7%	61	12.4%	45	10.9%	0.9	0.7-1.2	0.56
Monthly or less	62	6.9%	38	7.7%	24	5.8%	0.8	0.5-1.2	0.29
None (ref)	226	25.0%	118	24.0%	108	26.2%	1.0	-	-
ANY	678	73.8%	374	76.0%	304	73.8%	1.0	0.8-1.2	0.65
Vaginal wiping									
Daily	252	27.9%	135	27.4%	117	28.4%	0.9	0.7-1.2	0.54
Weekly	45	5.0%	24	4.9%	21	5.1%	1.2	0.7-1.8	0.51
Monthly or less	35	3.9%	19	3.9%	16	3.9%	1.0	0.6-1.9	0.92
None (ref)	572	63.3%	314	63.8%	258	63.3%	1.0	-	-
ANY	332	36.7%	178	36.2%	154	37.4%	1.0	0.8-1.2	0.82
Vaginal insertion (non-menstruation related)									
Daily	55	6.1%	27	5.5%	28	6.8%	1.2	0.8-1.8	0.50
Weekly	15	1.7%	7	1.4%	8	1.9%	1.4	0.6-3.8	0.45
Monthly or less	21	2.3%	13	2.6%	8	1.9%	0.7	0.3-1.8	0.51
None (ref)	813	89.9%	445	90.5%	368	89.3%	1.0	-	-
ANY	91	10.1%	47	9.6%	44	10.7%	1.1	0.8-1.7	0.52

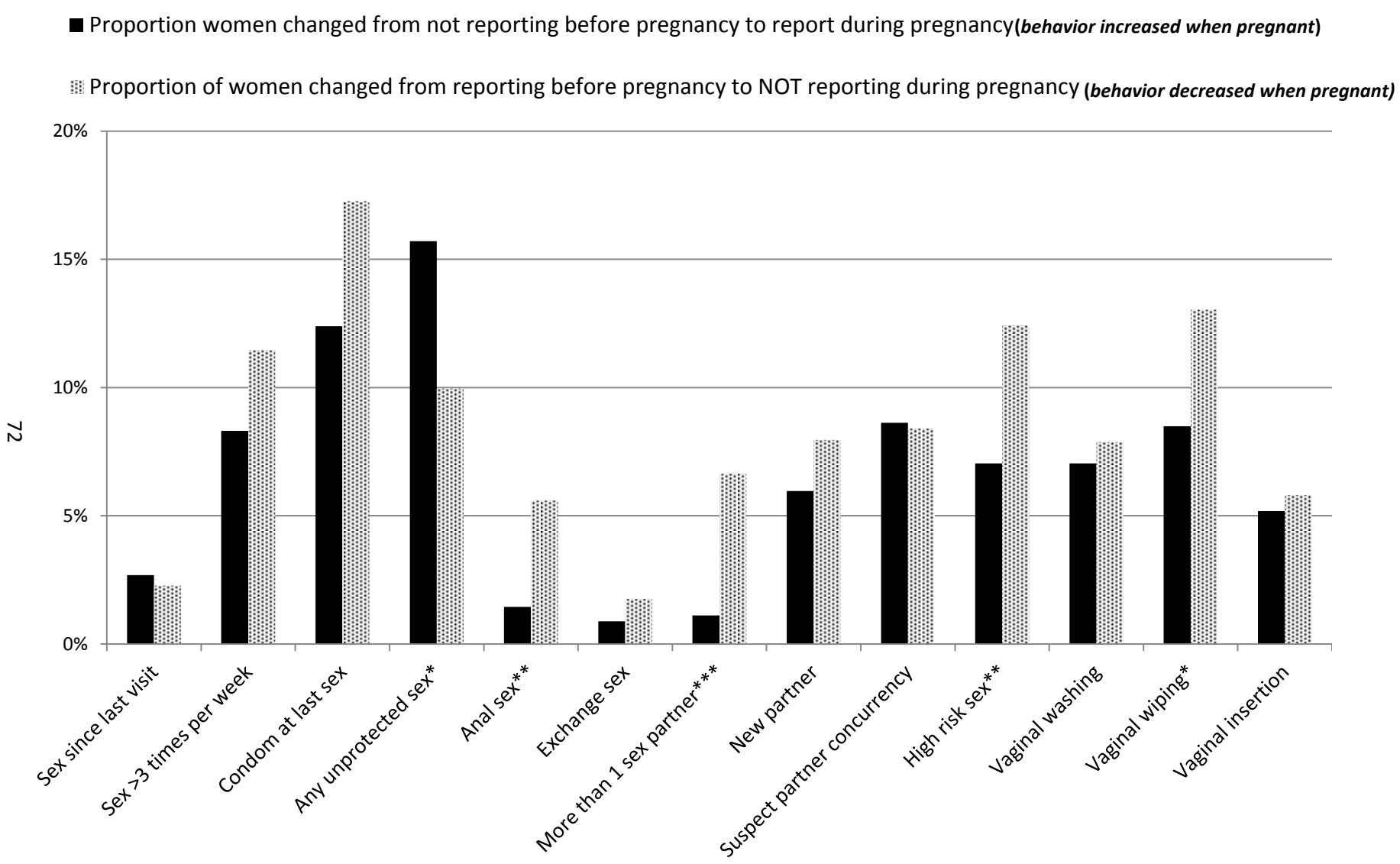
Table 3.4 Risk behaviors reported at 2,540 follow-up visits (unadjusted frequencies) and association (odds ratio) between hormonal contraceptive type and pregnant status, N=483 women (odds ratios adjusted for clustering with GEE)

Reported behaviors at follow-up	Total		Frequency of report at oral HC use visits		Frequency of report at injectable HC use visits		Frequency of report at no HC use visits		Frequency of report at pregnant visits	
	N	%	N	%	N	%	N	%	N	%
	2540	100.0 %	543	21.4%	165	6.5%	928	36.5%	904	35.6%
Any vaginal sex since last visit	2409	94.8%	534	98.3%	148	89.7%	878	94.6%	849	93.9%
Vaginal sex frequency per week										
mean (range)* (not or)	3.6 (0-10)		4.2 (0-10)		3.3 (0-10)		3.6 (0-10)		3.4 (0-10)	
≥3 times per week	1607	69.4%	1357	79.0%	102	68.5%	563	67.4%	585	66.6%
Condom use (male or female) at last sex	1654	68.7%	374	70.0%	97	65.5%	632	72.0%	551	64.9%
Unprotected sex (any report)	1646	68.3%	361	67.6%	105	71.0%	552	68.0%	74	74.0%
Anal sex since last visit	136	5.4%	34	6.3%	18	10.9%	52	5.6%	32	3.5%
Sex in exchange for money or drugs	79	3.3%	11	2.1%	5	3.3%	40	4.5%	23	2.7%
≥2 male sex partners since last visit	156	6.5%	18	3.4%	25	16.7%	87	9.8%	26	3.1%
New sex partner	264	10.9%	40	7.5%	23	15.4%	125	14.1%	76	9.0%
Suspects/knows male partner concurrency	573	23.7%	87	16.3%	46	30.9%	251	28.4%	189	22.3%
High risk sex (exchange, anal sex, >2 partners, sex with drugs/alcohol)	431	17.0%	81	14.9%	46	27.9%	189	20.4%	115	12.7%
Vaginal washing										
Daily	1424	56.1%	333	61.3%	90	54.6%	491	52.9%	510	56.4%
Weekly	306	12.1%	51	9.4%	22	13.3%	127	13.7%	106	11.7%
Monthly or less	224	8.8%	59	10.9%	14	8.5%	89	9.6%	62	6.9%
None (ref)	586	23.1%	100	18.4%	39	23.6%	221	23.8%	226	25.0%
ANY	1954	76.9%	443	81.6%	126	76.4%	707	76.2%	678	75.0%
Vaginal wiping										
Daily	795	31.3%	202	37.2%	54	32.7%	287	30.9%	525	27.9%
Weekly	158	6.2%	34	6.3%	10	6.1%	69	7.4%	45	5.0%
Monthly or less	117	4.6%	27	5.0%	13	7.9%	42	4.5%	35	3.9%
None (ref)	1470	57.9%	280	51.6%	88	53.3%	530	57.1%	572	63.3%
ANY	1070	42.1%	263	48.4%	77	46.7%	398	42.9%	332	36.7%
Vaginal insertion (non-menstruation related)										
Daily	173	6.8%	46	8.5%	13	7.9%	59	6.4%	55	6.1%
Weekly	50	2.0%	6	1.1%	3	1.8%	26	2.8%	15	1.7%
Monthly or less	75	3.0%	17	3.1%	6	3.6%	31	3.3%	21	2.3%
None (ref)	813	89.9%	474	87.3%	143	86.7%	812	87.5%	813	89.9%
ANY	298	11.7%	69	12.7%	22	13.3%	116	12.5%	91	10.1%

Table 3.4 (continued)

Reported behaviors at follow-up	OR pregnant vs. OC			OR pregnant vs. injectable			OR pregnant vs. no HC use		
	OR	95%CI	p-value	OR	95%CI	p-value	OR	95%CI	p-value
Any vaginal sex since last visit	**2.1**	**1.3-3.4**	**<0.01**	0.6	0.3-1.0	0.05	1.1	0.8-1.7	0.53
Vaginal sex frequency per week									
mean (range)* (not or)	**1.7**	**1.3-2.0**	**<0.0001**	1.1	0.8-1.5	0.73	**1.3**	**1.1-1.5**	**<0.01**
≥3 times per week	**1.5**	**1.2-1.9**	**<0.01**	1.0	0.7-1.5	0.83	**1.2**	**1.0-1.4**	**0.05**
Condom use (male or female) at last sex	1.3	1.0-1.6	0.05	1.0	0.7-1.5	0.95	**1.3**	**1.0-1.5**	**0.02**
Unprotected sex (any report)	**0.7**	**0.6-0.9**	**<0.01**	0.7	0.5-1.1	0.17	**0.7**	**0.6-0.9**	**<0.01**
Anal sex since last visit	**1.9**	**1.2-3.1**	**0.01**	**2.5**	**1.2-5.3**	**0.01**	**1.7**	**1.1-2.5**	**0.01**
Sex in exchange for money or drugs	1.1	0.7-1.8	0.74	1.0	0.3-3.1	0.97	1.4	1.0-1.9	0.07
≥2 male sex partners since last visit	**2.0**	**1.3-3.0**	**<0.01**	**3.5**	**1.5-8.3**	**<0.01**	**3.6**	**2.3-5.7**	**<0.0001**
New sex partner	1.0	0.7-1.5	0.88	1.6	0.9-2.7	0.11	**1.7**	**1.3-2.1**	**<0.01**
Suspects/knows male partner concurrency	1.0	0.8-1.2	0.73	1.1	0.7-1.7	0.57	1.2	1.0-1.5	0.06
High risk sex (exchange, anal sex, >2 partners, sex with drugs/alcohol)	**1.4**	**1.0-2.0**	**0.03**	**2.2**	**1.4-3.4**	**<0.01**	**1.8**	**1.4-2.2**	**<0.0001**
Vaginal washing									
Daily	**1.3**	**1.1-1.6**	**<0.01**	1.3	1.0-1.7	0.08	1.12	1.0-1.3	0.15
Weekly	1.1	0.8-1.5	0.51	1.2	0.8-1.8	0.27	1.14	0.9-1.4	0.22
Monthly or less	**1.6**	**1.1-2.4**	**0.01**	1.3	0.9-1.9	0.16	1.30	1.0-1.7	0.06
None (ref)	1.0	-	-	1.0	-	-	1.00	-	-
ANY	**1.3**	**1.1-1.6**	**0.01**	1.3	1.0-1.8	0.07	1.16	1.0-1.4	0.08
Vaginal wiping									
Daily	**1.6**	**1.3-2.0**	**<0.0001**	**1.5**	**1.1-2.1**	**<0.01**	**1.31**	**1.1-1.5**	**<0.01**
Weekly	**1.7**	**1.2-2.4**	**<0.01**	**2.0**	**1.2-3.3**	**<0.01**	**1.41**	**1.1-1.9**	**0.02**
Monthly or less	**1.8**	**1.1-2.8**	**0.01**	**2.2**	**1.1-4.3**	**0.02**	1.38	1.0-1.9	0.06
None (ref)	1.0	-	-	1.0	-	-	1.00	-	-
ANY	**1.7**	**1.5-2.1**	**<0.0001**	**1.8**	**1.3-2.4**	**<0.01**	**1.35**	**1.2-1.6**	**<0.0001**
Vaginal insertion (non-menstruation related)									
Daily	**1.5**	**1.0-2.1**	**0.04**	1.6	0.9-3.0	0.12	1.17	0.8-1.6	0.36
Weekly	1.1	0.5-2.1	0.87	1.1	0.3-3.6	0.87	1.63	0.9-3.0	0.10
Monthly or less	1.5	0.9-2.4	0.15	1.8	0.8-4.2	0.19	1.31	0.8-2.1	0.27
None (ref)	1.0	-	-	1.0	-	-	1.00	-	-
ANY	1.5	1.1-1.9	<0.01	1.7	1.0-2.7	0.05	1.23	0.9-1.6	0.13

Figure 3.2 Risk behaviors reported at two follow-up visits for each woman: non-pregnant and first pregnant visit, N=483



*p<0.05; **p<0.01; ***p<0.001

Table 3.5 Association between pregnancy status and reported risk behaviors and vaginal practices at follow-up visits, N=483 women, 2,540 visits (modified Poisson risk regression)

Risk behavior	Univariable			Adjusted		
	RR	95%CI	p-value	RR	95%CI	p-value
Any vaginal sex since last visit	0.8	0.6-1.0	0.05	0.84	0.7-1.1	0.12
Vaginal sex ≥3 times per week	**0.9**	**0.8-1.0**	**0.03**	**0.9**	**0.8-1.0**	**0.04**
Condom use (male or female) at last sex	**0.9**	**0.8-1.0**	**0.01**	**0.8**	**0.7-0.9**	**<0.01**
Unprotected sex (any report)	**1.3**	**1.1-1.5**	**<0.01**	**1.3**	**1.2-1.5**	**<0.0001**
Anal sex since last visit	**0.7**	**0.5-1.0**	**0.01**	**0.7**	**0.5-0.9**	**0.01**
Sex in exchange for money or drugs	0.8	0.6-1.2	0.30	0.8	0.6-1.2	0.27
≥2 male sex partners since last visit	**0.6**	**0.4-0.8**	**<0.01**	**0.5**	**0.3-0.7**	**<0.01**
New sex partner	0.8	0.7-1.0	0.07	**0.8**	**0.7-1.0**	**0.03**
Suspects/knows male partner concurrency	0.9	0.8-1.1	0.21	**0.9**	**0.8-1.0**	**0.10**
High risk sex (exchange, anal sex, ≥2 partners, new partner, new regular partner)	**0.8**	**0.6-0.9**	**<0.01**	**0.7**	**0.6-0.9**	**<0.01**
Vaginal washing						
Daily	0.9	0.8-1.1	0.29	0.9	0.8-1.1	0.32
Weekly	0.9	0.8-1.1	0.36	0.9	0.8-1.1	0.33
Monthly or less	**0.7**	**0.6-0.9**	**0.01**	**0.7**	**0.6-0.9**	**<0.01**
None (ref)	1.0	-	-	1.0	-	-
ANY	0.9	0.8-1.0	0.14	0.9	0.8-1.0	0.15
Vaginal wiping						
Daily	**0.9**	**0.8-0.9**	**<0.01**	**0.9**	**0.8-1.0**	**0.01**
Weekly	**0.8**	**0.6-1.0**	**0.04**	**0.8**	**0.6-1.0**	**0.04**
Monthly or less	0.8	0.6-1.1	0.13	0.8	0.6-1.1	0.14
None (ref)	1.0	-	-	1.0	-	-
ANY	**0.8**	**0.8-0.9**	**<0.01**	**0.8**	**0.8-0.9**	**<0.01**
Vaginal insertion (non-menstruation related)						
Daily	0.9	0.8-1.2	0.52	0.9	0.8-1.2	0.49
Weekly	0.9	0.5-1.4	0.57	0.9	0.5-1.4	0.57
Monthly or less	0.8	0.6-1.1	0.12	0.8	0.6-1.1	0.12
None (ref)	1.0	-	-	1.0	-	-
ANY	0.9	0.7-1.0	0.12	0.9	0.7-1.0	0.11

*Compares pregnant visits (reference) to non-pregnant/non-postpartum visits; adjusted models include age, randomization arm and city

Chapter 4

Incidence of sexually transmitted infections during pregnancy

Abstract

Background: Prevalence of sexually transmitted infections (STI) is high among pregnant women in certain settings. Few studies have documented STI incidence among pregnant women and whether pregnancy increases STI acquisition. We compared STI incidence among pregnant and non-pregnant person time among women in a randomized clinical trial.

Methods: Data came from the Methods for Improving Reproductive Health in Africa (MIRA) study, a randomized clinical trial conducted in South Africa and Zimbabwe from 2003-2006. Women 18-50 years of age were included in the analysis if they had at least one follow-up visit within 6 months of the date of enrollment that included pregnancy and STI testing. All visits included laboratory testing for four STI (chlamydia, gonorrhea, trichomoniasis, and HIV) and self-reporting of sexual behaviors and vaginal practices. Logistic models were used to examine differences in reported behaviors at follow-up visits by pregnancy status. Cox proportional hazards models for individual STI were fitted using pregnancy status as a time-varying exposure and reported sexual behaviors and vaginal practices as time-varying covariates in adjusted models. Non-pregnant months were further divided by use of hormonal contraceptive methods, with months not using hormonal contraception used as the reference group.

Results: 4,549 women from the MIRA study were included in this analysis (91.9% of all enrolled), 766 (16.8%) of whom had a lab confirmed pregnancy during follow-up. Median follow-up time was 18 months [interquartile range (IQR): 12-24]. During pregnancy, most women continued to be sexually active but reported less sex. Compared to women who were pregnant, women who were not pregnant reported more condom use at last sex (odds ratio(OR) 1.5;95%CI 1.3-1.7) and also more high risk sexual behaviors (OR 1.5;95%CI 1.3-1.8). Vaginal practices also appeared to decrease in frequency during pregnancy. In multivariable models comparing pregnant periods to non-pregnant periods when women were not using hormonal contraception, pregnant women were not at higher risk for acquiring STIs.

However, in analysis of risk in the periods when women became pregnant, they were also at increased risk of chlamydia (adjusted hazards ratio (aHR)1.9;95%CI 1.3-2.8).

Conclusions: In this analysis, we found differences in sexual risk behaviors and vaginal practices depending on a woman's pregnancy status among women enrolled in an RCT in southern Africa. Pregnancy did not increase risk of STI acquisition. However, during the time periods when women became pregnant they were also at higher risk for chlamydia infection. Greater efforts are needed to help pregnant women avoid STIs in order to protect their own health and the health of their children.

Background

High prevalence of sexually transmitted infections (STI) has been observed in some populations of pregnant women. Data from routine screening in antenatal care settings in sub-Saharan Africa have shown prevalence of bacterial STIs, including chlamydia, gonorrhea and syphilis, to be as high as 15%.[10,22,23,88] Viral STIs (HIV, HSV-2 and HPV) are also very common with up to 30% of pregnant women infected with HIV in southern Africa and almost half of all women under 30 years of age infected with Herpes Simplex Virus type 2 (HSV-2) across sub-Saharan Africa.[2,90] Pregnant adolescents have been found to have very high STI prevalence; a study from Portugal found more than 11% of pregnant teenagers to have trichomonasis and almost 5% to be infected with gonorrhea.[128] Among incarcerated pregnant women in the Los Angeles, 11% were infected with chlamydia and gonorrhea prevalence was 3%.[129]

STIs can cause chronic pain as well as permanent infertility in women who do not receive treatment. STIs are of particular concern during pregnancy as they can lead to adverse pregnancy outcomes including stillbirth and low birth weight which jeopardize the health of women and their infants.[4,10,17] STIs, primarily HIV, also contribute significantly to maternal mortality;[130] HIV is the leading cause of maternal mortality in high prevalence settings with infection increasing risk of death for pregnant women and mothers up to six-fold.[18] An additional concern for pregnant women infected with STIs is mother-to-child transmission (MTCT), which can cause serious impairment in children, including blindness and developmental delays.[7] MTCT of HIV and HSV-2 causes lifelong infection and mortality in infected children.[8,9,11,21] Data have also shown that incident infection with HIV and HSV-2 during pregnancy may confer higher risk of MTCT; however the extent to which MTCT is increased for incident infections from other STIs is not known.[66,67,84,131]

Despite evidence of high STI prevalence among some groups of pregnant women, there have been few examinations of the timing of STI acquisition in relation to pregnancy. Timing of infection is critical for understanding the etiology of STIs in pregnant women for establishing whether women may be at greater risk of acquiring STIs while they are pregnant. Studies examining STI incidence during pregnancy are few but have shown that acquisition of common infections continues during pregnancy.[26,69-71,73-75,77,78] A small number of studies have also shown that in settings with high HIV burden, women have high risk of infection during pregnancy.[80] Further, modeling studies have suggested high rates of new HIV infections early in pregnancy (or concurrent with conception).[132,133] Studies comparing incidence of STIs in pregnant and non-pregnant women are very limited and the question of whether pregnancy increases STI acquisition risk has only been examined in relation to HPV and HIV.[31,33,35,74] No increased risk for high risk HPV types was found in pregnant women in Holland,[74] however several studies have shown greater risk of HIV acquisition during pregnancy.[31,33,35]

More data are needed to understand whether pregnancy is a time of increased risk for STI acquisition, and if so, which STIs pregnant women are most likely to contract and what behavioral and biological factors may contribute to this increased risk. Pregnant women may have higher risk of acquiring STIs as a result of changes in sexual risk behaviors, particularly sex without a condom.[93,94] There have been few studies examining sexual behaviors of pregnant women or of behavioral changes that occur during pregnancy. Available data show that women continue to be sexually active during pregnancy, with diminishing frequency as pregnancy progresses.[27,29,30] Several studies have shown that pregnant women report lower use of condoms compared to women who are not pregnant.[30-33] Other behavioral risk factors, such as transactional sex in exchange for drugs or money and other types of high risk sex, including multiple concurrency of sexual partners and anal sex, are less commonly reported during pregnancy.[30,31,33,35] We reported similar findings in the previous chapter of this dissertation, with

women reporting more unprotected sex during pregnancy compared to non-pregnant periods, but less frequency of these other high risk behaviors.

In addition to sexual risk behaviors, vaginal practices, including douching, cleaning, wiping and insertion of materials and substances into the vagina, have also been studied as potential behavioral risk factors for STI acquisition, particularly HIV.[99,101] Women may engage in vaginal practices for hygienec purposes, pregnancy prevention, and with the goal of drying and/or tightening the vagina for sex.[100] Women in sub-Saharan Africa have reported particularly high rates of vaginal practices.[99] A recent large pooled analysis of data found that insertion of cloth or paper into the vagina and cleaning with soap were associated with increased HIV risk.[101] Limited data have also shown vaginal practices to be associated with incidence of chlamydia[102] and other STIs.[103] The mechanism through which vaginal practices increase STI and HIV risk has not been determined; it may be through abrasions to vaginal tissue or alteration of vaginal pH levels leading to bacterial vaginosis (BV) which has been estimated to increase women's susceptibility to HIV by up to 70%.[101,104] No studies (aside from this dissertation) have examined changes to vaginal practices during pregnancy, making it difficult to understand the potential impact of vaginal practices on STI risk specifically among pregnant women. Data from the previous chapter of this dissertation suggest that among women in South Africa and Zimbabwe, frequency of vaginal practices decreased somewhat during pregnancy.

Biological changes that occur during pregnancy could also impact women's STI risk including alternations in immune function and physiological changes to the cervix. Significant increases in concentrations of progesterone and estrogen during pregnancy may alter innate and acquired immune function in a number of ways [49-54] including by reducing the number of natural killer immune cells which protect against pathogens,[58,59] by contributing to inflammation of the genital tract,[60] and/or by increasing concentrations of chemokine receptor-5 cells in the cervix which are important co-receptors for HIV infection in the female genital tract.[62,104] Changes to cervical tissue could also increase STI risk

during pregnancy. Cervical ripening that occurs in preparation for delivery may contribute to risk by increasing exposure of more vulnerable endocervical cells to pathogens entering the lower female genital tract; cervical ectopy, a condition in which columnar epithelial tissue of the inner cervix protrudes to the outer cervix, may also occur more frequently during pregnancy and increase risk in the same way.[36-39,41,45,46] While data suggest biologically plausible ways through which pregnancy could increase STI risk, further studies are needed to explore and measure the impact of these potential pathways of risk.

In light of the significant consequences of STIs for women and children and the lack of data on whether and how pregnant women may be at greater risk for acquiring infections, it is critical to document whether pregnancy is a time of increased vulnerability and to understand the biological and behavioral risk factors for STI acquisition among pregnant women. This information could lead to more effective efforts to prevent STIs in women of reproductive age which would have a beneficial impact on women's health and the health of their children. In this analysis, we examined differences in sexual risk behaviors and vaginal practices according to pregnancy status, measured incidence of four common STIs among pregnant and non-pregnant women and compared STI acquisition based on pregnancy status. Data came from a randomized clinical trial conducted in South Africa and Zimbabwe from 2003-2006.

