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Evaluating individual and couple-level risk factors associated with HIV acquisition in HIV serodiscordant couples in Zambia: Alcohol use, fertility desire, and HIV acquisition from outside partners in an open cohort in Zambia

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Evaluating individual and couple-level risk factors associated with HIV acquisition in HIV serodiscordant couples in Zambia:
Alcohol use, fertility desire, and HIV acquisition from outside partners in an open cohort in Zambia

A dissertation submitted in partial satisfaction of the requirements

for the degree

Doctor of Philosophy in Epidemiology

by

Dvora Leah Davey

2016

ABSTRACT OF THE DISSERTATION

Evaluating individual and couple-level risk factors associated with HIV acquisition in HIV serodiscordant couples in Zambia: Alcohol use, fertility desire, and HIV acquisition from outside partners in an open cohort in Zambia.

by

Dvora Leah Davey

Doctor of Philosophy in Epidemiology

University of California, Los Angeles, 2016

Professor Pamina M. Gorbach, Chair

The high prevalence of serodiscordant relationships coupled with the high risk of transmission within those relationships across sub-Saharan African countries makes these couples a target for HIV prevention efforts. The development of appropriate and effective interventions for HIV prevention in serodiscordant couples must be based on accurate assessments of sexual risk behaviors, substance use, and fertility desires. My dissertation evaluates HIV acquisition and transmission in heterosexual HIV serodiscordant couples in Lusaka, Zambia.

My first study analyzed the role that alcohol use plays on sexual behavior and HIV acquisition in Zambian serodiscordant couples. Men who reported heavy drinking was associated with increased odds of having =>1 outside sexual partnership (adjusted odds ratio [aOR]=2.02; 95% CI=1.58, 2.57), and condomless sex with main partner in the past 3-months (aOR=1.61; 95% CI=1.28, 2.04). Women's heavy drinking was associated with increased odds of having =>1

outside partner (aOR=1.89, 95% CI=1.35, 2.64), and condomless sex with main partner in past 3-months (aOR=1.54; 95% CI=1.31, 1.82). Women who reported being drunk daily or almost daily was associated with increased HIV acquisition (aHR=3.71; 95% CI=0.90, 15.25). Men who reported being drunk weekly or daily/almost daily was associated with increased HIV acquisition (aHR=1.72; 95% CI= 1.01, 2.93), and transmission (aHR=1.42; 95% CI=0.97, 2.10).

My second study evaluated the incidence and predictors of HIV acquisition from an outside partner in serodiscordant couples in Zambia. Forty-five unlinked HIV infections occurred among women (1.85 per 100-couple-years [CY]) and 55 unlinked HIV infections occurred among men (1.82 per 100 CY). Risk of female unlinked infection was associated with baseline female alcohol consumption (aHR=5.44; 95% CI: 1.03, 28.73), recent genital ulcers and/or genital inflammation (aHR=6.09; 95% CI: 2.72, 13.64 and aHR=11.92; 95% CI: 5.60, 25.37, respectively). Risk of male unlinked HIV infection was associated with reporting being drunk weekly or daily/almost daily at baseline (aHR=3.52; 95% CI=1.19, 10.46), recent genital inflammation (aHR=8.52; 95% CI: 3.82, 19.03), genital ulceration (aHR=4.27; 95% CI: 2.05, 8.89), self-reporting =>1 outside partner (aHR=3.36; 95% CI: 1.53, 7.37).

My third study evaluated the effect of fertility desires on HIV acquisition among HIV serodiscordant couples in Zambia. Among a sub-set of 1,029 serodiscordant couples, 311 agreed that they wanted a child in the future (30.4%), and 368 agreed they did not want a child or did not know (36.0%), and 344 couples disagreed about having a child (33.6%), of which in 212 couples (61.6% of disagreeing couples) the man wanted a child but the woman did not, and in 132 couples (38.3% of disagreeing couples) the woman wanted a child but the man did not. The adjusted risk ratio for woman's HIV acquisition was 2.06 (95% CI=1.40, 3.03) among women who wanted a child, 1.75 (95% CI=1.07, 2.87) for men who wanted a child in the next 12-months, and 2.55 (95%

CI=1.32, 4.93) among couples who agreed that they wanted a child compared to couples who agreed they did not want a child.