Methods

Data source

The data for this analysis came from the Methods for Improving Reproductive Health in Africa (MIRA) study (see chapter 2 for more information).

Study procedures

Detailed study procedures are described in chapter 2 including ethical approvals. For this analysis, we examined the association between pregnancy status and risk of acquiring one of four STIs: chlamydia, gonorrhea, trichomoniasis and HIV. Women enrolled in the MIRA trial 18-50 years of age were included in the analysis if they had at least one follow-up visit that occurred within 6 months of the date of enrollment that included both pregnancy and STI testing. For the statistical analysis, pregnancy status was treated as a time-varying exposure and selected sexual risk behaviors and vaginal practices were treated as time-varying covariates.

Participant inclusion criteria for analysis

Only women included in the final analysis of the MIRA trial who were between the ages of 18 and 50 years of age at enrollment were included in this analysis. All women were HIV-negative at enrollment. Women who tested positive for treatable STIs at enrollment, including chlamydia, gonorrhea, trichomonasis and syphilis, were not excluded from the analysis, however women who were negative at enrollment but tested positive for these infections at the 2 week study visit after enrollment were excluded. Women were also excluded from the analysis if they did not have a first study visit within 6 months of the date of their enrollment in order to avoid missing information during gaps in follow-up time.

Follow-up visit inclusion criteria

For the analyses, only scheduled quarterly follow-up visits were examined (off-schedule, additional study visits were excluded). Visits that occurred more than 6 weeks after a woman gave birth were also excluded as incidence of STIs during the post-partum period may differ from other non-pregnant periods.[107] In addition, visits that took place more than 6 months from the prior visit were excluded in order to ensure that visit schedules were relatively standardized and to improve the ability to attribute pregnancy and STIs to a given time period. Finally, visits that occurred after an HIV diagnosis for women who seroconverted were excluded as behaviors may have changed as a result of the HIV diagnosis.

Exposure measurement: pregnancy and hormonal contraceptive use status

As in the previous analysis, pregnancy status was ascertained through laboratory testing and as reported by women at scheduled follow-up visits. Women were considered pregnant at a quarterly study visit if they had a lab positive human chorionic gonadotropin (HCG) urine pregnancy test (laboratory results determined pregnant status regardless of the woman's knowledge of the pregnancy prior to the visit). For the main analysis women were considered non-pregnant at visits with a negative pregnancy test even at visits when they reported pregnancies during the interval between the current and previous visits (visits with a negative pregnancy test but report of miscarriages or terminations between visits were also classified as non-pregnant). Pregnancy status at a given visit was applied to determine exposure status during the interval of time prior to that visit; i.e. if a woman was pregnant at her third quarterly visit, she was considered to have been pregnant for the full period of time between the second and third quarterly visit. For all analyses, women who attended a visit within 6 weeks of giving birth were considered pregnant at the study visit because they would have been pregnant for most of the time between the study visits.

For a planned additional analysis, we distinguished time between visits based on whether women were pregnant for the full amount of time from those when women became pregnant. Visits when a woman had a lab confirmed pregnancy test and had been pregnant (lab confirmed) at the previous visit were considered "established" pregnancies (the woman was pregnant for the full period of time between visits). Visits when women had lab confirmed pregnancy tests but at the previous visits had not been pregnancy were classified as "newly" pregnant indicating that the pregnancy occurred at some point between the previous and current visit. The purpose of this analysis was to distinguish between behavior change and incident infections that may have occurred at or near the time of conception from those that occurred during established, ongoing pregnancies. (For a sensitivity analysis, we also examined a broader definition of pregnancy status which included all lab confirmed and self-reported pregnancies in order to examine whether inclusion of miscarriages, terminations and other self-reported pregnancies would alter results.)

In addition to classifying women by pregnancy status at study visits, visits that were categorized as non-pregnant were further classified according to the type of hormonal contraceptive used which was self-reported by women at all visits. Hormonal contraceptive use at visits was categorized into three groups: oral contraceptives (including combined estrogen and progesterone and progesterone-only pills), injectable hormonal contraceptives (Net-En and Depo Provera), and no use of hormonal contraceptives which included women who reported only use of condoms, diaphragm and natural methods or no method.

Outcome: STI incidence

There were four STI outcomes examined in this analysis: chlamydia, gonorrhea, trichomoniasis and HIV. At all quarterly study visits participants were tested for all four STIs. Diagnosis of chlamydia, gonorrhea and trichomoniasis was assessed using urine samples with DNA PCR testing (Roche

Pharmaceuticals, Branchburg, NJ, USA). HIV testing was conducted with two rapid tests on whole blood samples from finger prick or venipuncture; Determine HIV-1/2 (Abbot Laboratories, Tokyo, Japan) and Oraquick (Orasure Technologies, Bethlehem, PA, USA). Discordant rapid tests were confirmed with ELISA. PCR testing of stored blood samples taken at enrollment were tested at the time of a positive HIV test during follow-up to ensure HIV-negative status at enrollment (women found to be HIV-infected at enrollment were excluded). Assays were performed at laboratories in Zimbabwe and South Africa which were monitored for quality control during the study. All women testing positive for HIV were referred for care and treatment services and continued follow-up in the study. Women with positive tests for curable STIs were given treatment at the study site.

For the analysis, the time to first incident infection of each of the four STIs was examined (incident infection was defined as the first positive test after a previous negative test) using a Cox proportional hazards model. STI infection outcomes were assigned to the quarterly study visit with positive test results and matched with the behavioral data that was self-reported at the same visit corresponding to their report on the period *prior* to the visit. Positive STI test results from non-quarterly visits (when women sought additional services generally due to symptoms) were carried forward to the subsequent quarterly study visit in order to be matched with the corresponding reported behavioral data from the same visit interval.

Covariates

We examined both time-fixed and time-varying hypothesized confounders in the analysis. The time-fixed covariates were age at enrollment, education (completion of high school), intervention arm (diaphragm or control), contraceptive use at enrollment, binary variables indicating any high risk sexual behaviors and high partner risk at enrollment. High risk sex at enrollment and during follow-up was indicated with women's self-report of any of the following: anal sex, sex in exchange for drugs or money,

two or more sexual partners, a new sexual partner or sex under the influence of drugs or alcohol. Male partner high risk sexual behavior (only measured at enrollment) which was reported by women, was indicated by known HIV-infected status, being away from home for more than one month in past year, use of drugs during sex and if the woman knew or suspected her male partner of having other sex partners. Time-varying covariates (measured at all quarterly study visits) included any reported vaginal sex and frequency of sexual activity between study visits, use of condom at last sex act, any unprotected sex (without a condom) between visits, sex in exchange for money or drugs, two or more sex partners, having a new sex partner, suspecting or knowing that a male partner had other partners and a binary indicator variable indicating any high risk sexual risk behaviors (exchange sex, anal sex, 2 or more partners, new sex partner, sex under the influence of drugs or alcohol). Vaginal practices, categorized as any report of washing, wiping or inserting paper products into the vagina (not for menstruation), were also treated as time-varying covariates and examined by frequency (daily, weekly, monthly or no report) and as a binary variable indicating any report.

Statistical analysis

Enrollment characteristics

Descriptive statistics were used to compare demographic characteristics and frequency of reported sexual behaviors and vaginal practices at enrollment for women 18-50 years of age included in the analysis according to whether they had at least one pregnancy during follow-up. Women with lab positive pregnancies were compared to women with no reported or lab-confirmed pregnancies for characteristics and behaviors reported at enrollment using chi-squared tests for categorical variables and Wilcoxon tests for continuous variables. We also examined differences between women included in the analysis, categorized as above, and those excluded from the analysis due to age or a greater than 6

month time lag between enrollment and their first quarterly visit in order to determine any potential bias in the exclusion of participants.

Association between pregnancy status and reported behaviors at follow-up visits

Descriptive statistics were used to compare reported sexual behaviors and vaginal practices at quarterly follow-up visits according to pregnancy status and non-pregnant visits with or without HC-use. To compare frequency of reported behaviors and measure associations between pregnancy (and HC-use) status at visits, general estimating equations (GEE) models with robust standard errors were used to account for multiple observations per participants. Unadjusted (univariable) logistic regression GEE models were fitted with pregnancy status modeled as the independent variable (pregnant visits were the referent group) and sexual risk behavior and vaginal practice were treated as dependent variables. Crude odds ratios with 95% confidence intervals are reported for each sexual risk behavior and vaginal practice. For modeling dependent variables with more than two categories (such as vaginal practice frequency), individual logistic GEE models were run comparing each categorical level of the variable to a common reference level for the variable (for example three individual models were run comparing daily, weekly and monthly vaginal washing to the reference category of “no vaginal washing”).

We also conducted the same analyses distinguishing between established and new pregnancies to examine differences in behaviors based on known pregnancy. For the analysis of behaviors reported at visits during established pregnancies vs newly pregnancy visits, the referent group for was established pregnancy visits. We further conducted sensitivity analysis repeating these models using the broader definition of pregnancy that included self-reported pregnancies.

STI incidence rate calculations

To estimate incidence of STIs during pregnant and non-pregnant periods, each of the four STIs was examined separately with follow-up time for participants calculated from the date of enrollment in

the study to the first diagnosis of each STI or the end of study follow-up (in this analysis). In order to calculate incidence rates, follow-up time at risk was estimated by summing the intervals of time between visits for each participant. For intervals between visits that culminated in a positive test (incident STI infection), the midpoint between the last visit when the participant was negative and the visit with the positive test was used to estimate follow-up time at risk for that visit interval. Incidence rates were calculated for each of the exposure groups: pregnant, non-pregnant/oral HC, non-pregnant/injectable HC and non-pregnant/no HC use. Incidence rates (in person years) for each exposure group were calculated by dividing the corresponding follow-up time at risk for each exposure into the number of cases that occurred when women were in the corresponding exposure groups. In addition, overall incidence of each STI using all incident infections and all follow-up time, regardless of exposure group, was also calculated.

Modeling of incidence of STIs and comparison between pregnant and non-pregnant periods

Cox proportional hazards models were fitted to examine the relationship between pregnancy status and incidence of each of the four STIs separately and a composite measure of first incidence infection with any of the four STIs. Pregnancy was treated as a time-varying exposure and sexual risk behaviors and vaginal practices were treated as time-varying covariates; demographic and enrollment characteristics were modeled as time-fixed covariates. Univariable and multivariable Cox proportional hazards models were fitted using time-fixed and time-varying covariates accounting for different follow-up time per participant. Adjusted models included the following a priori hypothesized confounders: time-fixed demographic variables (age, study location, education and randomization arm), as well as enrollment characteristics (contraceptive use, report of high risk sex, having a high risk partner and having an STI including chlamydia, gonorrhea, trichomonasis or HSV-2) as well time-varying sexual risk behaviors and vaginal practices (see above). In a separate analysis, multivariable models were adjusted for covariates that were found to result in a greater than 10% change in the beta estimate for pregnancy

status on the outcomes; no differences were found between those models, thus the models using a priori covariates are reported. Study location was also tested for potential effect measure modification on the multiplicative scale using a cross-product term to examine whether the relationship between pregnancy and STI incidence differed by study location.

We further implemented these models comparing established and newly pregnant status. Finally, we conducted two additional sensitivity analyses of these models using the broader definition of pregnancy as well as a complete case analysis including only those women who attended all scheduled follow-up visits in order to examine potential biases associated with follow-up time that was not accounted for (gaps as a result of missed follow-up visits).

Impact of location on enrollment characteristics, reported behaviors at follow-up and differences in incidence

In addition to examining whether location was an effect modifier in the relationship between pregnancy status and STI outcomes as described above, location was also examined to determine differences in demographic characteristics and reported sexual risk behaviors at the time of enrollment and at study follow-up visits. Unadjusted logistic regression models were used to examine differences at enrollment among individual women, and at all follow-up visits models used GEE to account for unequal numbers of visits attended by participants. Incidence rates of each of the four STIs by pregnancy status was calculated for each of the study locations using the methods described above.

Results

Of the 4,948 women analyzed in the MIRA trial, 4,935 were 18-50 years of age at enrollment and 4,552 had at least one follow-up visit with STI and pregnancy testing that was within six months of the date of enrollment and prior to HIV infections (Figure 4.1). 382 women did not have a follow-up visit within six months of enrollment and were excluded; four additional women were excluded because they tested positive for chlamydia or trichomoniasis at the two week study visit. Among the 4,549 women included in the analysis (91.9% of all enrolled), 766 (16.8%) women had a lab confirmed pregnancy and of the 24,337 visits included in the analysis, 1,609 (6.6%) were during a pregnancy. Median follow-up time among women included in the analysis was 18 months [interquartile range (IQR): 12-24]; among women with pregnancies, median follow-up was 15 months [IQR: 12-21] and among those with no pregnancies, it was 18 months [IQR: 12-24] (p=0.47).

Enrollment: demographic characteristics, reported sexual risk behaviors and vaginal practices

At enrollment, women who went on to have pregnancies during follow-up were more likely to be from Zimbabwe (compared to South Africa) and were younger; the median age for women who had a pregnancy was 25 years (range: 18-47) whereas the median age of women who did not have a pregnancy was 28 years (range: 18-50) (p<0.0001) (Table 4.1). Women with pregnancies during follow-up were more likely to have completed high school and were less like to have children at enrollment. There were no differences between women with and without pregnancies in other demographic characteristics, including employment and marital status or cohabitation with male partners at enrollment. Women who had pregnancies during follow-up were more like to report use of oral contraception and to have had 2 or more sexual partners at enrollment compared to women who did not have pregnancies (Table 4.1). Women without pregnancies were more likely to report injectable hormonal contraceptives (including Net-En and Depo Provera) and to be infected with HSV-2 at

enrollment compared to those who had pregnancies during follow-up. There were no other differences in reported sexual behaviors, male partner characteristics or STI status at enrollment.

In a separate analysis shown in Appendix 4.1, enrollment characteristics were examined to identify differences between women with pregnancies during follow-up, those without, as and the 386 women aged 18-50 years who were excluded from the analysis. Among the excluded women, 62 (16.1%) had a lab confirmed pregnancy during follow-up. Excluded women were younger than women who did not have pregnancies but somewhat older than women with pregnancies (Appendix 4.1). Excluded women as a group were also more likely than women without pregnancies and less likely than women with pregnancies to have completed high school; they were also more likely than women with pregnancies to have had a previous pregnancy. Women who were excluded reported significantly more sex per week than either group of women included in the analysis and were more likely to have a male partner of the same age (Appendix 4.1).

Association between pregnancy status and behaviors reported at follow-up visits

Women were pregnant at 1,609 (6.6%) of follow-up visits included in the analysis. At the remaining non-pregnant visits: 8,375 (34.4%) included report of oral contraceptives, at 6,028 (24.8%) women reported injectable HC use and at 8,325 (34.2%) women reported no HC use (including condoms, natural or no methods). Compared to visits during pregnancy, women were more likely to report any vaginal sex and $\geq$3 sex acts per week at non-pregnant visits when they were using oral HC (any sex: OR 1.7; 95%CI 1.3-2.3) and less likely when using no HC (any sex: OR 0.6; 95%CI 0.5-0.8) (Table 4.2). At all non-pregnant visits, regardless of HC type, women were significantly more likely to report last sex with a condom and less likely to report any unprotected sex (without a condom) compared to visits when women were pregnant; the odds of reporting sex without a condom between visits were 30% lower when women were not pregnant and using oral HC compared to pregnant visits (OR 0.7; 95%CI

0.6-0.8). Women using injectables and those not using HC were almost twice as likely to report two or more sex partners and more frequently reported new sex partners between visits (Table 4.2). High risk sex (anal sex, sex in exchange for drugs or money, two or more sexual partners or a new sexual partner) was more commonly reported during periods when women were using injectables or no contraception compared to when women were pregnant (Table 4.2).

Vaginal washing appeared to be more common at visits when women were using oral HC compared to pregnant visits overall and on a daily and monthly basis. However, the odds of vaginal washing did not differ between visits during pregnancy and when women were using injectables or reported no HC use (Table 4.2). Vaginal wiping was significantly more common at visits women were not pregnant, regardless of the type of HC use or when they were not using any HC; at visits with no HC use, the odds of vaginal wiping were 30% higher than at pregnant visits (OR 1.3;95%CI 1.1-1.4) and were 40% higher at visits with reported oral HC use (OR 1.4;95%CI 1.2-1.5). Non-pregnant status was also associated with increased frequency and overall reporting of vaginal insertion of paper products compared to visits when women were pregnant (Table 4.2).

In the analysis distinguishing between established and new pregnancies, 800 (3.3%) of all visits occurred were newly pregnant visits and 809 (3.3%) were during established pregnancies (Table 4.4). At newly pregnant visits, only 24.3% of women knew about their pregnancies before the visit while most (75.6%) learned of their pregnancy at the visit. Newly pregnant women were more than twice as likely to report any vaginal sex compared to women with established pregnancies (OR 2.3;95%CI 1.6-3.1) but were significantly less likely to report unprotected sex during the visit interval when they became pregnant (OR 0.7;95%CI 0.6-0.9). During newly pregnant visits, women were more likely to report two or more sexual partners and were more likely to report high risk sex compared to established pregnancy visits (OR 1.3;95%CI 1.1.-1.7). These findings were similar to the main analysis which showed that non-pregnant women were more likely to report high risk sex and concurrency. Vaginal practices, washing,

wiping and insertion, were also more commonly reported by women when they were newly pregnant compared to during established pregnancy visits (Table 4.4).

Results from the sensitivity analysis using the broader definition of pregnancy led to 1,744 (7.2%) visits being classified as occurring during pregnancy (Table 4.3) and did not differ from the main analysis.

STI Incidence rate

The overall incidence rate of chlamydia in the 4,949 women included in the analysis was 6.7 per 100 person years (py) (Table 4.5). Chlamydia incidence was 9.9/100py during pregnancy, 10.1/100py during injectable HC use and 4.1/100py during oral HC use. Gonorrhea incidence across all follow-up time was lower overall (2.7/100py) but was highest during periods when women were pregnant at 4.9/100py and lowest when women reported oral HC (1.6/100py). Trichomoniasis incidence was the most common of all the STI measured; the incidence was 7.1/100py overall, and was highest during pregnancy 9.2/100py. HIV incidence among the women included in this analysis was 3.9/100py across all follow-up periods with the highest incidence occurring when women reported using injectable HC (4.8/100py) and when they were not using any HC (4.4/100py). The HIV incidence rate during pregnancy was 3.8/100py and 2.6/100py when women were using oral HC (Table 4.5).

In the analysis which separated visits during established pregnancies from first pregnancy visits, incidence of chlamydia was lower during established pregnancies, 3.8/100py, compared to first pregnant visits when it was very high, 15.4/100py (Table 4.5). For gonorrhea however, the opposite was true, most infections occurred during established pregnancies (6.2/100py) whereas the incidence rate at newly pregnant visits was 3.8/100py. Trichomoniasis incidence was similar among women with established pregnancies and for those who were newly pregnant with both periods somewhat higher than the overall incidence. HIV incidence was lower among women with established pregnancies,

3.0/100py, compared to those with new pregnancies who had the highest HIV incidence among all exposure groups, 4.6/100py (Table 4.5).

Sensitivity analysis using the broader definition of pregnancy did not change these results substantially (Table 4.5). Trichomoniasis and HIV incidence rates were slightly higher in these models, increasing to 9.6/100py and 4.4/100py, respectively.

Modeling of incidence of STIs and comparison between pregnant and non-pregnant periods

In unadjusted models, pregnancy was associated with an increased risk of chlamydia compared to periods when women were not pregnant and reported no HC use (hazard ratio (HR) 1.5;95%CI 1.1-2.2). In multivariable adjusted models however, the effect of pregnancy on chlamydia incidence was attenuated and the effect was no longer significant (Table 4.5a). Pregnancy did not appear to increase the hazards of gonorrhea, trichomoniasis or HIV infection (Tables 4.5b-d). Neither oral nor injectable HC use was associated with increased hazards of any of the four STIs (Tables 4.5a-d). There also was no association found between pregnancy status and the combined STI composite of incidence of any of the four STIs (Table 4.5e). Location was not found to be an effect modifier of the relationship between pregnancy and STI risk in adjusted models.

Other factors that appeared to be associated with increased hazard of chlamydia infection in adjusted models included being from South Africa (compare to women in Zimbabwe), younger age (aHR 18-25 vs. $\geq$35 5.6;95%CI 3.8-8.2), having an STI at enrollment (aHR 1.5;95%CI 1.2-1.9), reporting unprotected sex since last visit (aHR 1.3;95%CI 1.0-1.7) and suspecting or knowing a male partner to have other sexual partners (aHR 1.3;95%CI 1.0-1.6) (Table 4.5a). The same factors were associated with gonorrhea incidence in adjusted models. For trichomonasis, only women in Johannesburg, South Africa appeared at higher risk compared to women in Zimbabwe (aHR 1.2;95%CI 1.2-2.0) but age was not associated with increased hazard (Table 4.5c). After adjustment, HIV infection was associated with being

from Johannesburg (aHR 2.0;95%CI 1.4-2.9), younger age (aHR 18-24 vs ≥35 2.6;95%CI 1.7-3.9), use of injectable contraceptives at enrollment (aHR 1.8;95%CI 1.0-3.1), STI at enrollment (aHR 2.1;95%CI 1.5-2.8) and suspecting or knowing of partner concurrency (aHR 1.5;95%CI 1.1-2.0) (Table 4.5d).

Vaginal practices did not appear to be associated with risk of chlamydia, gonorrhea or trichomoniasis in unadjusted or adjusted models (Table 4.5a-c). Vaginal wiping was associated with increased hazard of HIV (aHR 1.5;95%CI 1.1-2.0)(Table4.5d).

In the analysis distinguishing between established and new pregnancies, women who were newly pregnant had almost twice the hazard of incident chlamydia infection compared to non-pregnant women who were not using HC (aHR 1.9;95%CI 1.3-2.8) (Table 4.6a). Established pregnancy status did not increase chlamydia risk compared to non-pregnant time. Hazard of incident gonorrhea, trichomoniasis and HIV incidence did not appear to be higher during either established or new pregnancies compared to when women were not pregnant women and not using HC (Table 4.6b-d). There also was no association found between new or established pregnancy status and the combined STI composite of incidence of any of the four STIs (Table 4.6e)

The two sensitivity analyses, using the broader definition of pregnancy status (Appendix 4.4a-d) and the complete case analysis (Appendix 4.54a-d), to examine any biases introduced by visit gaps, did not show any differences from the main analysis.

Impact of location on enrollment characteristics, reported behaviors at follow-up and differences in incidence

The analysis of differences in characteristics at the time of enrollment is show in Appendix 4.6 and found that women from Zimbabwe were more likely to be younger than 24 years of age (OR 0.6; 95%CI 0.5-0.7) and to use oral contraceptive compared to women in either Durban or Johannesburg. At enrollment, women in Durban were least likely to report high risk sexual behaviors (OR 0.7; 95%CI 0.6-

0.9), while women in Zimbabwe were least likely to report that their male partners as high risk (Appendix 4.6). At follow-up visits, Zimbabwean women were most likely to be pregnant and to report use of oral contraceptives at visits when they were not pregnant. Women in Durban and Johannesburg reported less sex between visits compared to women in Zimbabwe, as well as more condom use but also more high risk behaviors such as sex with multiple partners and new sexual partners (high risk sex reported by women in Johannesburg compared to women in Zimbabwe OR 1.9; 95%CI 1.7-2.1) (Appendix 4.6). All three types of vaginal practices were more commonly reported by women from Zimbabwe compared to women in Durban.

Incidence of the four STIs measured in the study also varied by location with Zimbabwe showing the lowest incidence for all (Appendix 4.7). Chlamydia incidence was 3.6 /100py in Zimbabwe compared to 10.3/100py in Durban and 10.8/100py in Johannesburg. Women in Durban and Johannesburg had higher incidence of gonorrhea at 4.3/100py and 3.8/100py, respectively, whereas incidence in Zimbabwe was 1.5/100py. The rate of trichomoniasis incidence was also lower in Zimbabwe compared to Durban and Johannesburg. Finally, HIV incidence was highest in Durban at 6.8/100py compared to 3.2/100py in Johannesburg and 2.6/100py in Zimbabwe (Appendix 4.7).

Discussion

In this secondary analysis of data from an RCT conducted in South Africa and Zimbabwe, we examined the association between pregnancy status and incidence of four common STIs: chlamydia, gonorrhea, trichomoniasis and HIV. The analysis included 4,549 women 18-50 years of age who attended over 24,300 study visits. Roughly 17% (N=766) of women had a pregnancy during follow-up and attended 1,609 visits (7% of all study visits) while pregnant. We found that during pregnancy, most women continued to be sexually active but reported less overall sex than non-pregnant women. We also found that reporting of condom use was lower during pregnancy but other types of high risk sexual behaviors, such as multiple sexual partners, sex in exchange for drugs or money and anal sex, were less commonly reported during pregnancy. Vaginal practices also appeared to decrease in frequency during pregnancy. We also found that incidence of all four measured STIs continued during pregnancy and that in periods when women became pregnant they appeared to be a high risk for acquiring chlamydia, trichomoniasis and HIV. Finally, in examining the association between pregnancy status and STI risk, we found that in multivariable models adjusted for demographic and time-varying self-reported behavioral risk factors and vaginal practices, pregnancy was not associated with STI risk. However in visit intervals when women became pregnant, they appeared to be at higher risk for contracting chlamydia compared to non-pregnant periods.