In conclusion, heavy alcohol use plays an important role in linked and unlinked HIV acquisition and transmission in serodiscordant couples. The strongest predictors of unlinked infection in serodiscordant relationships were alcohol use, genital inflammation and ulceration prior to infection. Further, women were particularly vulnerable when they or their partner wanted to have a child. These findings will help improve the implementation of HIV prevention and safer conception services among heterosexual serodiscordant couples in Southern Africa and beyond.

The dissertation of Dvora Leah Davey is approved.

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ABBREVIATIONS

Acquired Immunodeficiency Syndrome (AIDS)

Adjusted Hazard Ratio (aHR)

Adjusted Odds Ratio (aOR)

Adjusted Risk Ratio (aRR)

Antiretroviral therapy (ART)

AUDIT (Alcohol Use Disorders Identification Test)

Confidence Interval (CI)

Couples HIV Testing and Counseling (CHTC)

Couple year (CY)

Hazard ratio (HR)

HIV Prevention Trials Network (HPTN)

Human Immunodeficiency Virus (HIV)

Institutional Review Boards (IRB)

Inverse probability treatment weights (IPTW)

Marginal structural models (MSM)

Male HIV-positive/Female HIV-negative couple (M+F-)

Male HIV-negative/Female HIV-positive couple (M-F+)

Odds Ratio (OR)

Pre-exposure Prophylaxis (PrEP)

Prevention of mother to child transmission (PMTCT)

Risk Ratios (RR)

Rwanda Zambia HIV Research Group (RZHRG)

Sexually Transmitted Infection (STI)

Simian Immunodeficiency Virus (SIV)

Standard Deviation (SD)

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My appreciation also extends to my Doctoral Committee including Drs. Javanbakht, Comulada and Lee for their guidance and support of this dissertation. Each brings her or his own strengths and subject matter expertise to my project, shaping its content and making it a stronger dissertation. I also worked closely with Dr. Allen's research team at Emory University, including Dr. Kristin Wall, Dr. Ilene Brill, Amanda Tichacekm and Htee Khu for their assistance and support. I truly appreciated their capacities and professionalism, and learned a lot from each along the way.

I dedicate my work to my husband, Andrew, and my loving kids Ayden and Sophia (who was born during my first quarter at UCLA), without whom this dissertation would never have been completed. To my kids, you continue to bless me with a life of joy in the hours when my computer was closed. To Andrew, who moved across the world, moving mountains to enable me to chase and fulfill my dream, I will always be indebted to you. Thank you for your support, encouragement and sacrifice as you allowed me to chase my dream.

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SELECT PUBLICATIONS

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Joseph Davey D, Wang D, Billings J, Adachi K, Klausner J. "Systematic review of curable sexually transmitted infections among pregnant women in low and middle-income countries, 2010-2015." Sexually Transmitted Disease (STD), April, 2016 (In Press).

Joseph Davey D, Acosta Ldel R, Gupta P, Konda KA, Caceres CF, Klausner JD. Probable Syphilitic Aortitis Documented by Positron Emission Tomography. Sex Transm Dis. 2016 Mar;43(3):199-200. doi: 10.1097/OLQ.0000000000000411. PubMed PMID: 26859808.

Bustamante MJ, Konda KA, **Joseph Davey D**, León SR, Calvo GM, Salvatierra J, Brown B, Caceres CF, Klausner JD. HIV self-testing in Peru: questionable availability, high acceptability but potential low linkage to care among men who have sex with men and transgender women. Int J STD AIDS. 2016 Jan 29. pii: 0956462416630674. PubMed PMID: 26826160.

Wall K, Karita E, **Joseph Davey D**, De Clercq F, Kestelyn E, Allen S. "Influence network effectiveness in promoting couples' HIV voluntary counselling and testing in Kigali, Rwanda." AIDS. 2012 Jan 14; 26(2):217.

Submitted manuscripts:
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Chapter I. Introduction and background

Despite declining rates HIV incidence in sub-Saharan Africa, a large proportion of incident HIV infections occur in the context of stable HIV serodiscordant heterosexual relationships. The high prevalence of serodiscordant relationships coupled with the high risk of transmission within those relationships makes serodiscordant couples a key target for global HIV prevention efforts. To improve the development of appropriate and effective interventions for HIV prevention among serodiscordant couples, we analyzed factors associated with HIV transmission and acquisition in a long running Zambian cohort of HIV serodiscordant couples.

The purpose of this dissertation is to study three individual and couple-level factors associated with HIV transmission and acquisition in serodiscordant couples in Zambia. The first study examines the association between alcohol use and HIV transmission and acquisition among men and women in serodiscordant couples. The second study evaluates the association between fertility desire and HIV transmission and acquisition among men and women in serodiscordant couples. The third study examines factors associated with HIV acquisition from sex partners outside of the partnership. This project uses data from the Heterosexual Transmission of HIV Study conducted by the Rwanda Zambia HIV Research Group (RZHRG), which was an open prospective cohort which enrolled 3,049 adult heterosexual serodiscordant couples recruited from couples counseling and testing sites in Lusaka, Zambia. Data collection began in January 1995 and ended in December 2012.

1.1 HIV Epidemiology

HIV is spread primarily through sexual contact, with most new infections occurring in individuals of reproductive age [1]. A large proportion of incident HIV infections in sub-Saharan

Africa occur in the context of stable HIV serodiscordant (in which one partner is HIV-positive and the other is HIV-negative) heterosexual relationships [1-3]. The high prevalence of HIV serodiscordant relationships coupled with the high risk of transmission within those relationships (with a median of 11.1 incident HIV infections per 100 person-years) across African countries makes serodiscordant couples a key target for global HIV prevention efforts [4, 5]. The development of appropriate and effective interventions for HIV prevention among serodiscordant couples must be based on accurate assessments of sexual risk behaviors, substance use, and fertility desires in the context of such couples.

Improved understanding of the drivers of HIV infection over long periods of time in large, high-risk cohorts is critical to develop, target, and deliver high-impact prevention interventions. Factors with uncertain or debated influence on HIV transmission and acquisition at the individual level (including hormonal contraception [6-8], pregnancy and post-partum periods [9-11], concurrent sexual partnerships [13-18], alcohol use [19-22], and age discordancy [23-25]) should be further examined in the context of sexual partnerships. Specifically, little is known about the behaviors and risk in uninfected partners of serodiscordant couples. Learning one is in a HIV serodiscordant relationship may turn the HIV uninfected partner to seek outside partners, potentially of unknown HIV serostatus who might be perceived at lower risk of HIV infection than their primary HIV infected partner. Understanding the behaviors with primary and outside partners, and the association with HIV acquisition ('unlinked infections'), will inform HIV risk reduction messages for serodiscordant couples and be important for the design of HIV prevention strategies for uninfected partners.

Specific to the partnership or couple level, attempts to conceive may increase the risk of HIV transmission or reinfection [24, 25]. Further research has shown the burden of unplanned

pregnancy on HIV-positive and serodiscordant couples in Africa [9-11]. Despite this risk, the reproductive health concerns of serodiscordant couples has received little attention. This research gap is of concern because most serodiscordant couples are of child-bearing age, and with the widespread use of ART, life expectancy and sexual activity continue to increase.

Further, heavy alcohol use is a widespread problem among those at risk for and living with HIV, and its use by at least one partner can affect transmission risks to both partners, as well as ART adherence and disease progression of the infected partner whether or not they are the heavy drinker [28-31]. Alcohol elevates sexual risks through multiple channels including its interaction with personality characteristics and drinking environments, and its effects on decision making that can impact both members of a sexual partnership in which heavy drinking occurs. Further, excess alcohol consumption may lead to immune deficiency, causing increased susceptibility to certain diseases [31]. Finally, the high rate of seroconversion among these couples is only partially mitigated by ART in real-world settings [26, 27].

1.1.1 Epidemiology of HIV worldwide and in Africa

Globally, an estimated 35.3 million (32.2-38.8) people were living with HIV worldwide in 2012. There were 2.3 million (1.9-2.7) new HIV infections globally, showing a 33% decline in the number of infections from 3.4 (3.1-3.7) million in 2001. Specifically, incident HIV infections among adults in sub-Saharan Africa has declined by 34% since 2001 [54]. Despite these gains, the HIV epidemic continues to disproportionately affect sub-Saharan Africa, where 70% of all new HIV infections occurred in 2012. HIV prevalence within sub-Saharan Africa varies by region, as does the prevalence of HIV serodiscordant couples. Half to two-thirds of HIV infected adults in a cohabitating relationship in Africa have an HIV uninfected partner [4, 5], and women are equally likely as men to be the HIV infected member in a serodiscordant couple [5]. HIV incidence in

studies with HIV serodiscordant couples ranges from 2.0 to 11.8/100 person-years depending on the type of study and accompanying services made available to the couples such as couples HIV counseling and testing, ongoing counseling, ART, male circumcision, and free condoms [4, 5].