At enrollment, the women who went on to have pregnancies during follow-up were younger, somewhat more educated, had lower parity and were more likely to be using oral contraceptives compared to injectable contraception. These findings are in keeping with other previous reports from HIV prevention trials,[134-136] including a review of data from ten vaginal microbicide trials.[137] In addition, women with pregnancies during follow-up were more likely to be infected with HSV-2 at enrollment which is likely a result of their younger age, as prevalence of HSV-2, particularly in sub-Saharan Africa

(SSA), increases with age.[2] We did not find other significant differences at enrollment between women who went on to have pregnancies during follow-up compared to those who did not.

In the examination of sexual risk behaviors and vaginal practices, pregnant women reported somewhat less sex but were more likely to report sex without a condom and less likely to report high risk sexual behaviors that are associated with STI acquisition. These findings are in keeping with previous studies[29-33] and are evidence of the complicated picture of pregnant women's STI risk. In the analysis distinguishing between established and new pregnancies, newly pregnant women were much more likely to report sexual activity compared to during established pregnancies but were significantly less likely to report unprotected sex. This finding is interesting given that newly pregnant women must have had some vaginal sex without a condom in order to become pregnant and it may suggest biases in reporting of condom use by women based on pregnancy status. Over reporting of condom use is known to be common in studies,[114,115,117,118] however factors associated with differential reporting of sexual risk behaviors, including pregnancy status, have not been established. If women who are not pregnant or do not know they are pregnant are more likely to report condom use compared to pregnant women, which our data suggest, this could lead to differential misclassification of this important predictor of STI acquisition. Further studies are needed to understand and measure the potential biases associated with differential misreporting of condom use by pregnancy status.

We found higher incidence of chlamydia, gonorrhea and trichomoniasis when women were pregnant compared to the overall incidence rate for all follow-up visits. There are few data with which to compare our findings on chlamydia, gonorrhea and trichomoniasis as there have not been many previous analyses examining incidence of STIs during follow-up studies of pregnant women. The HIV incidence we report during pregnancy, 3.8 per 100 person years, is similar to three previous reports from cohorts in sub-Saharan Africa; pregnant women in our study had higher incidence than that reported from Rakai, Uganda (2.3 per 100 person)[35] and found in women in Uganda and Zimbabwe (1.6

per 100 person years)[33] but very similar to infection rates in a multi-country study (conduct in seven SSA countries) which found incidence during pregnancy to be 3.6 per 100 person year.[31] These incidence rates are in keeping with previous studies of HIV infection in non-pregnant women in Southern African women which have found incidence ranging from 3.0 to 6.5 per 100 person years.[138-140] Our data are further confirmation that women in Southern Africa face high HIV infection risk and that they continue to be at risk during pregnancy.

Our analysis did not find that pregnancy increased the risk for acquiring chlamydia, gonorrhea, trichomoniasis or HIV in adjusted models. There are few data with which to compare these findings as there have been no previous studies measuring increased risk of chlamydia, gonorrhea and trichomoniasis during pregnancy. Our findings showing no increased risk of HIV is in keeping with three previous analyses which used follow-up data from prospective cohorts and RCTs to examine increased risk during pregnancy and also found no effect of pregnancy in adjusted models.[31-33] One further study, conducted in Rakia, Uganda in over 2,100 women did find increased risk[35] however this finding has not been replicated.

While the focus of this analysis was on evaluating whether pregnancy increased STI risk, we also examined other factors that were associated with acquisition of infections. Women from South Africa appeared to have significantly higher STI incidence of all four infections which has previously been reported from this study.[141] Younger age (18-25 compared to women 35 and older) was also strongly associated with incidence of chlamydia, gonorrhea and HIV which is in keeping with previous analyses.[138,142] Other demographic and enrollment factors, including education, contraceptive use (at enrollment), and having a high risk partner were not significant predictors of risk. Having an STI at enrollment was a strong and significant predictor of risk for all STI outcomes in this analysis which may suggest, in part, that women's male partners were infected and may have re-infected them over the course of the study (the study did not include partner notification or testing). Sexual risk behaviors

reported by women over the course of follow-up in general did not appear to be good predictors of risk. In particular reporting of unprotected sex and condom use at last sex were inconsistent in predicting risk – while associated with increased risk of chlamydia and gonorrhea, they were not associated with trichomoniasis or HIV incidence. This inconsistency is in keeping with previous findings from other studies[117] and as noted above, reporting of these factors appeared to be systematically different among pregnant and non-pregnant women which may contribute to the conflicting findings for the four STIs.

One risk factor that was independently associated with increased risk for all STI outcomes was women suspecting or knowing that male partners had other sexual partners. In our study, women reported that male partners were more likely to have other sexual partners (known or suspected) during non-pregnant periods compared to pregnancy periods. This finding is not consistent with a previous analysis from Malawi in which women reported more suspected male partner concurrency during pregnancy.[30] While sexual concurrency is thought to be one of the most important risk factors for STI acquisition,[143,144] reported knowledge of a partner's concurrency has not been widely reported as a predictor of STI incidence. In a small study in Jamaica, women's report of male partner concurrency was found to be associated with women's STI prevalence.[145] These data suggest that women may have some information about their own STI risk and that despite this, may be unwilling or unable to protect themselves from infection. This finding warrants further examination in order to understand the reasons that women continue to have unprotected sex with male partners who are having concurrent partnerships.

Vaginal practices were also examined as risk factors for STI infection and were not found to consistently increase risk, although vaginal wiping was associated with HIV incidence in our study. At enrollment and during follow-up a high proportion of women reported vaginal practices: at enrollment, more than 80% of women reported some vaginal washing with more than 60% reported daily washing. Vaginal wiping and insertion (not associated with menstruation) were also reported by 57% and 21% of

women, respectively, at enrollment. These findings confirm previous reports of high rates of vaginal practices among women of reproductive age in sub-Saharan Africa.[99] This analysis (along with the previous chapter of this dissertation) provides some of the first data on changes in vaginal practices during pregnancy, which appear to decrease. The finding that vaginal wiping was associated with HIV risk is consistent with a recent large pooled analysis[101] but differ from two previous studies which found douching to be associated with higher incidence of chlamydia, gonorrhea and trichomoniasis.[102,103] One reason that vaginal practices may not have been associated with increased acquisition of other STIs could in part be a result of the observed decrease in vaginal practices over the course of the trial which was reported in a previous analysis.[126]

Our analysis is unique in distinguishing STIs acquired during established pregnancies from those contracted during the visit intervals in which women became pregnant. We found that chlamydia risk was much higher in visit intervals when women became pregnant – 15.4/100py compared to 3.8/100py during established pregnancy visits, with newly pregnant status conferring almost twice the hazards when compared to non-pregnant women (aHR 1.9;95%CI 1.3-2.8). We also found that women had significantly higher risk of chlamydia infection during periods when they become pregnant compared to non-pregnant periods. These finding are novel but not necessarily surprising – in periods when women became pregnant they were, by definition, having at least some sex not protected by condoms thus exposing them to STIs from infected partners. There are few previous studies that have examined concurrency of incident pregnancy and STI acquisition. These findings suggest that some women may be taking risks with regard to STIs in order to become pregnant and that partners of younger women, who were more likely to become pregnant, are more likely to be infected with STIs, specifically chlamydia. Further studies are needed to explore the reasons women are engaging in unprotected sex and what efforts can be made to improve condom use in order to protect women from STIs.

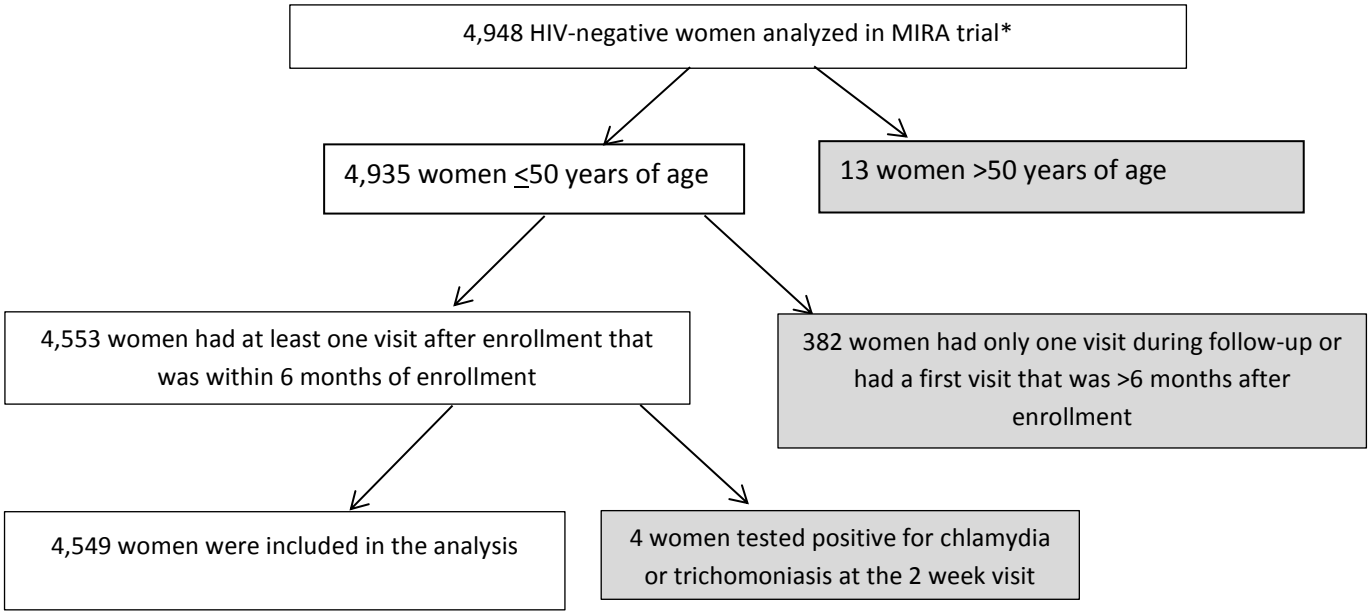
This study has a number of strengths. This analysis is one of only a few longitudinal studies to examine whether women are at greater risk of acquiring STIs during pregnancy. While several previous studies have looked at pregnancy as a risk factor for HIV, there are no previous examinations of increased risk of chlamydia, gonorrhea and trichomoniasis with prospective follow-up including periods when women were not pregnant. Previous studies have also not included data on vaginal practices which are considered important risk factors for HIV and other STI infections. The analysis is further strengthened by using data from a rigorously conducted RCT with high rates of follow-up and study completion. Our statistical analysis also allowed for changes in behaviors by including time-varying covariates.

The analysis also has some important limitations. One of the main limitations is that women self-reported sexual risk behaviors and vaginal practices and we did not have the means through which to validate any information. As noted above, we observed differential reporting of unprotected sex by women when they were newly pregnant suggesting that there may be systematic biases in this important risk. The impact of this potential bias most likely would bias our results towards the null by making pregnant women appear to have more unprotected sex and thus reducing any real effect of pregnancy on STI outcomes after adjustment for this misclassified condom use variables. Unfortunately we cannot know the extent of the bias or the impact on our results. Women also self-reported contraceptive use and may also be misclassified according to what type of hormonal contraceptives they were using. This misclassification could have had an impact on our results as conflicting data have shown that women using injectable progesterone-only hormonal contraceptives are at increased risk for STIs, including chlamydia, gonorrhea and HIV.[51,119,120] If women who were using injectable HC were classified as not using any HC and injectable HC use increase STI risk, this could have biased our results towards the null masking any increased risk from pregnancy. Another limitation is that we did not have data on male partner STI status or risk behaviors collected directly from men, all data were based on

women's knowledge of male partner behaviors which may have been inaccurate or incomplete. Finally, our statistical analysis included a multilevel exposure variable (pregnancy and HC use status) which led to multiple comparisons which could have increased the likelihood of a type error in finding statistical significance.

This analysis provides important information about women's risk of acquiring sexually transmitted infections, including HIV. Overall we did not find that pregnancy increased the risk for acquiring chlamydia, gonorrhea, trichomoniasis or HIV. Women were more likely to acquire chlamydia in periods when they become pregnant. Younger age, having had an STI previously and knowledge of a partner's concurrency were important risk factors for STI incidence. We also presented novel information about changes in sexual risk behaviors and vaginal practices during pregnancy. These data are important for understanding STI risk among women of reproductive age and may contribute to prevention efforts which would benefit women's health, as well as the health of their children.

Figure 4.1 Patient flow diagram



5,045 women were randomized in the trial: 3 were excluded because they were underage, 3 women discontinued on date of randomization, 72 had no HIV results post-enrollment and 19 were found to have been infected with HIV at baseline (after testing positive during follow-up)

Table 4.1 Characteristics at enrollment of 4,549 MIRA participants with and without pregnancies during follow-up

Characteristics at enrollment	Total		Women with no pregnancies		Women with lab confirmed pregnancies		p value
	N	%	N	%	N	%	
	4549	100.0%	3783	83.2%	766	16.8%	
Study location							
Harare	2255	49.6%	1847	48.9%	408	53.3%	**0.02**
Durban	1372	30.2%	1173	30.9%	199	26.0%	
Johannesburg	922	20.3%	763	20.2%	159	20.8%	
Age							
Median age (range)	27 (18-50)		28 (18-50)		25 (18-47)		**<0.0001**
18-24	1725	37.9%	1342	35.5%	383	50.0%	**<0.0001**
25-34	1782	39.2%	1472	38.9%	310	40.5%	
≥35	1042	22.9%	969	25.6%	73	9.5%	
Completed high school (11 yrs school)	2347	51.6%	1904	50.3%	443	57.8%	**<0.01**
Paid employment	1039	22.4%	883	23.4%	156	20.4%	0.07
Married	2695	59.3%	2245	59.4%	450	58.8%	0.75
Lives with male partner	3081	67.7%	2572	68.0%	509	66.5%	0.33
Number or previous pregnancies							
None	387	8.5%	280	7.4%	107	14.0%	**<0.0001**
1 previous pregnancy	1446	31.8%	1151	30.4%	295	38.5%	
2+ previous pregnancies	2716	59.7%	2352	62.2%	364	47.5%	
Current birth control (not mutually exclusive)							
Combined pills	991	21.8%	770	20.4%	221	28.9%	**<0.0001**
Injectable hormones	1129	24.8%	1029	27.2%	100	13.1%	**<0.0001**
Progesterone only pills	663	14.6%	566	15.0%	97	12.7%	0.10
Intrauterine device	16	0.4%	15	0.4%	1	0.1%	0.47
Condoms (male or female) (only)	1723	37.9%	1419	37.5%	304	39.7%	0.25
None (natural, withdrawal, tradition)	247	5.4%	194	5.1%	53	6.9%	0.05
Regular sexual partner	1852	40.7%	1537	40.6%	315	41.1%	0.44
Age at first sex (mean and range)	18 (10-31		18 (10-31)		18 (11-27)		0.36
Sex acts per week							
None	1877	41.3%	1551	41.0%	326	42.6%	0.65
1-2 per week	275	6.1%	227	6.0%	48	6.3%	
≥3 per week	2397	52.7%	2005	53.0%	392	51.2%	
Number of sexual partners in past 3 months							
None	273	6.0%	249	6.6%	24	3.1%	**<0.0001**
1 partner	3877	85.5%	3222	85.4%	655	85.5%	
2+	387	8.5%	300	8.0%	87.0	11.4%	
Condom use (male or female) at last sex	3200	70.4%	2653	70.1%	547	71.4%	0.48
Condom use (vaginal sex) in last three months							
Never	1338	29.5%	1132	30.0%	206	26.9%	0.16
Sometimes	1767	38.9%	1449	38.4%	318	41.5%	
Every time	1433	31.6%	1191	31.6%	242	31.6%	
Exchange for money or drugs past 3 months	360	7.9%	303	8.0%	57	7.4%	0.59
Anal sex (ever)	669	14.8%	53	14.7%	116	15.1%	0.74
Sex while intoxicated	168	26.7%	136	26.8%	32	26.0%	0.85

Table 4.1 continued

Characteristics at enrollment	Total		Women with no pregnancies		Women with lab confirmed pregnancies		p value
	N	%	N	%	N	%	
High risk (>2 partners, exchange, anal, sex w/ drugs/alcohol)	1059	23.3%	874	23.1%	185	24.2%	0.53
Partner age							
Same age (+/-5 years)	2510	57.8%	2077	57.7%	433	58.2%	0.11
Younger (more than 5 years)	50	1.2%	47	1.3%	3	0.4%	
Older (more than 5 years)	1785	41.1%	1477	41.0%	308	41.4%	
Partner tested HIV positive							
Yes	156	3.4%	135	3.6%	21	2.7%	0.24
No/don't know	4380	96.6%	3635	96.4%	745	97.3%	
Partner away from home >2 months	490	10.9%	413	10.9%	77	10.1%	0.49
Partner circumcised	976	21.5%	805	21.4%	171	22.3%	0.70
Suspects/knows partner concurrency	1392	30.7%	1159	30.7%	233	30.4%	0.86
Physical or verbal abuse	821	33.0%	693	33.0%	128	33.4%	0.86
Partner used force to get sex	244	9.8%	208	9.9%	36	9.4%	0.76
Sex w/ partner with drugs or alcohol	1622	35.9%	1351	36.0%	271	35.5%	0.80
Partner high risk (HIV+, away >1 month, other partners, drugs)	2836	62.3%	2363	62.5%	473	61.8%	0.71
Vaginal washing							
Daily	2778	61.2%	2289	61.0%	480	62.7%	0.59
Weekly	658	14.5%	558	14.8%	100	13.1%	
Monthly or less	320	7.1%	263	7.0%	57	7.4%	
None	781	17.2%	652	17.3%	129	16.8%	
ANY	3756	82.6%	3119	82.5%	637	83.2%	0.80
Vaginal wiping							
Daily	1832	40.4%	1525	40.5%	307	40.1%	0.55
Weekly	435	9.6%	353	9.4%	82	10.7%	
Monthly or less	293	6.5%	239	6.3%	54	7.1%	
None	1977	43.6%	1654	43.9%	323	42.2%	
ANY	2560	56.3%	2117	56.0%	443	57.8%	0.43
Vaginal insertion (non-menstruation related)							
Daily	598	13.2%	504	13.4%	94	12.3%	0.83
Weekly	159	3.5%	130	3.5%	29	3.8%	
Monthly or less	189	4.2%	156	4.1%	33	4.3%	
None	3589	79.1%	2979	79.0%	610	79.6%	
ANY	946	20.8%	790	20.9%	156	20.4%	0.58
Diaphragm arm	2273	50.0%	1890	50.0%	383	50.0%	0.99
Positive for STI (enrollment)							
Chlamydia	195	4.3%	155	4.1%	40	5.2%	0.16
Gonorrhea	34	0.8%	29	0.8%	5	0.7%	0.74
Trichomoniasis	166	3.7%	133	3.5%	33	4.3%	0.29
HSV-2	2656	58.4%	2254	59.6%	402	52.5%	**<0.01**

Table 4.2 Risk behaviors reported at follow-up visits by 4,549 women and the association with pregnancy status (unadjusted odds ratios) (N=24,337 visits)

Reported behaviors at follow-up	Total		Pregnant visits		Oral HC use visits		Injectable HC use visits		No HC use visits	
	N	%	N	%	N	%	N	%	N	%
	24337	100.0%	1609	6.6%	8375	34.4%	6028	24.8%	8325	34.2%
Any vaginal sex since last visit	22933	94.2%	1520	94.5%	8172	97.6%	5637	93.5%	7604	91.3%
Sex ≥3 times per week	15948	65.5%	1045	65.0%	6049	72.2%	3908	64.8%	4946	59.4%
Condom use (male or female) at last sex	16856	69.3%	993	61.7%	5812	69.4%	4122	68.4%	5929	71.2%
Unprotected sex (any report)	14334	58.9%	1172	72.8%	4991	59.6%	3663	60.8%	4508	54.1%
Sex in exchange for money or drugs	728	3.0%	39	2.4%	259	3.1%	195	3.2%	235	2.8%
2 or more male sex partners since last visit	1584	6.5%	72	4.5%	254	3.0%	452	7.5%	806	9.7%
New sex partner	2831	11.6%	159	9.9%	720	8.6%	846	14.0%	1106	13.3%
Suspects/knows partner concurrency	6064	24.9%	365	22.7%	1647	19.7%	1684	27.9%	2368	28.5%
High risk sex*	4415	18.1%	244	15.2%	1268	15.1%	1240	20.6%	1663	20.0%
Vaginal washing										
Daily	13312	54.7%	893	55.5%	4938	59.0%	3150	52.3%	4331	52.0%
Weekly	2819	11.6%	198	12.3%	742	8.9%	756	12.5%	1123	13.5%
Monthly or less	2197	9.0%	130	8.1%	837	10.0%	549	9.1%	681	8.2%
None (ref)	5901	24.3%	380	23.6%	1839	22.0%	1547	25.7%	2135	25.7%
ANY	18328	75.3%	1221	75.9%	6517	77.8%	4455	73.9%	6135	73.7%
Vaginal wiping										
Daily	7096	29.2%	460	28.6%	2759	32.9%	1579	26.2%	2298	27.6%
Weekly	1581	6.5%	90	5.6%	456	5.4%	413	6.9%	622	7.5%
Monthly or less	1223	5.0%	72	4.5%	448	5.4%	317	5.3%	386	4.6%
None (ref)	14329	58.9%	979	60.9%	4693	56.0%	3693	61.3%	4964	59.6%
ANY	9900	40.7%	622	38.7%	3663	43.7%	2309	38.3%	3306	39.7%
Vaginal insertion (non-menstruation related)										
Daily	1823	7.5%	92	5.7%	696	8.3%	414	6.9%	621	7.5%
Weekly	530	2.2%	30	1.9%	173	2.1%	119	2.0%	208	2.5%
Monthly or less	798	3.3%	37	2.3%	293	3.5%	182	3.0%	286	3.4%
None (ref)	21076	86.6%	1442	89.6%	7194	85.9%	5285	87.7%	7155	85.9%
ANY	3151	13.0%	159	9.9%	1162	13.9%	715	11.9%	1115	13.4%

Table 4.2 continued

Reported behaviors at follow-up	OR pregnant vs. OC			OR pregnant vs. injectable			OR pregnant vs. no HC use		
	OR	95%CI	p value	OR	95%CI	p value	OR	95%CI	p value
Any vaginal sex since last visit	**1.7**	**1.3-2.3**	**<0.0001**	0.8	0.6-1.1	0.12	0.6	0.5-0.8	**<0.01**
Sex ≥3 times per week	**1.5**	**1.3-1.7**	**<0.0001**	0.9	0.8-1.1	0.42	0.8	0.8-0.9	**0.01**
Condom use (male or female) at last sex	**1.3**	**1.1-1.5**	**<0.01**	**1.3**	**1.2-1.5**	**<0.0001**	**1.5**	**1.3-1.7**	**<0.0001**
Unprotected sex (any report)	**0.7**	**0.6-0.8**	**<0.0001**	**0.7**	**0.6-0.8**	**<0.0001**	**0.6**	**0.5-0.7**	**<0.0001**
Sex in exchange for money or drugs	1.5	1.0-2.1	**0.04**	1.5	1.0-2.2	0.05	1.4	1.0-2.0	0.07
2 or more male sex partners since last visit	1.1	0.8-1.5	0.65	**2.0**	**1.4-2.7**	**<0.0001**	**2.5**	**1.9-3.4**	**<0.0001**
New sex partner	1.0	0.8-1.2	0.92	**1.4**	**1.2-1.7**	**<0.01**	**1.5**	**1.2-1.8**	**<0.0001**
Suspects/knows male partner concurrency	1.0	0.9-1.3	0.80	**1.3**	**1.2-1.6**	**<0.0001**	**1.3**	**1.1-1.4**	**<0.01**
High risk sex*	1.1	1.0-1.3	0.10	**1.5**	**1.2-1.8**	**<0.0001**	**1.5**	**1.3-1.8**	**<0.0001**
Vaginal washing									
Daily	1.2	1.0-1.3	0.01	1.1	1.0-1.2	0.16	1.1	0.9-1.2	0.36
Weekly	1.0	0.8-1.2	0.97	1.1	0.9-1.3	0.32	1.1	1.0-1.3	0.15
Monthly or less	**1.5**	**1.2-1.8**	**<0.01**	1.2	1.0-1.5	0.08	1.1	0.9-1.4	0.37
None (ref)	1.0	-	-	1.0	-	-	1.0	-	-
ANY	**1.2**	**1.1-1.3**	**<0.01**	1.1	1.0-1.3	0.10	1.1	1.0-1.2	0.09
Vaginal wiping									
Daily	**1.3**	**1.2-1.5**	**<0.0001**	1.1	1.0-1.3	0.11	**1.2**	**1.1-1.4**	**<0.01**
Weekly	1.2	1.0-1.5	0.13	1.3	1.0-1.6	0.03	**1.4**	**1.1-1.7**	**<0.01**
Monthly or less	**1.4**	**1.1-1.7**	**0.02**	1.2	0.9-1.6	0.18	1.1	0.9-1.5	0.30
None (ref)	1.0	-	-	1.0	-	-	1.0	-	-
ANY	**1.3**	**1.2-1.5**	**<0.0001**	**1.2**	**1.0-1.3**	**0.01**	**1.3**	**1.1-1.4**	**<0.0001**
Vaginal insertion (non-menstruation related)									
Daily	**1.5**	**1.2-1.9**	**<0.0001**	1.3	1.0-1.6	0.05	**1.4**	**1.1-1.8**	**<0.01**
Weekly	1.4	1.0-2.1	0.08	1.2	0.8-1.8	0.47	**1.5**	**1.0-2.2**	**0.04**
Monthly or less	**1.6**	**1.2-2.7**	**<0.01**	1.3	0.9-1.9	0.11	**1.6**	**1.2-2.8**	**<0.01**
None (ref)	1.0	-	-	1.0	-	-	1.0	-	-
ANY	**1.5**	**1.3-1.8**	**<0.0001**	**1.3**	**1.0-1.5**	**0.02**	**1.5**	**1.2-1.8**	**<0.0001**

*High risk sex: exchange, anal sex, >2 partners, sex with drugs/alcohol

Table 4.3. Risk behaviors reported at follow-up visits by 4,549 women and the association with pregnancy status distinguishing established and new pregnancies* (unadjusted odds ratios) (N=24,337 visits)

**Established pregnancies defined as those identified at previous visit while newly pregnant is first visit of pregnancy*

Reported behaviors at follow-up	Total		Established pregnant visits		Newly pregnant visits		Oral HC use visits		Injectable HC use visits		No HC use visits	
	N	%	N	%	N	%	N	%	N	%	N	%
	24337	100.0%	809	3.3%	800	3.3%	8376	34.4%	6028	24.8%	8325	34.2%
Any vaginal sex since last visit	22933	94.2%	754	93.2%	766	95.8%	8173	97.6%	5636	93.5%	7604	91.3%
Sex ≥3 times per week	15948	65.5%	500	61.8%	545	68.1%	6050	72.2%	3907	59.4%	4946	59.4%
Condom use (male or female) at last sex	16856	69.3%	507	62.7%	486	60.8%	5812	69.4%	4122	68.4%	5929	71.2%
Unprotected sex (any report)	14334	58.9%	582	71.9%	590	73.8%	4992	59.6%	3662	60.8%	4508	54.1%
Sex in exchange for money or drugs	728	3.0%	22	2.7%	17	3.0%	259	3.1%	195	3.2%	235	2.8%
2 or more male sex partners since last visit	1584	6.5%	20	2.5%	52	6.5%	254	3.0%	452	7.5%	806	9.7%
New sex partner	2831	11.6%	68	8.4%	91	11.4%	720	8.6%	846	14.0%	1106	13.3%
Suspects/knows partner concurrency	6064	24.9%	169	20.9%	196	24.5%	1648	19.7%	1638	27.9%	2368	28.5%
High risk sex*	4415	18.1%	109	13.5%	135	16.9%	1268	15.1%	1240	20.6%	1663	20.0%
Vaginal washing												
Daily	13312	54.7%	461	57.0%	432	54.0%	4938	59.0%	3150	52.3%	4331	52.0%
Weekly	2819	11.6%	92	11.4%	106	13.3%	742	8.9%	756	12.5%	1123	13.5%
Monthly or less	2197	9.0%	57	7.1%	73	9.1%	837	10.0%	549	9.1%	681	8.2%
None (ref)	5901	24.3%	198	24.5%	182	22.8%	1839	22.0%	1547	25.7%	2135	25.7%
ANY	18328	75.3%	610	75.4%	611	76.4%	6518	77.8%	4454	73.9%	6135	73.7%
Vaginal wiping												
Daily	7096	29.2%	237	29.3%	223	27.9%	2759	32.9%	1579	26.2%	2298	27.6%
Weekly	1581	6.5%	44	5.4%	46	5.8%	456	5.4%	413	6.9%	622	7.5%
Monthly or less	1223	5.0%	30	3.7%	42	5.3%	448	5.4%	317	5.3%	386	4.6%
None (ref)	14329	58.9%	497	61.4%	482	60.3%	4693	56.0%	3693	61.3%	4964	59.6%
ANY	9900	40.7%	311	38.4%	311	38.9%	3663	43.7%	2309	38.3%	3306	39.7%
Vaginal insertion (non-menstruation related)												
Daily	1823	7.5%	48	5.9%	44	5.5%	696	8.3%	414	6.9%	621	7.5%
Weekly	530	2.2%	15	1.9%	15	1.9%	173	2.1%	119	2.0%	208	2.5%
Monthly or less	798	3.3%	17	2.1%	20	2.5%	293	3.5%	182	3.0%	286	3.4%
None (ref)	21076	86.6%	728	90.0%	714	89.3%	7194	85.9%	5285	87.7%	7155	85.9%
ANY	3151	13.0%	80	9.9%	79	9.9%	1162	13.9%	715	11.9%	1115	13.4%

Table 4.3 continued

Reported behaviors at follow-up	OR established pregnant vs. newly pregnant			OR established pregnant vs. OC use			OR established pregnant vs. injectable			OR established pregnant vs. no HC use		
	OR	95%CI	p value	OR	95%CI	p value	OR	95%CI	p value	OR	95%CI	p value
Any vaginal sex since last visit	**2.3**	**1.6-3.1**	**<0.0001**	**1.8**	**1.6-2.7**	**0.01**	1.1	0.8-1.4	0.76	0.2	0.6-1.1	0.13
Sex ≥3 times per week	**1.8**	**1.6-2.1**	**<0.0001**	**1.5**	**1.3-1.8**	**<0.0001**	1.2	1.0-1.4	0.05	1.1	0.9-1.2	0.51
Condom use (male or female) at last sex	1.2	0.9-1.4	0.11	0.8	0.7-1.0	0.09	1.2	1.0-1.5	0.05	**1.4**	**1.1-1.6**	**<0.01**
Unprotected sex (any report)	**0.7**	**0.6-0.9**	**<0.01**	1.1	0.9-1.4	0.16	**0.7**	**0.6-0.9**	**<0.01**	**0.6**	**0.5-0.8**	**<0.0001**
Sex in exchange for money or drugs	1.3	0.9-2.0	0.22	0.8	0.5-1.3	0.38	1.3	0.8-2.1	0.22	1.3	0.8-1.9	0.29
2 or more male sex partners since last visit	**2.0**	**1.1-3.5**	**0.02**	**2.5**	**1.4-4.5**	**<0.01**	**3.6**	**2.0-6.5**	**<0.0001**	**4.7**	**2.6-8.3**	**<0.0001**
New sex partner	1.2	0.9-1.5	0.19	**1.4**	**1.1-1.8**	**0.03**	**1.7**	**1.3-2.2**	**<0.0001**	**1.8**	**1.4-2.9**	**<0.0001**
Suspects/knows male partner concurrency	1.0	0.9-1.2	0.69	1.1	0.9-1.3	0.32	**1.4**	**1.2-1.7**	**<0.01**	**1.3**	**1.1-1.6**	**<0.01**
High risk sex*	**1.3**	**1.1-1.7**	**0.01**	1.3	1.1-1.7	0.03	**1.7**	**1.4-2.2**	**<0.0001**	**1.8**	**1.4-2.2**	**<0.0001**
Vaginal washing												
Daily	**1.2**	**1.1-1.4**	**0.02**	1.0	0.9-1.2	0.63	1.1	0.9-1.3	0.16	1.1	0.9-1.2	0.32
Weekly	1.1	0.9-1.3	0.61	1.1	0.9-1.4	0.36	1.2	0.9-1.4	0.19	1.2	0.9-1.5	0.10
Monthly or less	1.7	1.3-2.2	<0.0001	1.3	1.0-1.8	0.04	1.4	1.1-1.8	0.01	1.3	1.0-1.7	0.06
None (ref)												
ANY	**1.3**	**1.1-1.5**	**<0.01**	1.1	0.9-1.3	0.24	1.2	1.0-1.4	0.05	1.2	1.0-1.3	0.05
Vaginal wiping												
Daily	**1.4**	**1.2-1.7**	**<0.0001**	1.1	0.9-1.3	0.15	1.2	1.0-1.4	0.36	**1.3**	**1.1-1.5**	**<0.01**
Weekly	1.2	0.9-1.7	0.12	1.1	0.8-1.5	0.54	**1.4**	**1.0-1.8**	**0.04**	**1.4**	**1.1-1.9**	**0.01**
Monthly or less	**1.7**	**1.2-2.4**	**<0.01**	**1.5**	**1.1-2.2**	**0.04**	**1.5**	**1.1-2.2**	**0.03**	1.4	1.0-2.0	0.05
None (ref)												
ANY	**1.5**	**1.3-1.7**	**<0.0001**	**1.2**	**1.0-1.4**	**.03**	**1.3**	**1.1-1.5**	**<0.01**	**1.4**	**1.2-1.6**	**<0.0001**
Vaginal insertion (non-menstruation related)												
Daily	**1.6**	**1.2-2.2**	**<0.01**	1.1	0.7-1.6	0.73	1.3	0.9-1.8	0.11	**1.5**	**1.1-2.0**	**0.02**
Weekly	1.4	0.8-2.4	0.18	1.0	0.5-1.9	0.95	1.2	0.7-2.0	0.56	**1.5**	**0.9-2.6**	**0.12**
Monthly or less	**1.8**	**1.2-2.7**	**0.01**	1.2	0.7-2.1	0.56	1.5	0.9-2.3	0.10	**1.8**	**1.8-2.8**	**0.01**
None (ref)												
ANY	**1.6**	**1.3-2.1**	**<0.01**	1.1	0.8-1.4	0.61	**1.3**	**1.0-1.7**	**0.04**	**1.6**	**1.2-2.0**	**<0.01**

*High risk sex: exchange, anal sex, ≥2 partners, sex with drugs/alcohol

Table 4.4 Incidence rates of four STIs by pregnancy and hormonal contraceptive exposure group

	Overall			Pregnant visits			Non-pregnant & oral HC use visits			Non-pregnant & injectable HC use visits			Non-pregnant & no HC use		
	Cases	Person years	**IR p/Pty**	Cases	Person years	**IR p/yr**	Cases	Person years	**IR p/yr**	Cases	Person years	**IR p/yr**	Cases	Person years	**IR p/yr**
Chlamydia	400	5935	**6.7**	38	383	**9.9**	84	2066	**4.1**	146	1440	**10.1**	132	2045	**6.5**
Gonorrhea	165	6130	**2.7**	20	406	**4.9**	34	2114	**1.6**	49	1508	**3.2**	62	2103	**2.9**
Trichomoniasis	420	5935	**7.1**	36	390	**9.2**	115	2040	**5.6**	109	1469	**7.4**	160	2036	**7.9**
HIV	240	6199	**3.9**	16	417	**3.8**	56	2127	**2.6**	74	1530	**4.8**	94	2124	**4.4**

Additional analysis distinguishing between established pregnancies (pregnant for full visit interval) vs newly pregnant

	Overall			Established pregnant visits			Newly pregnant visits			Non-pregnant & oral HC use visits			Non-pregnant & injectable HC use visits			Non-pregnant & no HC use		
	Cases	Person years	**IR p/yr**	Cases	Person years	**IR p/yr**	Cases	Person years	**IR p/yr**	Cases	Person years	**IR p/yr**	Cases	Person years	**IR p/yr**	Cases	Person years	**IR p/yr**
Chlamydia	400	5935	**6.7**	7	182	**3.8**	31	201	**15.4**	84	2067	**4.1**	146	1440	**10.1**	132	2045	**6.5**
Gonorrhea	165	6130	**2.7**	12	194	**6.2**	8	212	**3.8**	34	2114	**1.6**	49	1508	**3.2**	62	2103	**2.9**
Trichomoniasis	420	5935	**7.1**	17	183	**9.3**	19	207	**9.2**	115	2040	**5.6**	109	1469	**7.4**	160	2036	**7.9**
HIV	240	6199	**3.9**	6	200	**3.0**	10	217	**4.6**	56	2127	**2.6**	74	1530	**4.8**	94	2124	**4.4**

Table 4.5a Unadjusted and adjusted hazard ratios estimating the relationship between pregnancy status and incident chlamydia infections (N=4,549)

	Unadjusted relative hazard (HR) of incident **chlamydia**			Adjusted relative hazard (HR)* of incident **chlamydia**		
	HR	95%CI	p value	HR	95%CI	p value
Pregnancy exposure group						
Pregnant	**1.5**	**1.1-2.2**	**0.02**	1.2	0.8-1.8	0.28
Not pregnant/oral contraceptive use	**0.6**	**0.5-0.9**	**<0.01**	0.9	0.6-1.3	0.53
Not pregnant/injectable contraceptive	**1.6**	**1.2-2.0**	**<0.01**	1.3	1.0-1.7	0.07
Not pregnant/no hormonal contraceptives	1.0	-	NA	1.0	-	NA
Time fixed covariates						
Study location						
Harare, Zimbabwe	1.0	-	NA	1.0	-	NA
Johannesburg, South Africa	**2.7**	**2.3-3.6**	**<0.0001**	**2.4**	**1.7-3.2**	**<0.0001**
Durban, South Africa	**2.9**	**2.2-3.7**	**<0.0001**	**2.5**	**1.8-3.5**	**<0.0001**
Age						
18-24	**5.0**	**3.5-7.1**	**<0.0001**	**5.6**	**3.8-8.2**	**<0.0001**
25-34	**2.1**	**1.4-3.1**	**<0.01**	**2.4**	**1.7-3.6**	**<0.0001**
≥35	1.0		NA	1.0		NA
Completed high school (11 years school)	1.5	1.2-1.8	<0.01	1.1	0.9-1.3	0.51
Diaphragm randomization arm (ref: control)	1.2	1.0-1.5	0.07	1.2	0.9-1.5	0.12
Contraceptive use at enrollment						
None	1.0	-	NA	1.0	-	NA
Oral contraceptives	**0.7**	**0.5-0.9**	**0.03**	1.0	0.7-1.7	0.86
Injectable contraceptives	1.4	1.0-2.1	0.05	1.0	0.7-1.5	0.95
Other (diaphragm, IUD, condoms, vasectomy)	1.2	0.8-1.7	0.35	1.1	0.8-1.6	0.59
High risk sex at enrollment	1.3	1.0-1.6	0.03	1.2	0.9-1.5	0.08
Partner high risk at enrollment	1.3	1.0-1.5	0.03	1.1	0.8-1.3	0.66
STI at enrollment**	1.2	0.9-1.5	0.07	**1.5**	**1.2-1.9**	**<0.01**
Time varying covariates						
Vaginal sex since last visit (ref:none)	0.9	0.6-1.4	0.58	1.0	0.6-1.6	0.95
≥3 Vaginal sex acts per week (ref:0-2)	0.9	0.8-1.2	0.19	1.2	0.9-1.5	0.13
No condom use at last sex	1.1	0.8-1.3	0.62	0.9	0.7-1.2	0.54
Unprotected sex since last visit	**1.4**	**1.1-1.7**	**<0.01**	**1.3**	**1.0-1.7**	**0.04**
High risk sex^	**1.7**	**1.3-2.1**	**<0.0001**	1.2	0.9-1.6	0.09
Suspects/knows partner concurrency	**1.6**	**1.3-2.0**	**<0.0001**	**1.3**	**1.0-1.6**	**0.04**
Vaginal washing (any reported)	1.0	0.8-1.2	0.86	0.9	0.7-1.2	0.68
Vaginal wiping (any reported)	0.9	0.7-1.1	0.35	0.9	0.7-1.2	0.51
Insertion of paper products (any reported)	1.1	0.9-1.5	0.38	1.3	0.9-1.7	0.12

*Multivariable models were adjusted for all covariates listed in the table

**STIs at enrollment: chlamydia, gonorrhea, trichomoniasis, HSV-2

^High risk sex: exchange, anal sex, >2 partners, new partner, new regular partner

Table 4.5b Unadjusted and adjusted hazard ratios estimating the relationship between pregnancy status and incident gonorrhea infections (N=4,549)

	Unadjusted relative hazard (HR) of incident **gonorrhea**			Adjusted relative hazard (HR)* of incident **gonorrhea**		
	HR	95%CI	p value	HR	95%CI	p value
Pregnancy exposure group						
Pregnant	1.6	0.9-2.6	0.07	1.5	0.9-5.2	0.14
Not pregnant/oral contraceptive use	0.6	0.4-0.8	0.01	0.8	0.5-1.4	0.47
Not pregnant/injectable contraceptive	1.1	0.8-1.6	0.65	0.9	0.6-1.5	0.73
Not pregnant/no hormonal contraceptives	1.0	-	NA	1.0	-	NA
Time fixed covariates						
Study location						
Harare, Zimbabwe	1.0	-	NA	1.0	-	NA
Johannesburg, South Africa	**2.9**	**2.0-4.2**	**<0.0001**	**2.2**	**1.4-3.6**	**<0.01**
Durban, South Africa	**2.6**	**1.7-3.9**	**<0.0001**	**2.2**	**1.3-3.8**	**<0.01**
Age						
18-24	**4.2**	**2.5-7.06**	**<0.0001**	**5.2**	**3.0-9.2**	**<0.0001**
25-34	**1.8**	**1.0-3.2**	**0.04**	**2.3**	**1.3-4.2**	**0.01**
≥35	1.0	-	NA	1.0	-	NA
Completed high school (11 years school)	1.3	0.9-1.8	0.09	1.0	0.7-1.4	0.87
Diaphragm randomization arm (ref: control)	0.9	0.7-1.3	0.69	1.1	0.8-1.5	0.70
Contraceptive use at enrollment						
None	1.0	-	NA	1.0	-	NA
Oral contraceptives	0.9	0.5-1.7	0.73	1.7	0.8-3.8	0.16
Injectable contraceptives	1.6	0.9-3.0	0.14	1.4	0.7-2.8	0.31
Other (diaphragm, IUD, condoms, vasectomy)	1.8	0.9-3.3	0.07	1.7	0.9-3.1	0.11
High risk sex at enrollment	0.8	0.5-1.2	0.22	0.7	0.5-1.0	0.06
Partner high risk at enrollment	**1.8**	**1.3-2.5**	**<0.01**	1.4	0.9-2.0	0.07
STI at enrollment**	**1.9**	**1.3-2.7**	**<0.01**	**2.2**	**1.5-3.1**	**<0.0001**
Time varying covariates						
Vaginal sex since last visit (ref:none)	1.0	0.5-2.0	1.00	1.3	0.6-2.7	0.52
≥3 Vaginal sex acts per week (ref:0-2)	**0.7**	**0.5-0.9**	**0.03**	0.8	0.6-1.2	0.30
No condom use at last sex	**0.6**	**0.4-0.9**	**0.02**	**0.6**	**0.4-0.9**	**0.01**
Unprotected sex since last visit	1.1	0.8-1.5	0.47	1.2	0.9-1.8	0.25
High risk sex^	**1.9**	**1.4-2.7**	**<0.01**	**1.5**	**1.1-2.2**	**0.02**
Suspects/knows partner concurrency	**2.4**	**1.8-3.3**	**<0.0001**	**1.8**	**1.3-2.5**	**<0.01**
Vaginal washing	1.1	0.8-1.6	0.64	1.1	0.8-1.7	0.59
Vaginal wiping	1.0	0.7-1.4	0.93	1.0	0.7-1.5	0.80
Insertion of paper products	0.9	0.5-1.4	0.57	0.9	0.5-1.5	0.71

*Multivariable models were adjusted for all covariates listed in the table

**STIs at enrollment: chlamydia, gonorrhea, trichomoniasis, HSV-2

^High risk sex: exchange, anal sex, >2 partners, new partner, new regular partner

Table 4.5c Unadjusted and adjusted hazard ratios estimating the relationship between pregnancy status and incident trichomoniasis infections (N=4,549)

	Unadjusted relative hazard (HR) of incident **trichomoniasis**			Adjusted relative hazard (HR)* of incident **trichomoniasis**		
	HR	95%CI	p value	HR	95%CI	p value
Pregnancy exposure group						
Pregnant	1.1	0.8-1.6	0.51	1.3	0.9-1.9	0.24
Not pregnant/oral contraceptive use	**0.7**	**0.6-0.9**	**0.01**	0.9	0.7-6-1.3	0.65
Not pregnant/injectable contraceptive	0.9	0.7-1.2	0.60	1.0	0.7-1.4	0.98
Not pregnant/no hormonal contraceptives	1.0	-	NA	1.0	-	NA
Time fixed covariates						
Study location						
Harare, Zimbabwe	1.0	-	NA	1.0	-	NA
Johannesburg, South Africa	**1.7**	**1.4-2.2**	**<0.0001**	**1.5**	**1.2-2.0**	**<0.01**
Durban, South Africa	1.3	0.9-1.7	0.07	1.3	0.9-1.8	0.14
Age						
18-24	1.0	0.8-1.2	0.75	1.2	0.9-1.6	0.12
25-34	**0.7**	**0.6-0.9**	**0.01**	0.9	0.7-1.2	0.46
≥35	1.0	-	NA	1.0	-	NA
Completed high school (11 years school)	**0.7**	**0.6-0.9**	**<0.01**	0.8	0.7-1.0	0.06
Diaphragm randomization arm (ref: control)	0.9	0.8-1.1	0.41	0.9	0.8-1.2	0.36
Contraceptive use at enrollment						
None	1.0	-	NA	1.0	-	NA
Oral contraceptives	**0.5**	**0.4-0.7**	**<0.0001**	0.8	0.6-1.2	0.31
Injectable contraceptives	**0.7**	**0.5-0.9**	**0.01**	0.8	0.5-1.1	0.12
Other (diaphragm, IUD, condoms, vasectomy)	**0.6**	**0.4-0.8**	**<0.01**	**0.6**	**0.4-0.8**	**<0.01**
High risk sex at enrollment	1.2	0.9-1.5	0.07	1.1	0.9-1.4	0.27
Partner high risk at enrollment	**1.4**	**1.1-1.7**	**<0.01**	1.2	0.9-1.5	0.11
STI at enrollment**	**2.0**	**1.6-2.4**	**<0.0001**	**1.8**	**1.4-2.3**	**<0.0001**
Time varying covariates						
Vaginal sex since last visit (ref:none)	0.8	0.6-1.2	0.33	0.9	0.6-1.5	0.78
≥3 Vaginal sex acts per week (ref:0-2)	**0.8**	**0.6-0.9**	**0.03**	0.9	0.7-1.2	0.40
No condom use at last sex	1.1	0.8-1.3	0.65	1.0	0.8-1.4	0.79
Unprotected sex since last visit	1.2	0.9-1.4	0.11	1.1	0.9-1.4	0.46
High risk sex^	**1.4**	**1.1-1.8**	**<0.01**	1.1	0.9-1.4	0.31
Suspects/knows partner concurrency	**1.5**	**1.2-1.8**	**<0.01**	**1.3**	**1.0-1.6**	**0.03**
Vaginal washing	1.0	0.8-1.28	0.93	1.0	0.8-1.3	0.98
Vaginal wiping	1.0	0.8-1.3	0.64	0.9	0.7-1.16	0.51
Insertion of paper products	**1.4**	**1.1-1.8**	**0.02**	1.3	0.9-1.8	0.06

*Multivariable models were adjusted for all covariates listed in the table

**STIs at enrollment: chlamydia, gonorrhea, trichomoniasis, HSV-2

^High risk sex: exchange, anal sex, >2 partners, new partner, new regular partner

Table 4.5d Unadjusted and adjusted hazard ratios estimating the relationship between pregnancy status and incident HIV infections (N=4,549)

	Unadjusted relative hazard (HR) of incident **HIV**			Adjusted relative hazard (HR)* of incident **HIV**		
	HR	95%CI	p value	HR	95%CI	p value
Pregnancy exposure group						
Pregnant	0.8	05-1.3	0.36	0.7	0.4-1.2	0.20
Not pregnant/oral contraceptive use	**0.6**	**0.4-0.8**	**<0.01**	0.8	0.5-1.3	0.39
Not pregnant/injectable contraceptive	1.1	0.8-1.5	0.67	0.8	0.6-1.2	0.27
Not pregnant/no contraceptive use	1.0	-	NA	1.0	-	NA
Time fixed covariates						
Study location						
Harare, Zimbabwe	1.0	-	NA	1.0	-	NA
Johannesburg, South Africa	**2.7**	**2.0-3.5**	**<0.0001**	**2.0**	**1.7-2.9**	**<0.01**
Durban, South Africa	1.3	0.9-1.9	0.18	1.0	0.6-1.6	0.95
Age						
18-24	**2.1**	**1.4-3.0**	**<0.0001**	**2.6**	**1.7-3.9**	**<0.0001**
25-34	1.3	0.9-2.0	0.14	**1.7**	**1.1-2.5**	**0.02**
≥35	1.0	-	NA	1.0	-	NA
Completed high school (11 years school)	1.2	0.9-1.5	0.19	1.1	0.9-1.5	0.33
Diaphragm randomization arm (ref: control)	1.0	0.8-1.3	0.98	1.0	0.8-1.3	0.92
Contraceptive use at enrollment						
None	1.0	-	NA	1.0	-	NA
Oral contraceptives	0.8	0.5-1.4	0.53	1.3	0.7-2.5	0.37
Injectable contraceptives	**1.8**	**1.1-3.0**	**0.03**	**1.8**	**1.0-3.1**	**0.04**
Other (diaphragm, IUD, condoms, vasectomy)	1.4	0.9-2.4	0.17	1.5	0.9-2.5	0.16
High risk sex at enrollment	1.1	0.8-1.5	0.56	1.1	0.8-1.5	0.47
Partner high risk at enrollment	**1.6**	**1.2-2.1**	**<0.01**	1.3	0.9-1.7	0.12
STI at enrollment**	**2.0**	**1.5-2.7**	**<0.0001**	**2.1**	**1.5-2.8**	**<0.0001**
Time varying covariates						
Vaginal sex since last visit (ref:none)	1.1	0.6-1.9	0.86	1.1	0.6-2.1	0.76
≥3 Vaginal sex acts per week (ref:0-2)	0.8	0.6-1.0	0.07	0.9	0.6-1.2	0.31
No condom use at last sex	1.0	0.7-1.3	0.98	0.9	0.7-1.3	0.14
Unprotected sex since last visit	1.3	0.9-1.6	0.09	1.3	0.9-1.7	0.15
High risk sex^	**1.5**	**1.1-2.1**	**0.01**	1.1	0.8-1.6	0.42
Suspects/knows partner concurrency	**2.0**	**1.5-2.6**	**<0.0001**	**1.5**	**1.1-2.0**	**0.01**
Vaginal washing	1.1	0.8-1.4	0.72	0.9	0.7-1.3	0.56
Vaginal wiping	**1.4**	**1.1-1.8**	**0.01**	**1.5**	**1.1-2.0**	**0.01**
Insertion of paper products	1.3	0.9-1.8	0.18	1.1	0.8-1.7	0.50

*Multivariable models were adjusted for all covariates listed in the table

**STIs at enrollment: chlamydia, gonorrhea, trichomoniasis, HSV-2

^High risk sex: exchange, anal sex, >2 partners, new partner, new regular partner

Table 4.5e Unadjusted and adjusted hazard ratios estimating the relationship between pregnancy status and first incidence of any of the four STIs (chlamydia, gonorrhea, trichomoniasis or HIV) (N=4,549)

	Unadjusted relative hazard (HR) of incidence of any STI			Adjusted relative hazard (HR)* of incidence of any STI		
	HR	95%CI	p value	HR	95%CI	p value
Pregnancy exposure group						
Pregnant	1.2	0.9-1.5	0.18	1.1	0.9-1.4	0.38
Not pregnant/oral contraceptive use	0.7	0.6-0.8	<0.0001	0.8	0.7-1.0	0.12
Not pregnant/injectable contraceptive	1.1	1.0-1.3	0.12	1.0	0.9-1.3	0.67
Not pregnant/no hormonal contraceptives	1.0	-	NA	1.0	-	NA
Time fixed covariates						
Study location						
Harare, Zimbabwe	1.0	-	NA	1.0	-	NA
Johannesburg, South Africa	2.1	1.8-2.5	<0.0001	1.8	1.5-2.2	<0.0001
Durban, South Africa	1.8	1.5-2.1	<0.0001	1.6	1.3-2.0	<0.0001
Age						
18-24	1.8	1.5-2.2	<0.0001	2.3	1.9-2.8	<0.0001
25-34	1.1	0.9-1.3	0.56	1.3	1.1-1.6	0.01
≥35	1.0		NA	1.0	-	NA
Completed high school (11 years school)	1.1	0.9-1.2	0.35	1.0	0.9-1.1	0.89
Diaphragm randomization arm (ref: control)	1.0	0.9-1.1	0.96	1.0	0.9-1.2	0.88
Contraceptive use at enrollment						
None	1.0	-	NA	1.0	-	NA
Oral contraceptives	0.6	0.5-0.8	<0.01	1.0	0.8-1.4	0.77
Injectable contraceptives	1.0	0.8-1.3	0.94	0.9	0.7-1.2	0.51
Other (diaphragm, IUD, condoms, vasectomy)	0.9	0.7-1.1	0.41	0.9	0.7-1.2	0.51
High risk sex at enrollment	1.1	0.9-1.2	0.33	1.0	0.9-1.2	0.85
Partner high risk at enrollment	1.4	1.2-16	<0.0001	1.2	1.0-1.3	0.03
STI at enrollment**	1.6	1.4-1.9	<0.0001	1.8	1.5-2.0	<0.0001
Time varying covariates						
Vaginal sex since last visit (ref:none)	0.9	0.7-1.1	0.31	1.0	0.8-1.4	0.83
≥3 Vaginal sex acts per week (ref:0-2)	0.8	0.7-0.9	<0.01	1.0	0.8-1.1	0.72
No condom use at last sex	1.0	0.9-1.1	0.88	0.9	0.8-1.1	0.31
Unprotected sex since last visit	1.2	1.1-1.4	<0.01	1.2	1.0-1.4	0.01
High risk sex^	1.4	1.2-1.7	<0.0001	1.2	1.0-1.3	0.08
Suspects/knows partner concurrency	1.6	1.4-1.8	<0.0001	1.3	1.1-1.5	<0.01
Vaginal washing	1.0	0.9-1.2	0.91	1.0	0.8-1.1	0.70
Vaginal wiping	1.0	0.9-1.2	0.73	1.0	0.9-1.2	0.95
Insertion of paper products	1.2	1.0-1.4	0.12	1.2	1.0-1.4	0.13

*Other contraceptives at enrollment: diaphragm, IUD, condoms, male partner vasectomy
**STIs at enrollment: chlamydia, gonorrhea, trichomoniasis, HSV-2
***High risk sex: exchange, anal sex, >2 partners, new partner, new regular partner

Table 4.6a Unadjusted and adjusted hazard ratios estimating the relationship between pregnancy status and incident chlamydia infection distinguishing new and established pregnancies (N=4,549)

	Unadjusted relative hazard (HR) of incident **chlamydia**			Adjusted relative hazard (HR)* of incident **chlamydia**		
	HR	95%CI	p value	HR	95%CI	p value
Pregnancy exposure group						
Pregnant (established)	0.6	0.3-1.3	0.17	0.5	0.2-1.1	0.07
Newly pregnant	**2.4**	**1.6-3.5**	**<0.0001**	**1.9**	**1.3-2.8**	**<0.01**
Not pregnant/oral contraceptive use	**0.6**	**0.5-0.8**	**<0.01**	0.9	0.6-1.3	0.58
Not pregnant/injectable contraceptive	**1.6**	**1.2-2.0**	**<0.01**	1.3	0.9-1.7	0.08
Not pregnant/no hormonal contraceptives	1.0	-	NA	1.0	-	NA
Time fixed covariates						
Study location						
Harare, Zimbabwe	1.0	-	NA	1.0	-	NA
Johannesburg, South Africa	**2.7**	**2.3-3.6**	**<0.0001**	**2.4**	**1.7-3.2**	**<0.0001**
Durban, South Africa	**2.9**	**2.2-3.7**	**<0.0001**	**2.5**	**1.8-3.4**	**<0.0001**
Age						
18-24	**5.0**	**3.5-7.1**	**<0.0001**	**5.6**	**3.8-8.3**	**<0.0001**
25-34	**2.1**	**1.4-3.1**	**<0.01**	**2.5**	**1.7-3.7**	**<0.0001**
≥35	1.0		NA	1.0	-	NA
Completed high school (11 years school)	**1.5**	**1.2-1.8**	**<0.01**	1.1	0.9-1.4	0.40
Diaphragm randomization arm (ref: control)	1.2	1.0-1.5	0.07	1.2	0.9-1.4	0.15
Contraceptive use at enrollment						
None	1.0	-	NA	1.0	-	NA
Oral contraceptives	**0.7**	**0.5-0.9**	**0.03**	1.0	0.7-1.6	0.89
Injectable contraceptives	1.4	1.0-2.1	0.05	1.0	0.6-1.4	0.86
Other (diaphragm, IUD, condoms, vasectomy)	1.2	0.8-1.7	0.35	1.1	0.8-1.6	0.65
High risk sex at enrollment	**1.3**	**1.0-1.6**	**0.03**	1.2	1.0-1.6	0.06
Partner high risk at enrollment	**1.3**	**1.0-1.5**	**0.03**	1.0	0.8-1.3	0.69
STI at enrollment**	1.2	0.9-1.5	0.07	**1.5**	**1.2-1.8**	**<0.01**
Time varying covariates						
Vaginal sex since last visit (ref:none)	0.9	0.6-1.4	0.58	1.0	0.6-1.6	0.98
≥3 Vaginal sex acts per week (ref:0-2)	0.9	0.8-1.2	0.19	1.2	0.9-1.5	0.19
No condom use at last sex	1.1	0.8-1.3	0.62	0.9	0.7-1.2	0.55
Unprotected sex since last visit	**1.4**	**1.1-1.7**	**<0.01**	**1.3**	**1.0-1.7**	**0.04**
High risk sex^	**1.7**	**1.3-2.1**	**<0.0001**	1.2	1.0-1.2	0.10
Suspects/knows partner concurrency	**1.6**	**1.3-2.0**	**<0.0001**	**1.3**	**1.0-1.6**	**0.04**
Vaginal washing	1.0	0.8-1.2	0.86	1.0	0.7-1.2	0.15
Vaginal wiping	0.9	0.7-1.1	0.35	0.9	0.7-1.2	0.47
Insertion of paper products	1.1	0.9-1.5	0.38	1.3	0.9-1.7	0.12

*Multivariable models were adjusted for all covariates listed in the table

**STIs at enrollment: chlamydia, gonorrhea, trichomoniasis, HSV-2

^High risk sex: exchange, anal sex, >2 partners, new partner, new regular partner

Table 4.6b Unadjusted and adjusted hazard ratios estimating the relationship between pregnancy status and incident gonorrhea infection distinguishing new and established pregnancies (N=4,549)

	Unadjusted relative hazard (HR) of incident **gonorrhea**			Adjusted relative hazard (HR)* of incident **gonorrhea**		
	HR	95%CI	p value	HR	95%CI	p value
Pregnancy exposure group						
Pregnant (established)	1.9	1.0-3.6	0.04	1.8	0.9-3.4	0.08
Newly pregnant	1.3	0.6-2.7	0.52	1.2	0.6-2.5	0.68
Not pregnant/oral contraceptive use	**0.6**	**0.4-0.8**	**0.01**	0.8	0.5-1.4	0.46
Not pregnant/injectable contraceptive	1.1	0.8-1.6	0.66	0.9	0.6-1.4	0.69
Not pregnant/no hormonal contraceptives	1.0	-	NA	1.0	-	NA
Time fixed covariates						
Study location						
Harare, Zimbabwe	1.0	-	NA	1.0	-	NA
Johannesburg, South Africa	**2.9**	**2.0-4.2**	**<0.0001**	**2.2**	**1.4-3.6**	**<0.01**
Durban, South Africa	**2.6**	**1.7-3.9**	**<0.0001**	**2.3**	**1.3-3.8**	**<0.01**
Age (changed due to small number of outcomes)						
18-24	**4.2**	**2.5-7.06**	**<0.0001**	**5.3**	**3.0-9.3**	**<0.0001**
25-34	**1.8**	**1.0-3.2**	**0.04**	**2.3**	**1.3-4.2**	**0.01**
≥35	1.0	-	NA	1.0	-	NA
Completed high school (11 years school)	1.3	0.9-1.8	0.09	1.0	0.7-1.4	0.91
Diaphragm randomization arm (ref: control)	0.9	0.7-1.3	0.69	1.1	0.8-1.4	0.76
Contraceptive use at enrollment						
None	1.0	-	NA	1.0	-	NA
Oral contraceptives	0.9	0.5-1.7	0.73	1.7	0.8-3.8	0.15
Injectable contraceptives	1.6	0.9-3.0	0.14	1.4	0.7-2.8	0.31
Other (diaphragm, IUD, condoms, vasectomy)	1.8	0.9-3.3	0.07	1.7	0.9-3.2	0.10
High risk sex at enrollment	0.8	0.5-1.2	0.22	0.7	0.5-1.0	0.06
Partner high risk at enrollment	**1.8**	**1.3-2.5**	**<0.01**	1.4	1.0-2.0	0.06
STI at enrollment**	**1.9**	**1.3-2.7**	**<0.01**	**2.2**	**1.5-3.2**	**<0.0001**
Time varying covariates						
Vaginal sex since last visit (ref:none)	1.0	0.5-2.0	1.00	1.3	0.6-2.7	0.54
≥3 Vaginal sex acts per week (ref:0-2)	**0.7**	**0.5-0.9**	**0.03**	0.8	0.6-1.2	0.35
No condom use at last sex	0.6	0.4-0.9	0.02	0.6	0.4-0.9	0.01
Unprotected sex since last visit	1.1	0.8-1.5	0.47	1.2	0.9-1.7	0.27
High risk sex^	**1.9**	**1.4-2.7**	**<0.01**	**1.5**	**1.1-2.2**	**0.02**
Suspects/knows partner concurrency	**2.4**	**1.8-3.3**	**<0.0001**	**1.8**	**1.3-2.5**	**<0.01**
Vaginal washing	1.1	0.8-1.6	0.64	1.1	0.8-1.7	0.54
Vaginal wiping	1.0	0.7-1.4	0.93	1.0	0.7-1.5	0.83
Insertion of paper products	0.9	0.5-1.4	0.57	0.9	0.5-1.5	0.70

*Multivariable models were adjusted for all covariates listed in the table

**STIs at enrollment: chlamydia, gonorrhea, trichomoniasis, HSV-2

^High risk sex: exchange, anal sex, >2 partners, new partner, new regular partner

Table 4.6c Unadjusted and adjusted hazard ratios estimating the relationship between pregnancy status and incident trichomoniasis infection distinguishing new and established pregnancies (N=4,549)

	Unadjusted relative hazard (HR) of incident **trichomoniasis**			Adjusted relative hazard (HR)* of incident **trichomoniasis**		
	HR	95%CI	p value	HR	95%CI	p value
Pregnancy exposure group						
Pregnant (established)	1.1	0.7-1.8	0.69	1.2	0.7-2.0	0.49
Newly pregnant	1.1	0.7-1.8	0.57	1.2	0.8-2.0	0.39
Not pregnant/oral contraceptive use	**0.7**	**0.6-0.9**	**0.01**	0.9	0.6-1.3	0.56
Not pregnant/injectable contraceptive	0.9	0.7-1.2	0.60	1.0	0.7-1.3	0.80
Not pregnant/no hormonal contraceptives	1.0	-	NA	1.0	-	NA
Time fixed covariates						
Study location						
Harare, Zimbabwe	1.0	-	NA	1.0	-	NA
Johannesburg, South Africa	**1.7**	**1.4-2.2**	**<0.0001**	**1.5**	**1.2-2.0**	**<0.01**
Durban, South Africa	1.3	0.9-1.7	0.07	1.3	0.9-1.8	0.14
Age						
18-24	1.0	0.8-1.2	0.75	1.3	0.9-1.7	0.08
25-34	**0.7**	**0.6-0.9**	**0.01**	0.9	0.7-1.2	0.52
≥35	1.0	-	NA	1.0	-	NA
Completed high school (11 years school)	**0.7**	**0.6-0.9**	**<0.01**	0.8	0.7-1.0	0.06
Diaphragm randomization arm (ref: control)	0.9	0.8-1.1	0.41	0.9	0.8-1.1	0.49
Contraceptive use at enrollment						
None	1.0	-	NA	1.0	-	NA
Oral contraceptives	**0.5**	**0.4-0.7**	**<0.0001**	0.8	0.5-1.2	0.29
Injectable contraceptives	**0.7**	**0.5-0.9**	**0.01**	0.7	0.5-1.1	0.11
Other (diaphragm, IUD, condoms, vasectomy)	**0.6**	**0.4-0.8**	**<0.01**	**0.6**	**0.4-0.8**	**<0.01**
High risk sex at enrollment	1.2	0.9-1.5	0.07	1.1	0.9-1.4	0.28
Partner high risk at enrollment	**1.4**	**1.1-1.7**	**<0.01**	1.2	0.9-1.5	0.09
STI at enrollment**	**2.0**	**1.6-2.4**	**<0.0001**	**1.8**	**1.4-2.3**	**<0.0001**
Time varying covariates						
Vaginal sex since last visit (ref:none)	0.8	0.6-1.2	0.33	0.9	0.6-1.5	0.74
≥3 Vaginal sex acts per week (ref:0-2)	**0.8**	**0.6-0.9**	**0.03**	0.9	0.7-1.2	0.49
No condom use at last sex	1.1	0.8-1.3	0.65	1.0	0.8-1.4	0.84
Unprotected sex since last visit	1.2	0.9-1.4	0.11	1.1	0.7-1.4	0.44
High risk sex^	**1.4**	**1.1-1.8**	**<0.01**	1.1	0.9-1.4	0.31
Suspects/knows partner concurrency	**1.5**	**1.2-1.8**	**<0.01**	**1.3**	**1.0-1.6**	**0.03**
Vaginal washing	1.0	0.8-1.28	0.93	1.0	0.8-1.3	0.90
Vaginal wiping	1.0	0.8-1.3	0.64	0.9	0.7-1.2	0.51
Insertion of paper products	**1.4**	**1.1-1.8**	**0.02**	1.3	1.0-1.7	0.06

*Multivariable models were adjusted for all covariates listed in the table

**STIs at enrollment: chlamydia, gonorrhea, trichomoniasis, HSV-2

^High risk sex: exchange, anal sex, >2 partners, new partner, new regular partner

Table 4.6d Unadjusted and adjusted hazard ratios estimating the relationship between pregnancy status and incident HIV infection distinguishing new and established pregnancies (N=4,549)

	Unadjusted relative hazard (HR) of incident **HIV**			Adjusted relative hazard (HR)* of incident **HIV**		
	HR	95%CI	p value	HR	95%CI	p value
Pregnancy exposure group						
Pregnant (established)	0.6	0.3-1.3	0.18	0.5	0.2-1.2	0.13
Newly pregnant	1.0	0.5-1.9	1.00	0.9	0.5-1.8	0.82
Not pregnant/oral contraceptive use	**0.6**	**0.4-0.8**	**<0.01**	0.8	0.5-1.3	0.37
Not pregnant/injectable contraceptive	1.1	0.8-1.5	0.67	0.8	0.5-1.1	0.22
Not pregnant/no hormonal contraceptives	1.0	-	NA	1.0	-	NA
Time fixed covariates						
Study location						
Harare, Zimbabwe	1.0	-	NA	1.0	-	NA
Johannesburg, South Africa	**2.7**	**2.0-3.5**	**<0.0001**	**2.0**	**1.4-2.9**	**<0.01**
Durban, South Africa	1.3	0.9-1.9	0.18	1.0	0.6-1.5	0.95
Age						
18-24	**2.1**	**1.4-3.0**	**<0.0001**	**2.6**	**1.7-3.9**	**<0.0001**
25-34	1.3	0.9-2.0	0.14	**1.7**	**1.1-2.5**	**0.01**
≥35	1.0	-	NA	1.0	-	NA
Completed high school (11 years school)	1.2	0.9-1.5	0.19	1.2	0.9-1.6	0.22
Diaphragm randomization arm (ref: control)	1.0	0.8-1.3	0.98	1.0	0.8-1.3	0.95
Contraceptive use at enrollment						
None	1.0	-	NA	1.0	-	NA
Oral contraceptives	0.8	0.5-1.4	0.53	1.4	0.7-2.5	0.35
Injectable contraceptives	**1.8**	**1.1-3.0**	**0.03**	**1.8**	**1.1-3.2**	**0.03**
Other (diaphragm, IUD, condoms, vasectomy)	1.4	0.9-2.4	0.17	1.5	0.9-2.5	0.12
High risk sex at enrollment	1.1	0.8-1.5	0.56	1.1	0.8-1.5	0.56
Partner high risk at enrollment	**1.6**	**1.2-2.1**	**<0.01**	1.3	0.9-1.8	0.07
STI at enrollment**	**2.0**	**1.5-2.7**	**<0.0001**	**2.1**	**1.6-2.9**	**<0.0001**
Time varying covariates						
Vaginal sex since last visit (ref:none)	1.1	0.6-1.9	0.86	1.1	0.6-2.1	0.80
≥3 Vaginal sex acts per week (ref:0-2)	0.8	0.6-1.0	0.07	0.9	0.6-1.2	0.31
No condom use at last sex	1.0	0.7-1.3	0.98	0.9	0.7-1.3	0.73
Unprotected sex since last visit	1.3	0.9-1.6	0.09	1.2	0.9-1.7	0.18
High risk sex^	1.5	1.1-2.1	0.01	1.1	0.8-1.6	0.74
Suspects/knows partner concurrency	**2.0**	**1.5-2.6**	**<0.0001**	**1.5**	**1.1-1.9**	**0.01**
Vaginal washing	1.1	0.8-1.4	0.72	0.9	0.7-1.3	0.67
Vaginal wiping	**1.4**	**1.1-1.8**	**0.01**	**1.4**	**1.1-1.9**	**0.02**
Insertion of paper products	1.3	0.9-1.8	0.18	1.1	0.8-1.7	0.51

*Multivariable models were adjusted for all covariates listed in the table

**STIs at enrollment: chlamydia, gonorrhea, trichomoniasis, HSV-2

^High risk sex: exchange, anal sex, >2 partners, new partner, new regular partner

Table 4.6e Unadjusted and adjusted hazard ratios estimating the relationship between pregnancy status and first incidence of any of the four STIs distinguishing new and established pregnancies (N=4,549)

	Unadjusted relative hazard (HR) of incident **chlamydia**			Adjusted relative hazard (HR)* of incident **chlamydia**		
	HR	95%CI	p value	HR	95%CI	p value
Pregnancy exposure group						
Pregnant (established)	1.0	0.7-1.5	0.96	1.0	0.7-1.4	0.85
Newly pregnant	1.3	1.0-1.8	0.06	1.3	0.9-1.7	0.15
Not pregnant/oral contraceptive use	0.7	0.6-0.8	<0.0001	0.8	0.7-1.0	0.11
Not pregnant/injectable contraceptive	1.1	1.0-1.3	0.12	1.0	0.9-1.3	0.67
Not pregnant/no hormonal contraceptives	1.0	-	NA	1.0	-	NA
Time fixed covariates						
Study location						
Harare, Zimbabwe	1.0	-	NA	1.0	-	NA
Johannesburg, South Africa	2.1	1.8-2.5	<0.0001	1.8	1.5-2.2	<0.0001
Durban, South Africa	1.8	1.5-2.1	<0.0001	1.6	1.3-2.0	<0.0001
Age						
18-24	1.8	1.5-2.2	<0.0001	2.3	1.9-2.8	<0.0001
25-34	1.1	0.9-1.3	0.56	1.3	1.1-1.6	0.01
≥35	1.0		NA	1.0	-	NA
Completed high school (11 years school)	1.1	0.9-1.2	0.35	1.0	0.9-1.1	0.89
Diaphragm randomization arm (ref: control)	1.0	0.9-1.1	0.96	1.0	0.9-1.2	0.88
Contraceptive use at enrollment						
None	1.0	-	NA	1.0	-	NA
Oral contraceptives	0.6	0.5-0.8	<0.01	1.0	0.8-1.4	0.76
Injectable contraceptives	1.0	0.8-1.3	0.94	0.9	0.7-1.2	0.51
Other (diaphragm, IUD, condoms, vasectomy)	0.9	0.7-1.1	0.41	0.9	0.7-1.2	0.51
High risk sex at enrollment	1.1	0.9-1.2	0.33	1.0	0.9-1.2	0.85
Partner high risk at enrollment	1.4	1.2-16	<0.0001	1.2	1.0-1.3	0.03
STI at enrollment**	1.6	1.4-1.9	<0.0001	1.8	1.5-2.0	<0.0001
Time varying covariates						
Vaginal sex since last visit (ref:none)	0.9	0.7-1.1	0.31	1.0	0.8-1.4	0.84
≥3 Vaginal sex acts per week (ref:0-2)	0.8	0.7-0.9	<0.01	1.0	0.8-1.3	0.69
No condom use at last sex	1.0	0.9-1.1	0.88	0.9	0.8-1.1	0.31
Unprotected sex since last visit	1.2	1.1-1.4	<0.01	1.2	1.0-1.4	0.01
High risk sex^	1.4	1.2-1.7	<0.0001	1.2	1.0-1.3	0.08
Suspects/knows partner concurrency	1.6	1.4-1.8	<0.0001	1.3	1.1-1.5	<0.01
Vaginal washing	1.0	0.9-1.2	0.91	1.0	0.8-1.1	0.70
Vaginal wiping	1.0	0.9-1.2	0.73	1.0	0.9-1.2	0.94
Insertion of paper products	1.2	1.0-1.4	0.12	1.2	1.0-1.4	0.14

*Other contraceptives at enrollment: diaphragm, IUD, condoms, male partner vasectomy
**STIs at enrollment: chlamydia, gonorrhea, trichomoniasis, HSV-2
***High risk sex: exchange, anal sex, >2 partners, new partner, new regular partner

Chapter 5

Conclusion

Sexually transmitted infections (STI) pose significant threats to the health of women, primarily those of reproductive age who face infertility from untreated infections and poor birth outcomes, including mother-to-child transmission of some infections. Given the risks, it is critical to understand more about when and how women are contracting STIs and whether they are at greater risk of infection during pregnancy. This dissertation explored changes in women's sexual risk behaviors during pregnancy and examined whether women had higher STI incidence while pregnant.

In Chapter 2 of this dissertation, I examined existing evidence documenting STI acquisition among pregnant women and available data comparing pregnant women's risk to that of non-pregnant women. Very few studies were found documenting incidence of STIs during pregnancy and even fewer evaluated whether women are at higher risk during pregnancy. Although there is a good deal of prevalence data available for pregnant women, only 18 studies were found that reported STI incidence among pregnant women and just five studies compared STI risk in pregnant and non-pregnant women. From the limited available evidence it appears that women do continue to acquire STIs during pregnancy although incidence varied within and across infection types, populations and geographic settings. Overall the review in Chapter 1 revealed the paucity of data on women's STI risk with the few existing studies documenting outcomes only for chlamydia, gonorrhea, HPV, HSV-2 and HIV. No studies were found examining trichomoniasis and syphilis, STIs that cause adverse birth outcomes and can affect the long term health of women and children. The lack of data overall and on certain common STIs highlights the need for more studies on incidence among pregnant women. This information is needed to understand which infections pregnant women may be most vulnerable to, what behavioral and biological factors drive incidence during pregnancy and to identify whether pregnancy increases risk of STI acquisition.

The aim of Chapter 3 was to describe changes in sexual behaviors during pregnancy in order to examine what risk factors may impact STI incidence in pregnant women. The analysis used self-reported

sexual behavior and vaginal practice data collected over follow-up during the Methods for Improving Reproductive Health in Africa (MIRA) trial which was conducted in South Africa and Zimbabwe 2003-2006. The analysis used several different analytic approaches, including matched pairs and regression modeling to compare behaviors reported before and during pregnancies. The analysis found that pregnant women continued having sex but with lower frequency over the course of pregnancy and that pregnant women were more likely to report sex without a condom, but significantly less likely to engage in other risk factors for STI acquisition, including anal sex, concurrent sexual relationships and having new sex partners. These findings are similar to those reported from a small number of studies that have previously compared the sexual risk behaviors of pregnant and non-pregnant women, and present a complex picture of STI risk during pregnancy. The data suggest that pregnant women may have increased risk through unprotected sex but potentially lower risk resulting from fewer other risk behaviors.

Chapter 3 also reported changes in vaginal practices during pregnancy and found that wiping and insertion of paper or cloth into the vagina was less frequent when women were pregnant. This finding is both unique and interesting; no previous studies have examined changes in vaginal practices during pregnancy and the findings suggest that pregnant women may reduce their risk for STIs through this specific pathway. The reasons why women might engage in fewer vaginal practices while pregnant is not clear however previous studies have shown vaginal practices to be associated with transactional sex[100] and pregnant women report less sex in exchange for drugs and money which could, in part, help explain the decrease in vaginal practices during pregnancy. More studies are needed of vaginal practices during pregnancy to understand their potential impact on STI risk and also in relation to adverse birth outcomes.

The analysis in Chapter 3 also found that sexual risk behaviors and vaginal practices differed not just between pregnant and non-pregnant periods but were also dependent on the type of hormonal

contraceptives that were used during non-pregnant periods. Women using injectable contraceptives were most likely to report high risk sexual behaviors (anal sex, exchange sex, concurrency, new partners and drugs and alcohol use during sex) but were less likely to report vaginal practices compared to women using oral contraceptives. Although contraceptive use was not the focus of this dissertation, this information is relevant to increasing concerns regarding whether injectable contraceptives increase STI and HIV acquisition.[120]

Overall, the analysis in Chapter 3 is one of only a few studies to explore sexual risk behaviors that are known to be associated with STI incidence during pregnancy and is the only analysis to date examining the impact of pregnancy on vaginal practices. These data are therefore an important contribution to developing a better understanding of sexual behaviors and STI risk factors in women of reproductive age.

Finally, in Chapter 4, I examined the incidence of four common STIs (chlamydia, gonorrhea, trichomoniasis and HIV) and evaluated whether women were at increased risk of infection during pregnancy. The analysis included 4,549 women 18-50 years of age from the MIRA trial who attended over 24,300 study visits. Similar to Chapter 3, in this analysis sexual risk behavior and vaginal practices were compared during pregnant and non-pregnant periods but this analysis also included women who did not have pregnancies during follow-up (findings were similar to those reported in the previous chapter). The analysis in Chapter 4 also reported incidence rates of four STIs during pregnant and non-pregnant periods and found pregnant women had high incidence of chlamydia, gonorrhea and trichomoniasis. In multivariable models, adjusted for demographic and time-varying behavioral risk factors and vaginal practices, pregnant women did not appear to have higher incidence than non-pregnant women for chlamydia, gonorrhea, trichomonasis or HIV.

The analysis in Chapter 4 provides previously undocumented data on trichomoniasis incidence among pregnant women (which has not been previously reported), as well as new, higher quality evidence on incidence of chlamydia and gonorrhea during pregnancy (both of which have been previously reported but not from studies with rigorous follow-up). Overall the analysis found that women had continued incidence of STIs during pregnancy but that their risk did not appear higher than women who were not pregnant. This finding differs from some previous studies and is consistent with others, suggesting the need for further examination of this question.

The analysis in Chapter 4 also identified risk factors for STI incidence including younger age, being in settings with higher STI prevalence and having had an STI enrollment. Other risk factors were less consistent in their associations with STIs, including unprotected sex (without a condom), reporting of high risk sexual behaviors such as sex in exchange for drugs or money and anal sex. Knowledge of a male partner's concurrency was a strong predictor of acquisition of all STIs which is a factor warranting further study.

A unique aspect of the analysis in Chapter 4 is that we examined differences in sexual risk behaviors and STI risk for established pregnancies as well as for the periods when pregnancies occurred. We found that in periods when women became pregnant they were more likely to report sexual risk behaviors that would put them at greater risk for acquiring STIs with the exception of unprotected sex (without a condom) which they reported less frequently than women during established pregnancies. This finding has potentially important methodological implications as it suggests that there may be systematic bias in reporting of condom use, with women who are not pregnant or newly pregnant potentially over reporting use of condoms. Further exploration of the extent of this bias and greater understanding of the factors that lead to the potential over reporting are needed in light of the importance of this particular risk factor and in its use as a variable in statistical adjustment for STI incidence in most studies. Women were also found to be at greater risk for acquiring chlamydia during

periods when they became pregnant compared to when they were not pregnant. This finding is unique as previous analyses have not described the timing of STI incidence with regard to occurrence of pregnancies.

Although pregnancy was not found to increase risk for STI acquisition, we did find that pregnant women do have incident STIs which underscores the importance of identifying effective strategies to help prevent STIs in women of reproductive age in order to protect their own health and to reduce congenital and peri-natal STI infections in children. There is clear evidence with regard to HIV that incident infection during pregnancy dramatically increases the risk of MTCT, with an increasing proportion of peri-natal infections in children attributed to maternal HIV acquisition during pregnancy.[66,81-86] While there is no evidence regarding incident infection during pregnancy and MTCT risk in relation to other STIs, it is possible that, as with HIV, there may be higher risk of congenital infection with gonorrhea and chlamydia when women are infected during pregnancy. While this dissertation was not designed to answer this question, it is an important avenue for future research.

Unfortunately behavioral interventions to increase and improve the consistency of condom use have had limited impact generally, however it is important to note that pregnancy is a period when some women and their male partners may be highly motivated to take steps to reduce risk in order to protect their unborn child. Some promising evidence in this area was reported from the PartnerPlus Project in South Africa which showed that education and counseling improved reported condom use improved among pregnant women and their male partners.[98] In contrast, a more recent study of an intervention aimed at increasing condom use during pregnancy showed that while reported condom use increased, STI incidence among women attending antenatal care in South Africa did not improve[146], underscoring the challenges of reducing risk through behavioral interventions.

Going forward, more studies are needed to measure the incidence of STIs during pregnancy, specifically those that have not been previously well documented. More data are needed to assess whether pregnant women in certain populations may be at greater risk of acquiring infections, which types of infections they may be most vulnerable to and whether there are any biological pathways through which pregnancy increases risk for certain infections. Greater documentation of vaginal practices among pregnant women are also needed as these behaviors may have implications both for STI risk, specifically HIV, and could also be associated with adverse birth outcomes. And finally, as noted above, regardless of whether pregnancy increases risk, greater efforts are needed to identify effective behavioral interventions that can be implemented to help protect women and children from these infections.

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Appendices

Appendix Table 3.1 Characteristics at enrollment, MIRA subjects included in the analysis and those excluded, N=4,802

Characteristics at enrollment	Total		Women with no pregnancies		Women with lab pregnancies in analysis		Excluded women		p value
	N	%	N	%	N	%	N	%	
	4802	100.0%	3830	79.8%	483	10.1%	489	10.2%	
Study location									
Harare	2391	49.8%	1881	49.1%	274	56.7%	236	48.3%	0.0265
Durban	1434	29.9%	1165	30.4%	124	25.7%	145	29.7%	
Johannesburg	977	20.4%	784	20.5%	85	17.6%	108	22.1%	
Age									
Median age (range)	27	(18-45)	27	(18-45)	24	(18-44)	27	(18-45)	<0.0001
18-24	1891	39.4%	1388	36.3%	247	51.1%	256	52.4%	<0.0001
25-34	1939	40.4%	1551	40.5%	199	41.2%	189	38.7%	
≥35	971	20.2%	890	23.2%	37	7.7%	44	9.0%	
Completed high school (11 years school)	2550	53.1%	1987	51.9%	276	57.1%	287	58.7%	0.0030
Paid employment	1092	22.8%	888	23.2%	97	20.1%	107	21.9%	0.2739
Married	2809	58.5%	2250	58.8%	296	61.3%	263	53.8%	0.0466
Lives with male partner	3230	67.3%	2591	67.7%	337	69.8%	302	61.8%	0.0414
Number of previous pregnancies									
None	426	8.9%	294	7.7%	63	13.0%	69	14.1%	<0.0001
1 previous pregnancy	1572	32.7%	1178	30.8%	195	40.4%	199	40.7%	
2+	2804	58.4%	2358	61.6%	225	46.6%	221	45.2%	
Current birth control (not mutually exclusive)									
Combined pills	1070	22.3%	791	20.7%	149	30.9%	130	26.6%	<0.0001
Injectable hormones	1216	25.3%	1068	27.9%	64	13.3%	84	17.2%	<0.0001
Progesterone only pills	723	15.1%	600	15.7%	60	12.4%	63	12.9%	0.0623
Intrauterine device	16	0.3%	15	0.4%	1	0.2%	0	0.0%	0.6417
Condoms (male or female) (only)	1829	38.1%	1453	37.9%	182	37.7%	194	39.7%	0.7409
None (natural, withdrawal, tradition)	261	5.4%	192	5.0%	38	7.9%	31	6.3%	0.0218
Regular sexual partner	1989	41.4%	1577	41.2%	186	38.5%	226	46.2%	0.1066
Age at first sex (mean and range)	18	(10-31)	18	(10-31)	18	(11-26)	18	(12-27)	0.3681
Sex acts per week									
None	1924	40.1%	1522	39.7%	210	43.5%	192	39.3%	0.2698
1-2 per week	291	6.1%	229	6.0%	35	7.3%	27	5.5%	
≥3 per week	2587	53.9%	2079	54.3%	238	49.3%	270	55.2%	
Number of sexual partners in past 3 months									
None	274	5.7%	239	6.3%	11	2.3%	24	4.9%	0.0004
1 partner	4101	85.6%	3267	85.6%	427	8.4%	407	83.2%	
2+	415	8.7%	312	8.2%	45	9.3%	58	11.9%	
Condom use (male or female) at last sex	3388	70.6%	2701	70.5%	339	70.2%	348	71.2%	0.9407
Condom use (vaginal sex) in last three months									
Never	1359	29.3%	1123	29.4%	136	28.2%	136	27.8%	0.2103
Sometimes	1887	38.9%	1487	38.9%	185	38.3%	215	44.0%	
Every time	1509	31.9%	1209	31.7%	162	33.5%	138	28.2%	
Sex in exchange for money or drugs	382	8.0%	306	8.0%	38	7.9%	38	7.8%	0.9828
Anal sex (ever)	695	14.5%	550	14.4%	79	16.4%	66	13.5%	0.4142

Appendix 3.1 continued

Characteristics at enrollment	Total		Women with no pregnancies		Women with lab pregnancies in analysis		Excluded women		p value
Sex while intoxicated	174	26.3%	136	26.8%	20	27.8%	18	22.2%	0.6591
High risk (≥2 partners, exchange, anal, sex w/ drugs/alcohol)	1109	23.1%	879	23.0%	123	25.5%	107	21.9%	0.3725
Partner age									
Same age (+/-5 years)	2675	58.2%	2114	57.8%	277	58.9%	284	60.0%	0.4147
Younger (more than 5 years)	44	1.0%	38	1.0%	1	0.2%	5	1.1%	
Older (more than 5 years)	1878	40.9%	1502	41.1%	192	40.9%	184	40.9%	
Partner tested HIV positive									
Yes	162	3.4%	136	3.6%	14	2.9%	12	2.5%	0.3642
No/don't know	4626	96.6%	3680	96.4%	469	97.1%	477	97.6%	
Partner away from home >2 months	535	11.1%	425	11.1%	47	9.7%	63	12.9%	0.2880
Partner circumcised	1031	21.5%	821	21.5%	106	22.0%	104	21.3%	0.4253
Partner has other sexual partners (suspects/knows)	1464	30.6%	1164	30.5%	138	28.6%	162	33.1%	0.2971
Physical or verbal abuse	877	33.4%	715	33.3%	74	33.0%	88	34.8%	0.8856
Partner used force to get sex	259	9.9%	213	9.9%	21	9.4%	25	9.9%	0.9671
Sex w/ partner with drugs/alcohol	1711	35.9%	1368	36.0%	179	37.1%	164	33.7%	0.5034
Partner high risk (HIV+, away >1 month, other partners, drugs)	2990	62.3%	2386	62.3%	299	61.9%	305	62.4%	0.9852
Vaginal washing									
Daily	2939	61.4%	2331	61.1%	301	62.3%	307	62.8%	0.6048
Weekly	695	14.5%	562	14.7%	61	12.6%	72	14.7%	
Monthly or less	337	7.0%	268	7.0%	31	6.4%	38	7.8%	
None	819	17.1%	657	17.2%	90	18.6%	72	14.7%	
ANY	3983	83.0%	3173	82.9%	393	81.4%	417	85.3%	0.2533
Vaginal wiping									
Daily	1944	40.6%	1546	40.5%	195	40.4%	203	41.5%	0.6024
Weekly	458	9.6%	362	9.5%	56	11.6%	40	8.2%	
Monthly or less	303	6.3%	237	6.2%	30	6.2%	36	7.4%	
None	2085	43.5%	1673	43.8%	202	41.8%	210	42.9%	
ANY	2717	56.6%	2157	56.3%	281	58.8%	279	57.1%	0.7255
Vaginal insertion (non-menstruation related)									
Daily	622	13.0%	502	13.2%	58	12.0%	62	12.7%	0.9780
Weekly	163	3.4%	126	3.3%	18	373.0%	19	3.9%	
Monthly or less	197	4.1%	158	4.1%	20	4.1%	19	3.9%	
None	3804	79.5%	3028	79.4%	387	80.1%	389	79.6%	
ANY	998	20.8%	802	21.0%	96	19.9%	100	20.5%	0.8455
Diaphragm arm	2385	49.7%	1901	49.6%	231	47.8%	253	51.7%	0.4737
Positive for STI (enrollment)									
Chlamydia	215	4.5%	159	4.2%	28	5.8%	28	5.7%	0.0957
Gonorrhea	36	0.8%	30	0.8%	3	0.6%	3	0.6%	0.8659
Trichomoniasis	175	3.7%	137	3.6%	20	4.1%	18	3.7%	0.8233
HSV-2	2764	57.6%	2257	59.0%	235	48.7%	272	55.7%	<0.0001

Appendix 4.1 Enrollment characteristics enrollment of 4,935 MIRA participants included excluded in analysis

Characteristics at enrollment	Total		Women with no pregnancies		Women with lab confirmed pregnancies		Excluded women		p value
	N	%	N	%	N	%	N	%	
	4935	100.0%	3783	83.2%	766	15.5%	386	7.8%	
Study location									
Harare	2443	49.5%	1847	48.9%	408	53.3%	188	48.7%	0.06
Durban	1484	30.1%	1173	30.9%	199	26.0%	112	29.0%	
Johannesburg	1008	20.4%	763	20.2%	159	20.8%	86	22.3%	
Age									
Median age (range)	27 (18-50)		28 (18-50)		25 (18-47)		26 (18-49)		<0.0001
18-24	1891	38.3%	1342	35.5%	383	50.0%	166	43.0%	<0.0001
25-34	198	39.3%	1472	38.9%	310	40.5%	456	40.4%	
≥35	1106	22.4%	969	25.6%	73	9.5%	64	16.6%	
Completed high school (11 yrs school)	2556	51.8%	1904	50.3%	443	57.8%	209	54.2%	<0.01
Paid employment	1130	22.9%	883	23.4%	156	20.4%	91	23.6%	0.19
Married	2910	59.0%	2245	59.4%	450	58.8%	215	55.7%	0.38
Lives with male partner	3339	67.7%	2572	68.0%	509	66.5%	258	66.8%	0.02
Number or previous pregnancies									
None	429	8.7%	280	7.4%	107	14.0%	42	10.9%	<0.0001
1 previous pregnancy	1580	32.0%	1151	30.4%	295	38.5%	134	34.7%	
2+ previous pregnancies	2926	59.3%	2352	62.2%	364	47.5%	210	54.4%	
Current birth control (not mutually exclusive)									
Combined pills	1078	21.9%	770	20.4%	221	28.9%	87	22.5%	<0.0001
Injectable hormones	1226	24.9%	1029	27.2%	100	13.1%	97	25.1%	<0.0001
Progesterone only pills	723	14.7%	566	15.0%	97	12.7%	60	15.5%	0.23
Intrauterine device	17	0.3%	15	0.4%	1	0.1%	1	0.3%	0.79
Condoms (male or female) (only)	1860	37.7%	1419	37.5%	304	39.7%	137	35.5%	0.34
None (natural, withdrawal, tradition)	273	5.5%	194	5.1%	53	6.9%	26	6.7%	0.08
Regular sexual partner	2021	40.9%	1537	40.6%	315	41.1%	169	43.8%	0.01
Age at first sex (mean, range)	18 (10-31)		18 (10-31)		18 (11-27)		18 (12-26)		0.43
Sex acts per week									
None	1991	40.3%	1551	41.0%	326	42.6%	114	29.5%	<0.01
1-2 per week	302	6.1%	227	6.0%	48	6.3%	27	7.0%	
≥3 per week	2642	53.5%	2005	53.0%	392	51.2%	245	63.5%	
Number of sexual partners in past 3 months									
None	291	5.9%	249	6.6%	24	3.1%	18	4.7%	<0.01
1 partner	4212	85.5%	3222	85.4%	655	85.5%	335	86.8%	
2+	420	8.5%	300	8.0%	87.0	11.4%	33.0	8.6%	
Condom use (male or female) last sex	3466	70.2%	2653	70.1%	547	71.4%	266	68.9%	0.65
Condom use last three months									
Never	1455	29.6%	1132	30.0%	206	26.9%	117	30.3%	0.15
Sometimes	1930	39.2%	1449	38.4%	318	41.5%	163	42.2%	
Every time	1539	31.3%	1191	31.6%	242	31.6%	106	24.5%	
Exchange for money or drugs	394	8.0%	303	8.0%	57	7.4%	34	8.8%	0.72
Anal sex (ever)	713	14.5%	53	14.7%	116	15.1%	44	11.4%	0.19

Appendix 4.1 continued

Characteristics at enrollment	Total		Women with no pregnancies		Women with lab confirmed pregnancies		Excluded women		p value
	N	%	N	%	N	%			
Sex while intoxicated	177	26.1%	136	26.8%	32	26.0%	9	18.4%	0.43
High risk (>2 partners, exchange, anal, sex w/ drugs/alcohol)	1140	23.1%	874	23.1%	185	24.2%	81	21.0%	0.48
Partner age									
Same age (+/-5 years)	2747	58.2%	2077	57.7%	433	58.2%	237	63.7%	0.04
Younger (more than 5 years)	52	1.1%	47	1.3%	3	0.4%	2	0.5%	
Older (more than 5 years)	1918	40.7%	1477	41.0%	308	41.4%	133	35.8%	
Partner tested HIV positive									
Yes	165	3.4%	135	3.6%	21	2.7%	9	2.3%	0.25
No/don't know	4757	96.7%	3635	96.4%	745	97.3%	377	97.7%	
Partner away from home >2 months	549	11.1%	413	10.9%	77	10.1%	59	15.3%	0.02
Partner circumcised	1016	21.6%	805	21.4%	171	22.3%	87	22.5%	0.53
Suspects/knows partner concurrency	1505	30.6%	1159	30.7%	233	30.4%	113	29.3%	0.83
Physical or verbal abuse	903	33.3%	693	33.0%	128	33.4%	82	36.1%	0.63
Partner used force to get sex	265	9.8%	208	9.9%	36	9.4%	21	9.3%	0.92
Sex w/ partner under influence of drugs alcohol	1752	35.7%	1351	36.0%	271	35.5%	130	33.9%	0.71
Partner high risk (HIV+, away >1 month, other partners, drugs)	3067	62.2%	2363	62.5%	473	61.8%	231	59.8%	0.59
Vaginal washing									
Daily	3014	61.2%	2289	61.0%	480	62.7%	236	61.1%	0.85
Weekly	710	14.4%	558	14.8%	100	13.1%	52	13.5%	
Monthly or less	346	7.0%	263	7.0%	57	7.4%	26	6.7%	
None	83	17.3%	652	17.3%	129	16.8%	72	18.7%	
ANY	4070	82.5%	3119	82.5%	637	83.2%	314	81.4%	0.73
Vaginal wiping									
Daily	1989	40.4%	1525	40.5%	307	40.1%	157	40.7%	0.76
Weekly	470	9.6%	353	9.4%	82	10.7%	35	9.1%	
Monthly or less	313	6.4%	239	6.3%	54	7.1%	20	5.2%	
None	2151	43.7%	1654	43.9%	323	42.2%	174	45.1%	
ANY	2772	56.2%	2117	56.0%	443	57.8%	212	54.9%	0.61
Vaginal insertion (non-menstruation related)									
Daily	641	13.0%	504	13.4%	94	12.3%	43	11.2%	0.54
Weekly	167	3.4%	130	3.5%	29	3.8%	8	2.1%	
Monthly or less	203	4.1%	156	4.1%	33	4.3%	14	3.7%	
None	3908	79.4%	2979	79.0%	610	79.6%	319	83.1%	
ANY	1011	20.5%	790	20.9%	156	20.4%	67	17.4%	0.19
Diaphragm arm	2466	50.0%	1890	50.0%	383	50.0%	193	50.0%	1.00
Positive for STI (enrollment)									
Chlamydia	217	4.4%	155	4.1%	40	5.2%	22	5.7%	0.17
Gonorrhea	37	0.8%	29	0.8%	5	0.7%	3	0.8%	0.94
Trichomoniasis	181	3.7%	133	3.5%	33	4.3%	15	3.9%	0.55
HSV-2	2879	58.3%	2254	59.6%	402	52.5%	223	57.8%	<0.01

Appendix 4.2 Risk behaviors reported at follow-up visits by 4,549 women and the association with pregnancy status using the broader definition of pregnancy* (unadjusted odds ratios) (N=2,4337 visits)

**pregnancy defined as any lab confirmed or reported at visit (including miscarriages and terminations)*

Reported behaviors at follow-up	Total		Pregnant (lab confirmed & reported)		Oral HC use visits		Injectable HC use visits		No HC use visits	
	N	%	N	%	N	%	N	%	N	%
	24336	100.0%	1744	7.2%	8336	34.2%	6004	24.7%	8252	33.9%
Any vaginal sex since last visit	22933	94.2%	1648	94.5%	8134	91.6%	5615	93.5%	7536	91.3%
Sex ≥3 times per week	15948	65.5%	1135	65.1%	6021	72.2%	3893	64.8%	4899	65.5%
Condom use (male or female) at last sex	16856	69.3%	1085	62.2%	5789	69.5%	4107	68.4%	5875	71.2%
Unprotected sex (any report)	14334	58.9%	1262	72.4%	4965	59.6%	3647	60.7%	4459	54.0%
Sex in exchange for money or drugs	728	3.0%	41	2.4%	259	3.1%	195	3.3%	233	2.8%
2 or more male sex partners since last visit	1584	6.5%	85	4.9%	252	3.0%	449	7.5%	798	9.7%
New sex partner	2831	11.6%	174	10.0%	715	8.6%	844	14.1%	1098	13.3%
Suspects/knows partner concurrency	6064	24.9%	410	23.5%	1630	19.6%	1676	27.9%	2348	28.5%
High risk sex*	4415	18.1%	274	15.7%	1260	15.1%	1232	20.5%	1649	20.0%
Vaginal washing										
Daily	13312	54.7%	975	55.9%	4912	58.9%	3136	52.2%	4289	52.0%
Weekly	2819	11.6%	210	12.0%	739	8.9%	755	12.6%	1115	13.5%
Monthly or less	2197	9.0%	144	8.3%	835	10.0%	547	9.1%	671	8.1%
None (ref)	5901	24.3%	407	23.3%	1831	22.0%	1540	25.7%	2123	25.7%
ANY	18328	75.3%	1329	76.2%	6486	77.8%	4438	73.9%	6075	73.6%
Vaginal wiping										
Daily	7096	29.2%	504	28.9%	2743	32.9%	1575	26.2%	2274	27.6%
Weekly	1581	6.5%	95	5.5%	456	5.5%	410	6.8%	620	7.5%
Monthly or less	1223	5.0%	74	4.2%	448	5.4%	317	5.3%	384	4.7%
None (ref)	14329	58.9%	1063	61.0%	4670	56.0%	3676	61.2%	4920	59.6%
ANY	9900	40.7%	673	38.6%	3647	43.8%	2302	38.3%	3278	39.7%
Vaginal insertion (non-menstruation related)										
Daily	1823	7.5%	107	6.1%	689	8.3%	411	6.9%	616	7.5%
Weekly	530	2.2%	32	1.8%	173	2.1%	118	2.0%	207	2.5%
Monthly or less	798	3.3%	38	2.2%	293	3.5%	182	3.0%	285	3.5%
None (ref)	21076	86.6%	1559	89.4%	7162	85.9%	5265	87.7%	7090	85.9%
ANY	3151	13.0%	177	10.2%	1155	13.9%	711	11.8%	1108	13.4%

Appendix 4.2 continued

Reported behaviors at follow-up	OR pregnant vs. OC			OR pregnant vs. injectable			OR pregnant vs. no HC use		
	OR	95%CI	p value	OR	95%CI	p value	OR	95%CI	p value
Any vaginal sex since last visit	**1.8**	**1.4-2.3**	**<0.0001**	0.8	0.6-1.1	0.18	**0.6**	**0.5-0.8**	**<0.01**
Sex ≥3 times per week	**1.5**	**1.3-1.7**	**<0.0001**	0.9	0.8-1.1	0.42	**0.9**	**0.8-0.9**	**0.01**
Condom use (male or female) at last sex	**1.3**	**1.1-1.4**	**<0.01**	**1.3**	**1.1-1.5**	**<0.01**	**1.5**	**1.3-1.7**	**<0.0001**
Unprotected sex (any report)	**0.7**	**0.6-0.8**	**<0.0001**	**0.7**	**0.6-0.8**	**<0.0001**	**0.6**	**0.5-0.7**	**<0.0001**
Sex in exchange for money or drugs	**1.5**	**1.1-2.2**	**0.02**	**1.5**	**1.0-2.2**	**0.03**	**1.5**	**1.0-2.1**	**0.03**
2 or more male sex partners since last visit	1.0	0.8-1.3	0.94	**1.9**	**1.4-2.5**	**<0.0001**	**2.4**	**1.8-3.2**	**<0.0001**
New sex partner	1.0	0.8-1.2	0.97	**1.4**	**1.2-1.8**	**<0.01**	**1.5**	**1.2-1.8**	**<0.0001**
Suspects/knows male partner concurrency	1.0	0.8-1.1	0.51	**1.3**	**1.1-1.5**	**<0.01**	**1.2**	**1.1-1.4**	**<0.01**
High risk sex*	1.1	0.9-1.3	0.21	**1.4**	**1.2-1.7**	**<0.0001**	**1.5**	**1.3-1.7**	**<0.0001**
Vaginal washing									
Daily	**1.2**	**1.1-1.3**	**<0.01**	1.1	1.0-1.2	0.10	1.1	0.9-1.2	0.33
Weekly	1.0	0.9-1.2	0.90	1.1	1.0-1.3	0.19	1.1	1.0-1.3	0.09
Monthly or less	**1.4**	**1.2-1.7**	**<0.01**	1.2	0.9-1.4	0.16	1.1	0.9-1.3	0.62
None (ref)	1.0	-	-	1.0	-	-	1.0	-	-
ANY	**1.2**	**1.1-1.3**	**<0.01**	1.1	1.0-1.3	0.07	1.1	1.0-1.2	0.09
Vaginal wiping									
Daily	**1.3**	**1.2-1.5**	**<0.0001**	1.1	1.0-1.3	0.09	1.2	**1.1-1.4**	**<0.01**
Weekly	1.2	1.0-1.5	0.07	**1.3**	**1.1-1.7**	**0.02**	1.4	**1.1-1.8**	**<0.01**
Monthly or less	**1.4**	**1.1-1.8**	**<0.01**	1.3	1.0-1.7	0.06	1.2	1.0-1.5	0.12
None (ref)	1.0	-	-	1.0	-	-	1.0	-	-
ANY	**1.4**	**1.2-1.5**	**<0.0001**	**1.2**	**1.1-1.4**	**<0.01**	**1.3**	**1.1-1.4**	**<0.0001**
Vaginal insertion (non-menstruation related)									
Daily	**1.4**	**1.2-1.8**	**<0.01**	1.2	0.9-1.5	0.17	**1.3**	**1.1-1.7**	**0.01**
Weekly	1.5	1.0-2.1	0.05	1.2	0.8-1.8	0.41	**1.5**	**1.1-2.2**	**0.02**
Monthly or less	**1.8**	**1.3-2.5**	**<0.01**	1.4	1.0-2.1	0.05	**1.8**	**1.3-2.5**	**<0.01**
None (ref)	1.0	-	-	1.0	-	-	1.0	-	-
ANY	**1.5**	**1.3-1.8**	**<0.0001**	**1.2**	**1.0-1.5**	**0.03**	**1.5**	**1.2-1.8**	**<0.0001**

*High risk sex: exchange, anal sex, ≥2 partners, sex with drugs/alcohol

Appendix 4.3 Incidence rates of four STIs by pregnancy and hormonal contraceptive exposure group using broader definition of pregnancy (including all lab and reported pregnancies)

	Overall			Pregnant visits			Non-pregnant & oral HC use visits			Non-pregnant & injectable HC use visits			Non-pregnant & no HC use		
	Cases	Person years	**IR p/Pty**	Cases	Person years	**IR p/yr**	Cases	Person years	**IR p/yr**	Cases	Person years	**IR p/yr**	Cases	Person years	**IR p/yr**
Chlamydia	400	5935	**6.7**	40	416	**9.6**	84	2056	**4.1**	145	1434	**10.1**	131	2028	**6.5**
Gonorrhea	165	6130	**2.7**	22	442	**5.0**	34	2103	**1.6**	49	1501	**3.3**	60	2085	**2.9**
Trichomoniasis	420	5935	**7.1**	41	425	**9.6**	113	2030	**5.6**	108	1462	**7.4**	158	2017	**7.8**
HIV	240	6199	**3.9**	20	453	**4.4**	54	2117	**2.6**	73	1523	**4.8**	93	2105	**4.4**

Appendix 4.4a Unadjusted and adjusted hazard ratios estimating the relationship between pregnancy status and incident chlamydia infections using broader definition of pregnancy (all lab and reported) (N=4,549)

	Unadjusted relative hazard (HR) of incident **chlamydia**			Adjusted relative hazard (HR)* of incident **chlamydia**		
	HR	95%CI	p value	HR	95%CI	p value
Pregnancy exposure group						
Pregnant (lab+ and reported)	1.5	1.0-2.1	0.03	1.2	0.8-1.7	0.37
Not pregnant/oral contraceptive use	**0.6**	**0.5-0.9**	**<0.01**	0.9	0.6-1.3	0.55
Not pregnant/injectable contraceptive	**1.6**	**1.2-2.0**	**<0.01**	1.3	0.9-1.7	0.08
Not pregnant/no hormonal contraceptives	1.0	-	NA	1.0	-	NA
Time fixed covariates						
Study location						
Harare, Zimbabwe	1.0	-	NA	1.0	-	NA
Johannesburg, South Africa	**2.7**	**2.2-3.5**	**<0.0001**	**2.4**	**1.7-3.2**	**<0.0001**
Durban, South Africa	**2.9**	**2.2-3.7**	**<0.0001**	**2.5**	**1.8-3.5**	**<0.0001**
Age						
18-24	**5.0**	**3.5-7.1**	**<0.0001**	**5.6**	**3.8-8.2**	**<0.0001**
25-34	**2.1**	**1.4-3.1**	**<0.01**	**2.5**	**1.7-3.7**	**<0.0001**
≥35	1.0	-	NA	1.0	-	NA
Completed high school (11 years school)	**1.5**	**1.2-1.8**	**<0.01**	1.1	0.9-1.3	0.51
Diaphragm randomization arm (ref: control)	1.2	0.9-1.5	0.07	1.2	0.9-1.5	0.12
Contraceptive use at enrollment						
None	1.0	-	NA	1.0	-	NA
Oral contraceptives	**0.7**	**0.5-0.9**	**0.03**	1.0	0.7-1.7	0.88
Injectable contraceptives	**1.4**	**1.0-2.1**	**0.05**	1.0	0.7-1.5	0.94
Other (diaphragm, IUD, condoms, vasectomy)	1.2	0.8-1.7	0.35	1.1	0.8-1.6	0.60
High risk sex at enrollment	**1.3**	**1.0-1.6**	**0.03**	1.2	0.9-1.5	0.08
Partner high risk at enrollment	**1.3**	**1.0-1.5**	**0.03**	1.1	0.8-1.3	0.66
STI at enrollment**	1.2	0.9-1.50	0.07	**1.5**	**1.2-1.9**	**<0.01**
Time varying covariates						
Vaginal sex since last visit (ref:none)	0.9	0.6-1.6	0.58	1.0	0.6-1.6	0.95
≥3 Vaginal sex acts per week (ref:0-2)	0.9	0.8-1.2	0.54	1.2	0.9-1.5	0.13
No condom use at last sex	1.1	0.8-1.3	0.62	0.9	0.7-1.2	0.55
Unprotected sex since last visit	**1.4**	**1.1-1.7**	**<0.01**	**1.3**	**1.0-1.7**	**0.04**
High risk sex^	**1.7**	**1.3-2.1**	**<0.0001**	1.2	0.9-1.6	0.94
Suspects/knows partner concurrency	**1.6**	**1.3-2.0**	**<0.0001**	1.3	1.0-1.6	0.04
Vaginal washing	1.0	0.8-1.2	0.86	0.9	0.7-1.2	0.67
Vaginal wiping	0.9	0.7-1.1	0.35	0.9	0.7-1.2	0.51
Insertion of paper products	1.1	0.9-1.5	0.38	1.3	0.9-1.7	0.12

*Multivariable models were adjusted for all covariates listed in the table

**STIs at enrollment: chlamydia, gonorrhea, trichomoniasis, HSV-2

^High risk sex: exchange, anal sex, >2 partners, new partner, new regular partner

Table 4.4b Unadjusted and adjusted hazard ratios estimating the relationship between pregnancy status and incident gonorrhea infections using broader definition of pregnancy (all lab and reported) (N=4,549)

	Unadjusted relative hazard (HR) of incident **gonorrhea**			Adjusted relative hazard (HR)* of incident **gonorrhea**		
	HR	95%CI	p value	HR	95%CI	p value
Pregnancy exposure group						
Pregnant (lab+ and reported)	1.6	1.0-2.7	0.05	1.5	0.9-2.5	0.11
Not pregnant/oral contraceptive use	**0.6**	**0.4-0.9**	**0.01**	0.9	0.5-1.5	0.58
Not pregnant/injectable contraceptive	1.1	0.8-1.64	0.55	1.0	0.6-1.5	0.86
Not pregnant/no hormonal contraceptives	1.0	-	NA	1.0	-	NA
Time fixed covariates						
Study location						
Harare, Zimbabwe	1.0	-	NA	1.0	-	NA
Johannesburg, South Africa	**2.9**	**2.0-4.2**	**<0.0001**	**2.2**	**1.4-3.7**	**<0.01**
Durban, South Africa	**2.6**	**1.7-3.9**	**<0.0001**	**2.3**	**1.3-3.8**	**<0.01**
Age						
18-24	**4.2**	**2.5-7.1**	**<0.0001**	**5.2**	**2.9-9.1**	**<0.0001**
25-34	**1.8**	**1.0-3.2**	**0.04**	**2.3**	**1.9-4.1**	**0.01**
≥35	1.0	-	NA	1.0	-	NA
Completed high school (11 years school)	1.3	0.9-1.8	0.09	1.0	0.7-1.4	0.87
Diaphragm randomization arm (ref: control)	0.9	0.7-1.3	0.69	1.1	0.8-1.5	0.70
Contraceptive use at enrollment						
None	1.0	-	NA	1.0	-	NA
Oral contraceptives	0.9	0.5-1.7	0.73	1.7	0.8-3.7	0.17
Injectable contraceptives	1.6	0.9-3.0	0.14	1.4	0.7-2.8	0.33
Other (diaphragm, IUD, condoms, vasectomy)	1.8	0.9-3.3	0.07	1.7	0.9-3.1	0.11
High risk sex at enrollment	0.8	0.5-1.2	0.22	0.7	0.5-1.0	0.06
Partner high risk at enrollment	**1.8**	**1.3-2.5**	**<0.01**	1.4	0.9-2.0	0.07
STI at enrollment**	**1.9**	**1.3-2.7**	**<0.01**	**2.2**	**1.5-3.1**	**<0.0001**
Time varying covariates						
Vaginal sex since last visit (ref:none)	1.0	0.5-1.9	0.99	1.3	0.6-2.7	0.51
≥3 Vaginal sex acts per week (ref:0-2)	0.7	0.5-1.0	0.03	0.8	0.6-1.8	0.30
No condom use at last sex	**0.6**	**0.4-0.9**	**0.02**	**0.6**	**0.4-0.9**	**0.01**
Unprotected sex since last visit	1.1	0.8-1.5	0.47	1.2	0.9-1.8	0.25
High risk sex^	**1.9**	**1.4-2.7**	**<0.01**	**1.5**	**1.1-2.2**	**0.02**
Suspects/knows partner concurrency	**2.4**	**1.8-3.3**	**<0.0001**	**1.8**	**1.3-2.5**	**<0.01**
Vaginal washing	1.1	0.8-1.6	0.64	1.1	0.8-1.7	0.59
Vaginal wiping	1.0	0.7-1.4	0.93	1.0	0.7-1.5	0.71
Insertion of paper products	0.9	0.5-1.4	0.57	0.9	0.5-1.5	0.71

*Multivariable models were adjusted for all covariates listed in the table

**STIs at enrollment: chlamydia, gonorrhea, trichomoniasis, HSV-2

^High risk sex: exchange, anal sex, >2 partners, new partner, new regular partner

Table 4.4c Unadjusted and adjusted hazard ratios estimating the relationship between pregnancy status and incident trichomoniasis infections using broader definition of pregnancy (all lab and reported) (N=4,549)

	Unadjusted relative hazard (HR) of incident **trichomoniasis**			Adjusted relative hazard (HR)* of incident **trichomoniasis**		
	HR	95%CI	p value	HR	95%CI	p value
Pregnancy exposure group						
Pregnant (lab+ and reported)	1.2	0.8-1.7	0.35	1.3	0.9-1.9	0.15
Not pregnant/oral contraceptive use	**0.7**	**0.6-0.9**	**0.01**	0.9	0.6-1.3	0.57
Not pregnant/injectable contraceptive	0.9	0.7-1.9	0.59	1.0	0.7-1.4	0.97
Not pregnant/no hormonal contraceptives	1.0	-	NA	1.0	-	NA
Time fixed covariates						
Study location						
Harare, Zimbabwe	1.0	-	NA	1.0	-	NA
Johannesburg, South Africa	**1.7**	**1.4-2.2**	**<0.0001**	**1.5**	**1.2-2.0**	**<0.01**
Durban, South Africa	1.3	0.9-1.7	0.07	1.3	0.9-1.8	0.14
Age						
18-24	1.0	0.8-1.2	0.75	1.2	0.9-1.6	0.13
25-34	**0.7**	**0.6-0.9**	**0.01**	0.9	0.7-1.2	0.45
≥35	1.0	-	NA	1.0	-	NA
Completed high school (11 years school)	**0.7**	**0.6-0.9**	**<0.01**	0.8	0.7-1.0	0.06
Diaphragm randomization arm (ref: control)	0.9	0.8-1.1	0.41	0.9	0.8-1.2	0.56
Contraceptive use at enrollment						
None	1.0	-	NA	1.0	-	NA
Oral contraceptives	**0.5**	**0.4-0.7**	**<0.0001**	0.8	0.6-1.2	0.34
Injectable contraceptives	**0.7**	**0.5-0.9**	**0.01**	0.8	0.5-1.1	0.13
Other (diaphragm, IUD, condoms, vasectomy)	**0.6**	**0.4-0.8**	**<0.01**	**0.6**	**0.4-0.8**	**<0.01**
High risk sex at enrollment	1.2	0.9-1.5	0.07	1.1	0.9-1.4	0.27
Partner high risk at enrollment	**1.4**	**1.1-1.7**	**<0.01**	1.2	0.9-1.5	0.11
STI at enrollment**	**2.0**	**1.6-2.4**	**<0.0001**	**1.8**	**1.4-2.3**	**<0.0001**
Time varying covariates						
Vaginal sex since last visit (ref:none)	0.8	0.6-1.2	0.33	0.9	0.6-1.5	0.78
≥3 Vaginal sex acts per week (ref:0-2)	0.8	0.6-0.9	0.03	0.9	0.7-1.2	0.40
No condom use at last sex	1.1	0.8-1.3	0.65	1.0	0.8-1.4	0.80
Unprotected sex since last visit	1.2	0.9-1.4	0.11	1.1	0.9-1.4	0.47
High risk sex^	**1.4**	**1.1-1.8**	**<0.01**	1.1	0.9-1.4	0.31
Suspects/knows partner concurrency	**1.5**	**1.2-1.8**	**<0.01**	**1.3**	**1.0-1.6**	**0.03**
Vaginal washing	1.0	0.8-1.3	0.93	1.0	0.8-1.3	0.98
Vaginal wiping	1.0	0.9-1.3	0.64	0.9	0.7-1.2	0.51
Insertion of paper products	1.4	1.1-1.8	0.02	1.3	0.9-1.8	0.06

*Multivariable models were adjusted for all covariates listed in the table

**STIs at enrollment: chlamydia, gonorrhea, trichomoniasis, HSV-2

^High risk sex: exchange, anal sex, >2 partners, new partner, new regular partner

Table 4.4d Unadjusted and adjusted hazard ratios estimating the relationship between pregnancy status and incident HI infections using broader definition of pregnancy (all lab and reported) (N=4,549)

	Unadjusted relative hazard (HR) of incident **HIV**			Adjusted relative hazard (HR)* of incident **HIV**		
	HR	95%CI	p value	HR	95%CI	p value
Pregnancy exposure group						
Pregnant (lab+ and reported)	0.9	0.6-1.5	0.65	0.8	0.4-1.3	0.37
Not pregnant/oral contraceptive use	**0.6**	**0.4-0.8**	**<0.01**	0.8	0.5-1.2	0.27
Not pregnant/injectable contraceptive	1.1	0.8-1.4	0.71	0.8	0.6-1.2	0.23
Not pregnant/no hormonal contraceptives	1.0	-	NA	1.0	-	NA
Time fixed covariates						
Study location						
Harare, Zimbabwe	1.0	-	NA	1.0	-	NA
Johannesburg, South Africa	**2.7**	**2.0-3.5**	**<0.0001**	**2.0**	**1.4-2.9**	**<0.01**
Durban, South Africa	1.3	0.9-1.9	0.18	1.0	0.6-1.6	0.98
Age						
18-24	**2.1**	**1.4-3.0**	**<0.0001**	**2.6**	**1.7-3.9**	**<0.0001**
25-34	1.3	0.9-2.0	0.14	**1.7**	**1.1-2.5**	**0.02**
≥35	1.0	-	NA	1.0	-	NA
Completed high school (11 years school)	1.2	0.9-1.5	0.19	1.1	0.9-1.5	0.33
Diaphragm randomization arm (ref: control)	1.0	0.8-1.3	0.98	1.0	0.8-1.3	0.93
Contraceptive use at enrollment						
None	1.0	-	NA	1.0	-	NA
Oral contraceptives	0.8	0.5-1.4	0.53	1.4	0.7-2.6	0.32
Injectable contraceptives	**1.8**	**1.1-3.0**	**0.03**	**1.8**	**1.1-3.2**	**0.03**
Other (diaphragm, IUD, condoms, vasectomy)	1.4	0.9-2.4	0.17	1.5	0.9-2.5	0.15
High risk sex at enrollment	1.1	0.8-1.5	0.56	1.1	0.8-1.5	0.48
Partner high risk at enrollment	**1.6**	**1.2-2.1**	**<0.01**	1.3	0.9-1.7	0.12
STI at enrollment**	**2.0**	**1.5-2.7**	**<0.0001**	**2.1**	**1.5-2.8**	**<0.0001**
Time varying covariates						
Vaginal sex since last visit (ref:none)	1.1	0.6-1.9	0.86	1.1	0.5-2.1	0.76
≥3 Vaginal sex acts per week (ref:0-2)	0.8	0.6-1.0	0.07	0.9	0.6-1.2	0.32
No condom use at last sex	1.0	0.7-1.3	0.98	0.9	0.7-1.3	0.70
Unprotected sex since last visit	1.3	0.9-1.6	0.09	1.3	0.9-1.7	0.15
High risk sex^	**1.5**	**1.1-2.1**	**0.01**	1.1	0.8-1.6	0.42
Suspects/knows partner concurrency	**2.0**	**1.5-2.6**	**<0.0001**	**1.5**	**1.1-1.9**	**0.01**
Vaginal washing	1.1	0.8-1.4	0.72	0.9	0.7-1.3	0.56
Vaginal wiping	**1.4**	**1.1-1.8**	**0.01**	**1.5**	**1.1-2.0**	**0.01**
Insertion of paper products	1.3	0.9-1.8	0.18	1.1	0.8-1.7	0.48

*Multivariable models were adjusted for all covariates listed in the table

**STIs at enrollment: chlamydia, gonorrhea, trichomoniasis, HSV-2

^High risk sex: exchange, anal sex, >2 partners, new partner, new regular partner

Appendix 4.5a Unadjusted and adjusted hazard ratios estimating the relationship between pregnancy status and incident chlamydia infections using only those women with complete data (N=3,518)

	Unadjusted relative hazard (HR) of incident **chlamydia**			Adjusted relative hazard (HR)* of incident **chlamydia**		
	HR	95%CI	p value	HR	95%CI	p value
Pregnancy exposure group						
Pregnant	1.5	0.7-2.3	0.08	1.1	0.7-1.8	0.65
Not pregnant/oral contraceptive use	**0.6**	**0.5-0.9**	**<0.01**	0.8	0.5-1.9	0.26
Not pregnant/injectable contraceptive	**1.5**	**1.3-2.0**	**<0.01**	1.2	0.9-1.6	0.33
Not pregnant/no hormonal contraceptives	1.0	-	NA	1.0	-	NA
Time fixed covariates						
Study location						
Harare, Zimbabwe	1.0	-	NA	1.0	-	NA
Johannesburg, South Africa	**2.6**	**2.0-3.4**	**<0.0001**	**2.3**	**1.6-3.2**	**<0.0001**
Durban, South Africa	**2.7**	**2.1-3.6**	**<0.0001**	**2.4**	**1.7-3.4**	**<0.0001**
Age						
18-24	**5.4**	**3.6-8.0**	**<0.0001**	**6.1**	**4.0-9.4**	**<0.0001**
25-34	**2.4**	**1.6-3.6**	**<0.0001**	**2.8**	**1.8-4.4**	**<0.0001**
≥35	1.0	-	NA	1.0	-	NA
Completed high school (11 years school)	**1.5**	**1.2-1.9**	**<0.01**	1.1	0.9-1.4	0.30
Diaphragm randomization arm (ref: control)	1.2	0.9-1.5	0.09	1.1	0.9-1.4	0.31
Contraceptive use at enrollment						
None	1.0	-	NA	1.0	-	NA
Oral contraceptives	0.7	0.4-1.0	0.05	1.0	0.6-1.7	0.93
Injectable contraceptives	1.5	0.9-2.2	0.07	1.0	0.6-1.5	0.90
Other (diaphragm, IUD, condoms, vasectomy)	1.1	0.8-1.7	0.55	1.0	0.7-1.5	0.94
High risk sex at enrollment	**1.3**	**1.1-1.7**	**0.01**	1.3	1.0-1.4	0.06
Partner high risk at enrollment	**1.4**	**1.1-1.7**	**0.01**	1.1	0.9-1.4	0.32
STI at enrollment**	1.2	0.9-1.5	0.14	**1.5**	**1.2-1.9**	**<0.01**
Time varying covariates						
Vaginal sex since last visit (ref:none)	1.2	0.7-2.0	0.50	1.3	0.7-2.3	0.43
≥3 Vaginal sex acts per week (ref:0-2)	0.8	0.5-1.3	0.30	1.2	0.9-1.6	0.12
No condom use at last sex	1.2	0.9-1.5	0.19	1.1	0.8-1.5	0.57
Unprotected sex since last visit	**1.4**	**1.1-1.7**	**0.01**	1.2	0.9-1.6	0.13
High risk sex^	**1.7**	**1.3-2.2**	**<0.0001**	1.2	0.9-1.6	0.13
Suspects/knows partner concurrency	**1.7**	**1.3-2.1**	**<0.0001**	1.3	0.9-1.6	0.07
Vaginal washing	0.9	0.7-1.2	0.65	0.7	0.7-1.2	0.61
Vaginal wiping	0.8	0.7-1.1	0.15	0.8	0.7-1.1	0.18
Insertion of paper products	1.2	0.9-1.6	0.32	1.4	1.0-1.9	0.05

*Multivariable models were adjusted for all covariates listed in the table

**STIs at enrollment: chlamydia, gonorrhea, trichomoniasis, HSV-2

^High risk sex: exchange, anal sex, >2 partners, new partner, new regular partner

Appendix 4.5b Unadjusted and adjusted hazard ratios estimating the relationship between pregnancy status and incident gonorrhea infections using only those women with complete data (N=3,518)

	Unadjusted relative hazard (HR) of incident **gonorrhea**			Adjusted relative hazard (HR)* of incident **gonorrhea**		
	HR	95%CI	p value	HR	95%CI	p value
Pregnancy exposure group						
Pregnant	1.8	1.0-3.2	0.06	1.5	0.8-2.8	0.18
Not pregnant/oral contraceptive use	0.6	0.4-0.9	0.02	0.8	0.5-1.6	0.60
Not pregnant/injectable contraceptive	1.2	0.8-1.8	0.34	1.0	0.7-1.7	0.88
Not pregnant/no hormonal contraceptives	1.0	-	NA	1.0	-	NA
Time fixed covariates						
Study location						
Harare, Zimbabwe	1.0	-	NA	1.0	-	NA
Johannesburg, South Africa	3.1	2.1-4.7	<0.0001	2.6	1.5-4.5	<0.01
Durban, South Africa	2.7	1.8-4.3	<0.0001	2.4	1.4-4.3	<0.01
Age						
18-24	5.2	2.8-9.4	<0.0001	6.2	3.2-11.7	<0.0001
25-34	2.1	1.1-4.1	0.02	2.7	1.4-5.2	<0.01
≥35	1.0	-	NA	1.0	-	NA
Completed high school (11 years school)	1.4	1.0-2.0	0.04	1.1	0.7-1.5	0.73
Diaphragm randomization arm (ref: control)	1.0	0.7-1.4	0.85	1.0	0.7-1.5	0.89
Contraceptive use at enrollment						
None	1.0	-	NA	1.0	-	NA
Oral contraceptives	1.0	0.5-2.2	0.92	2.1	0.9-4.9	0.09
Injectable contraceptives	1.8	0.9-3.7	0.11	1.4	0.6-3.0	0.40
Other (diaphragm, IUD, condoms, vasectomy)	2.0	1.1-4.2	0.05	1.9	0.9-3.9	0.08
High risk sex at enrollment	0.8	0.5-1.2	0.22	0.6	0.4-0.9	0.04
Partner high risk at enrollment	1.8	1.2-2.6	0.00	1.4	0.9-2.1	0.08
STI at enrollment**	1.9	1.3-2.8	0.00	2.3	1.5-3.4	<0.0001
Time varying covariates						
Vaginal sex since last visit (ref:none)	1.0	0.5-2.0	0.91	1.1	0.5-2.6	0.75
≥3 Vaginal sex acts per week (ref:0-2)	0.8	0.5-1.1	0.13	0.9	0.6-1.4	0.75
No condom use at last sex	0.7	0.4-1.0	0.06	0.6	0.4-0.9	0.04
Unprotected sex since last visit	1.2	0.9-1.7	0.27	1.3	0.9-1.9	0.18
High risk sex^	2.1	1.5-3.0	<0.0001	1.6	1.1-2.4	0.01
Suspects/knows partner concurrency	2.4	1.7-3.4	<0.0001	1.7	1.2-2.5	<0.01
Vaginal washing	1.2	0.8-1.8	0.36	1.3	0.9-2.0	0.22
Vaginal wiping	0.9	0.7-1.3	0.71	1.0	0.7-1.4	0.82
Insertion of paper products	0.9	0.5-1.5	0.55	0.9	0.5-1.6	0.70

*Multivariable models were adjusted for all covariates listed in the table

**STIs at enrollment: chlamydia, gonorrhea, trichomoniasis, HSV-2

^High risk sex: exchange, anal sex, >2 partners, new partner, new regular partner

Appendix 4.5c Unadjusted and adjusted hazard ratios estimating the relationship between pregnancy status and incident trichomoniasis infections using only those women with complete data (N=3,518)

	Unadjusted relative hazard (HR) of incident **trichomoniasis**			Adjusted relative hazard (HR)* of incident **trichomoniasis**		
	HR	95%CI	p value	HR	95%CI	p value
Pregnancy exposure group						
Pregnant	1.1	0.7-1.7	0.60	1.3	0.8-2.0	0.33
Not pregnant/oral contraceptive use	0.7	0.5-0.9	<0.01	0.9	0.6-1.3	0.67
Not pregnant/injectable contraceptive	0.9	0.7-2.0	0.56	1.0	0.7-1.4	0.98
Not pregnant/no hormonal contraceptives	1.0	-	NA	1.0	-	NA
Time fixed covariates						
Study location						
Harare, Zimbabwe	1.0	-	NA	1.0	-	NA
Johannesburg, South Africa	1.9	1.5-2.4	<0.0001	1.7	1.3-2.3	<0.01
Durban, South Africa	1.4	1.1-1.9	0.02	1.4	1.0-2.0	0.05
Age						
18-24	0.9	0.7-1.2	0.40	1.2	0.9-1.6	0.28
25-34	0.7	0.6-0.9	0.02	0.9	0.7-1.3	0.70
≥35	1.0	-	NA	1.0	-	NA
Completed high school (11 years school)	0.8	0.6-0.9	0.01	0.8	0.7-1.1	0.12
Diaphragm randomization arm (ref: control)	0.9	0.7-1.1	0.35	0.9	0.7-1.1	0.43
Contraceptive use at enrollment						
None	1.0	-	NA	1.0	-	NA
Oral contraceptives	0.5	.04-0.7	<0.0001	0.8	0.5-1.2	0.23
Injectable contraceptives	0.6	0.5-0.9	0.01	0.7	0.5-1.0	0.07
Other (diaphragm, IUD, condoms, vasectomy)	0.6	0.4-0.8	<0.01	0.6	0.5-0.9	0.01
High risk sex at enrollment	1.2	0.9-1.6	0.08	1.1	0.9-1.4	0.36
Partner high risk at enrollment	1.4	1.1-1.8	<0.01	1.2	0.9-1.6	0.08
STI at enrollment**	2.0	1.5-2.5	<0.0001	1.8	1.4-2.3	<0.0001
Time varying covariates						
Vaginal sex since last visit (ref:none)	0.9	0.6-1.4	0.74	1.1	0.6-1.8	0.79
≥3 Vaginal sex acts per week (ref:0-2)	0.8	0.6-0.9	0.04	0.9	0.7-1.2	0.44
No condom use at last sex	1.0	0.8-1.3	0.98	1.0	0.8-1.4	0.96
Unprotected sex since last visit	1.1	0.9-1.4	0.27	1.1	0.8-1.4	0.59
High risk sex^	1.5	1.2-1.9	<0.01	1.2	0.9-1.6	0.16
Suspects/knows partner concurrency	1.5	1.2-1.9	<0.01	1.3	1.0-1.6	0.05
Vaginal washing	1.1	0.9-1.4	0.38	1.1	0.9-1.5	0.33
Vaginal wiping	1.1	0.9-1.3	0.51	0.9	0.7-1.2	0.47
Insertion of paper products	1.4	1.0-1.8	0.02	1.3	0.9-1.8	0.08

*Multivariable models were adjusted for all covariates listed in the table

**STIs at enrollment: chlamydia, gonorrhea, trichomoniasis, HSV-2

^High risk sex: exchange, anal sex, >2 partners, new partner, new regular partner

Appendix 4.5d Unadjusted and adjusted hazard ratios estimating the relationship between pregnancy status and incident HIV infections using only those women with complete data (N=3,518)

	Unadjusted relative hazard (HR) of incident **HIV**			Adjusted relative hazard (HR)* of incident **HIV**		
	HR	95%CI	p value	HR	95%CI	p value
Pregnancy exposure group						
Pregnant	1.1	0.6-1.9	0.82	0.9	0.5-1.6	0.78
Not pregnant/oral contraceptive use	0.6	0.4-0.9	0.01	0.8	0.5-1.3	0.34
Not pregnant/injectable contraceptive	1.1	0.8-1.5	0.48	0.8	0.5-1.1	0.19
Not pregnant/no hormonal contraceptives	1.0	-	NA	1.0	-	NA
Time fixed covariates						
Study location						
Harare, Zimbabwe	1.0	-	NA	1.0	-	NA
Johannesburg, South Africa	2.6	2.0-3.5	<0.0001	2.0	1.3-2.9	<0.01
Durban, South Africa	1.2	0.8-1.8	0.43	0.9	0.6-1.5	0.70
Age						
18-24	2.2	1.5-3.3	<0.0001	2.7	1.8-4.1	<0.0001
25-34	1.4	0.9-2.2	0.07	1.8	1.5-2.7	0.01
≥35	1.0	-	NA	1.0	-	NA
Completed high school (11 years school)	1.2	0.9-1.6	0.13	1.2	0.9-1.6	0.13
Diaphragm randomization arm (ref: control)	1.1	0.8-1.4	0.51	1.1	0.8-1.5	0.55
Contraceptive use at enrollment						
None	1.0	-	NA	1.0	-	NA
Oral contraceptives	0.9	0.5-1.6	0.79	1.5	0.8-2.9	0.24
Injectable contraceptives	1.9	1.1-3.3	0.02	2.0	1.1-3.6	0.02
Other (diaphragm, IUD, condoms, vasectomy)	1.4	0.8-2.4	0.22	1.5	0.9-2.5	0.17
High risk sex at enrollment	1.1	0.8-1.4	0.71	1.0	0.8-1.4	0.82
Partner high risk at enrollment	1.6	1.2-2.2	<0.01	1.3	1.0-1.8	0.06
STI at enrollment**	2.2	1.6-3.0	<0.0001	2.4	1.8-3.4	<0.0001
Time varying covariates						
Vaginal sex since last visit (ref:none)	1.2	0.6-2.5	0.58	1.3	0.6-2.6	0.49
≥3 Vaginal sex acts per week (ref:0-2)	0.8	0.6-1.1	0.79	0.9	0.6-1.6	0.31
No condom use at last sex	1.0	0.7-1.4	0.97	0.9	0.6-1.3	0.46
Unprotected sex since last visit	1.3	1.0-1.8	0.04	1.3	0.9-1.9	0.07
High risk sex^	1.6	1.2-2.2	<0.01	1.2	0.9-1.7	0.22
Suspects/knows partner concurrency	2.0	1.5-2.6	<0.0001	1.4	1.1-1.9	0.02
Vaginal washing	1.1	0.8-1.4	0.72	0.9	0.6-1.3	0.53
Vaginal wiping	1.5	1.1-1.9	0.01	1.5	1.1-2.0	0.01
Insertion of paper products	1.2	0.9-1.8	0.24	1.1	0.7-1.6	0.65

*Multivariable models were adjusted for all covariates listed in the table

**STIs at enrollment: chlamydia, gonorrhea, trichomoniasis, HSV-2

^High risk sex: exchange, anal sex, >2 partners, new partner, new regular partner

Appendix 4.6 Enrollment characteristics and risk behaviors reported at follow-up visits by 4,549 women and the association with location (N=24,337 visits)

	Total		Harare, Zimbabwe		Durban, South Africa		Johannesburg, South Africa	
	N	%	N	%	N	%	N	%
Enrollment characteristics	4549	100.0%	2255	49.6%	1372	30.1%	922	20.3%
Age								
18-24	1725	37.9%	816	36.2%	564	41.1%	345	37.4%
25-34	1782	39.2%	1029	45.6%	420	30.6%	333	36.1%
≥35	1042	22.9%	410	18.2%	388	28.3%	244	26.5%
Completed high school	2347	51.6%	1111	49.3%	589	42.9%	647	70.2%
Randomization arm	2271	50.0%	1134	50.3%	684	49.9%	453	49.2%
Contraceptive use enrollment								
None	474	10.4%	116	5.1%	244	17.8%	114	12.4%
Oral	1642	36.1%	1444	64.0%	87	6.3%	111	12.0%
Injectable	1129	24.8%	314	13.9%	534	38.9%	281	30.5%
Other*	1304	28.7%	381	16.9%	507	37.0%	416	45.1%
High risk sex	1059	23.3%	535	23.7%	257	18.7%	267	29.0%
Partner high risk	2836	62.3%	1333	59.1%	902	65.7%	601	65.2%
STI at enrollment**	2776	61.0%	1186	52.6%	972	70.9%	618	67.0%
	N	%	N	%	N	%	N	%
Follow-up visits	24337	100.0%	13018	53.5%	6743	27.7%	4576	18.8%
Pregnancy/HC use at visit								
Pregnant	1609	6.6%	875	6.7%	428	6.4%	306	6.7%
Not pregnant/oral HC	8375	34.4%	7358	56.5%	400	5.9%	617	13.5%
Not pregnant/injectable HC	6028	24.8%	2041	15.7%	2547	37.8%	1440	31.5%
Not pregnant/no HC	8325	34.2%	2744	21.1%	3368	50.0%	2213	48.4%
Any vaginal sex since last visit	22933	94.2%	12725	97.8%	6378	94.6%	3830	83.7%
Sex ≥3 times per week	15948	65.5%	9424	72.4%	4194	62.2%	2330	50.9%
Condom use last sex	16856	69.3%	9101	69.9%	4878	72.3%	2877	62.9%
Unprotected sex (any report)	14334	58.9%	7481	57.5%	3780	56.1%	3073	67.2%
Sex in exchange money/drugs	728	3.0%	445	3.4%	130	1.9%	153	3.3%
≥2 sex partners since last visit	1584	6.5%	215	1.7%	454	6.7%	915	20.0%
New sex partner	2831	11.6%	966	7.4%	1108	16.4%	757	16.5%
Partner has other sex partners (suspects/knows)	6064	24.9%	2572	19.8%	2202	32.7%	1290	28.2%
High risk sex***	4415	18.1%	1828	14.0%	1501	22.3%	1086	23.7%
Vaginal washing (any)	18328	75.3%	10072	77.4%	4770	70.7%	3486	76.2%
Vaginal wiping (any)	9900	40.7%	5653	43.5%	2599	38.5%	1648	36.0%
Vaginal insertion (any)	3151	13.0%	1760	13.5%	721	10.7%	670	14.6%

*Other contraceptives at enrollment: diaphragm, IUD, condoms, male partner vasectomy
**STIs at enrollment: chlamydia, gonorrhea, trichomoniasis, HSV-2
***High risk sex: exchange, anal sex, >2 partners, new partner, new regular partner

Appendix 4.6 continued

	OR Durban vs. Harare			OR Johannesburg vs. Harare		
	OR	**95%CI**	**p-value**	**OR**	**95%CI**	**p-value**
Enrollment characteristics						
Age						
18-24	**0.6**	**0.5-0.7**	**<0.0001**	**0.8**	**0.6-0.9**	**<0.01**
25-34	**1.4**	**1.1-1.6**	**<0.01**	**1.4**	**1.2-1.7**	**<0.01**
≥35	1.0	-	NA	1.0	-	NA
Completed high school	**0.8**	**0.7-0.9**	**<0.01**	**2.4**	**2.1-2.9**	**<0.0001**
Diaphragm randomization arm	1.0	0.9-1.1	0.80	1.0	0.8-1.1	0.63
Contraceptive use at enrollment						
None	1.0	-	NA	1.0	-	NA
Oral contraceptives	**<0.1**	**<0.1-0.1**	**<0.0001**	**0.1**	**0.1-0.2**	**<0.0001**
Injectable contraceptives	0.8	0.6-1.1	0.11	0.9	0.7-1.2	0.55
Other*	0.6	0.5-0.8	<0.01	1.1	0.8-1.5	0.48
High risk sex at enrollment	**0.7**	**0.6-0.9**	**<0.01**	**1.3**	**1.1-1.6**	**<0.01**
Partner high risk at enrollment	**1.3**	**1.2-1.5**	**<0.001**	**1.3**	**1.1-1.5**	**<0.01**
STI at enrollment**	**2.2**	**1.9-2.5**	**<0.001**	**1.8**	**1.6-2.2**	**<0.001**
Follow-up visits	**OR**	**95%CI**	**p-value**	**OR**	**95%CI**	**p-value**
Pregnancy/HC use at visit						
Pregnant	**0.3**	**0.3-0.4**	**<0.0001**	**0.4**	**0.3-0.4**	**<0.0001**
Non-pregnant/oral HC	**0.1**	**0.0-0.1**	**<0.0001**	**0.1**	**0.1-0.2**	**<0.001**
Non-pregnant/injectable HC	1.1	1.0-1.3	0.19	1.0	0.8-1.1	0.72
Non-pregnant/no HC	1.0	-	-	1.0	-	-
Any vaginal sex since last visit	**0.5**	**0.4-6**	**<0.0001**	**0.1**	**0.1-0.2**	**<0.0001**
Sex ≥3 times per week	**0.4**	**0.4-0.5**	**<0.0001**	**0.2**	**0.2-0.3**	**<0.0001**
Condom use last sex	**1.3**	**1.2-1.5**	**<0.0001**	**1.2**	**1.1-1.3**	**0.02**
Unprotected sex (any report)	0.9	0.8-1.0	0.08	**1.5**	**1.3-1.7**	**<0.0001**
Sex in exchange money/drugs	**0.5**	**0.4-0.7**	**<0.0001**	0.9	0.7-1.3	0.65
≥2 sex partners since last visit	**4.4**	**3.5-5.6**	**<0.0001**	**14.6**	**11.8-18.2**	**<0.0001**
New sex partner	**2.5**	**2.1-2.8**	**<0.0001**	**2.5**	**2.1-2.8**	**<0.0001**
Partner has other sex partners (suspects/knows)	**2.1**	**1.8-2.3**	**<0.0001**	**1.6**	**1.4-1.8**	**<0.0001**
High risk sex***	**1.7**	**1.5-1.9**	**<0.0001**	**1.9**	**1.7-2.1**	**<0.0001**
Vaginal washing (any)	**0.7**	**0.6-0.8**	**<0.0001**	1.0	0.8-1.1	0.58
Vaginal wiping	**0.8**	**0.7-0.9**	**<0.0001**	**0.7**	**0.7-0.8**	**<0.0001**
Vaginal insertion (any)	**0.7**	**0.6-0.9**	**<0.0001**	1.1	0.9-1.3	0.38

*Other contraceptives at enrollment: diaphragm, IUD, condoms, male partner vasectomy
**STIs at enrollment: chlamydia, gonorrhea, trichomoniasis, HSV-2
***High risk sex: exchange, anal sex, >2 partners, new partner, new regular partner

Appendix 4.7 Incidence rates of four STIs by pregnancy and hormonal contraceptive exposure group by location

Harare, Zimbabwe

	Overall			Pregnant visits			Non-pregnant & oral HC use visits			Non-pregnant & injectable HC use visits			Non-pregnant & no HC use		
	Cases	Person years	IR p/pyr	Cases	Person years	IR p/pyr	Cases	Person years	IR p/pyr	Cases	Person years	IR p/pyr	Cases	Person years	IR p/pyr
Chlamydia	116	3223	**3.6**	14	215	**3.6**	62	1825	**3.4**	20	500	**4.0**	20	683	**2.9**
Gonorrhea	48	3287	**1.5**	9	222	**4.1**	25	1859	**1.3**	3	512	**0.6**	11	694	**1.6**
Trichomoniasis	177	3158	**5.6**	20	214	**9.3**	95	1788	**5.3**	26	492	**5.3**	36	664	**5.4**
HIV	85	3307	**2.6**	8	225	**3.6**	48	1868	**2.6**	11	516	**2.1**	18	698	**2.6**

Durban, South Africa

	Overall			Pregnant visits (lab+ and reported)			Non-pregnant & oral HC use visits			Non-pregnant & injectable HC use visits			Non-pregnant & no HC use		
	Cases	Person years	IR p/pyr	Cases	Person years	IR p/pyr	Cases	Person years	IR p/pyr	Cases	Person years	IR p/pyr	Cases	Person years	IR p/pyr
Chlamydia	166	1618	**10.3**	12	98	**12.2**	4	100	**4.0**	76	603	**12.6**	74	817	**9.1**
Gonorrhea	73	1689	**4.3**	7	106	**6.6**	2	100	**2.0**	33	634	**5.2**	31	849	**3.7**
Trichomoniasis	162	1636	**9.9**	13	101	**12.9**	9	99	**9.1**	60	620	**9.7**	80	817	**9.8**
HIV	117	1715	**6.8**	5	111	**4.5**	4	102	**3.9**	48	645	**7.4**	60	857	**7.0**

Johannesburg, South Africa

	Overall			Pregnant visits (lab+ and reported)			Non-pregnant & oral HC use visits			Non-pregnant & injectable HC use visits			Non-pregnant & no HC use		
	Cases	Person years	IR p/pyr	Cases	Person years	IR p/pyr	Cases	Person years	IR p/pyr	Cases	Person years	IR p/pyr	Cases	Person years	IR p/pyr
Chlamydia	118	1094	**10.8**	12	71	**16.9**	18	142	**12.7**	50	336	**14.9**	38	546	**7.0**
Gonorrhea	44	1154	**3.8**	4	78	**5.1**	7	154	**4.5**	13	363	**3.6**	20	560	**3.6**
Trichomoniasis	81	1141	**7.1**	3	76	**3.9**	11	153	**7.2**	23	357	**6.4**	44	556	**7.9**
HIV	38	1177	**3.2**	3	81	**3.7**	4	158	**2.5**	15	369	**4.1**	16	569	**2.8**

Appendix 4.8 Post-hoc power calculations

Power calculations were performed in NCSS Power Analysis and Sample Size (PASS)© 2008 software for each of the four STI outcomes. Alpha was set at 0.05 and the sample size of 4,549 women was used for analyses in a Cox Proportional Hazards model. The overall incidence observed for each STI was used for the estimate of the overall event rate and the observed log of the hazard ratio was input for the comparison between pregnant and non-pregnant/no HC periods. Two estimates for the r-square of the exposure with the other covariates were used, 0.4 and 0.7 (indicating high correlation between the other exposure and the variables in the model which was observed in the analysis of pregnancy status predicting reported behaviors in Chapter 3) and the standard deviation was estimated using the proportion of outcomes in the overall group and the pregnancy-exposed group to give two estimates for each outcome. The results below indicate that the sample was adequate as evidenced by power >80% to detect differences in incidence for each of the STIs given high correlation between exposure and the other covariates in the model.

Chlamydia

Power	Sample Size (N)	Reg. Coef. (B)	S.D. of X1 (SD)	Event Rate (P)	R-Squared X1 vs Other X's (R2)	Two-Sided Alpha	Beta
1.00000	4549	0.4055	2.5000	0.0670	0.4000	0.05000	0.00000
1.00000	4549	0.4055	2.9000	0.0670	0.4000	0.05000	0.00000
1.00000	4549	0.4055	2.5000	0.0670	0.7000	0.05000	0.00000
1.00000	4549	0.4055	2.9000	0.0670	0.7000	0.05000	0.00000

Gonorrhea

Power	Sample Size (N)	Reg. Coef. (B)	S.D. of X1 (SD)	Event Rate (P)	R-Squared X1 vs Other X's (R2)	Two-Sided Alpha	Beta
1.00000	4549	0.4700	1.6000	0.0270	0.4000	0.05000	0.00000
1.00000	4549	0.4700	2.2000	0.0270	0.4000	0.05000	0.00000
0.99540	4549	0.4700	1.6000	0.0270	0.7000	0.05000	0.00460
0.99999	4549	0.4700	2.2000	0.0270	0.7000	0.05000	0.00001

Trichomoniasis

Power	Sample Size (N)	Reg. Coef. (B)	S.D. of X1 (SD)	Event Rate (P)	R-Squared X1 vs Other X's (R2)	Two-Sided Alpha	Beta
0.91255	4549	0.0953	2.5000	0.0710	0.4000	0.05000	0.08745
0.97831	4549	0.0953	3.0000	0.0710	0.4000	0.05000	0.02169
0.64997	4549	0.0953	2.5000	0.0710	0.7000	0.05000	0.35003
0.80353	4549	0.0953	3.0000	0.0710	0.7000	0.05000	0.19647

HIV

Power	Sample Size (N)	Reg. Coef. (B)	S.D. of X1 (SD)	Event Rate (P)	R-Squared X1 vs Other X's (R2)	Two-Sided Alpha	Beta
0.99210	4549	-0.2231	1.9000	0.0390	0.4000	0.05000	0.00790
0.99590	4549	-0.2231	2.0000	0.0390	0.4000	0.05000	0.00410
0.87129	4549	-0.2231	1.9000	0.0390	0.7000	0.05000	0.12871
0.90238	4549	-0.2231	2.0000	0.0390	0.7000	0.05000	0.09762