1.1.2 Epidemiology of HIV in Zambia

Zambia is one of the countries most affected by HIV in the world. The epidemic is generalized and cuts across gender, age, geographical and socio-economic status of the population. An estimated 1,100,000 [1,100,000 - 1,200,000] people were living with HIV in 2013, with an HIV prevalence rate of 12.5% [11.9% - 13.3%] among adults ages 15-49 [54]. Prevalence among females is at 16.1% against 12.3% in males. Urban-rural differentials also exist, with urban areas having a much higher prevalence rate of 20% compared with 10% for rural areas [54]. HIV is the leading cause of death for all ages in Zambia.

Over the past decade, Zambia has introduced and scaled high impact interventions in HIV prevention, care and treatment. As a result, HIV incidence has declined between 2002 and 2012, from a high of 16.1% in 2002 to 14.3% in 2007 and now 12.5% in 2012. Despite this decline, HIV continues to occur in the context of stable HIV serodiscordant heterosexual relationships [1-3]. In Zambia, an estimated 11% of all stable partnerships are serodiscordant. Of partnerships affected by HIV, 58% are serodiscordant, and 42% are concordant HIV-positive [4-5]. Cohabiting couples account for an estimated two-thirds of incident HIV infections [1].

The high prevalence of HIV serodiscordant relationships [4] coupled with the high risk of transmission within those relationships (with a median of 11.1 HIV transmissions per 100 person-years [5]) across Zambia, and other Southern African countries makes serodiscordant couples a key target for HIV prevention efforts. As a result, several African countries are adopting couples' HIV counseling and testing as an HIV prevention and care intervention [18]. Thus, more couples are

becoming aware of their HIV status with their partner, and many find out they are HIV serodiscordant. Appropriate and effective interventions for HIV prevention among serodiscordant couples depend on having accurate assessments of their sexual risk behaviors, substance use, and fertility desires.

1.1.3 Access to HIV Treatment in Zambia

Between 55-60% of Zambian adults requiring treatment were on ART in 2013 [43]. Zambia has adopted the WHO-recommended approach to couples in whom one person is HIV-positive by providing treatment regardless of their CD4 cell count [45]. However, despite this policy change, the high rate of seroconversion among serodiscordant couples is only partially mitigated by ART in real-world settings [3, 4], partly because of ongoing sex with outside, HIV-infected, partners. Therefore, primary prevention strategies are still needed for HIV-negative partners in serodiscordant couples, especially considering that within this cohort, over 1.83 incident infections from an outside sex partner occurred over 100 couple years.

ART significantly improves the health and survival of HIV-infected individuals [77, 78], and reduces their infectiousness and likelihood of transmitting HIV to one's sexual partners [76, 78-80]. HPTN 052, a multinational randomized clinical trial with HIV serodiscordant couples, demonstrated that early initiation of ART between CD4 counts of 350-550 cells/mm^3 reduced the risk of HIV transmission by 96% to the uninfected partner, compared to delayed ART initiation at CD4 of 250 cells/mm^3 [76]. The significant reductions in HIV transmission was also due to behavioral and biomedical interventions, such as quarterly viral load monitoring and adherence counseling, which enabled index partners' to be virally suppressed during the study.

As a result, WHO drafted guidelines on Couples HIV Testing and Counseling (CHTC) which recommended earlier ART initiation for serodiscordant couples to reduce transmission to

HIV-uninfected partners. However, primary prevention strategies are also needed for serodiscordant couples, as ART will not protect uninfected partners who seroconvert not from their HIV infected partner, but from outside or new partners, which accounted for 25-29% of HIV transmissions in HPTN 052 and the Partners in Prevention HSV/HIV Transmission Study [79], and 21% of seroconversions in our Zambian cohort (see study #2).

Another strategy to prevent HIV transmission to the uninfected partner is pre-exposure prophylaxis or PrEP. In the Partners PrEP Study, daily oral Tenofovir (TDF) and FTC/TDF were shown to have 62%-73% reduction in HIV acquisition among in East African heterosexual serodiscordant couples [83]. In contrast, FEM-PrEP, a trial of daily oral FTC/TDF in high-risk women, was stopped early due to lack of efficacy [85]. Similarly, the VOICE trial of TDF was also stopped early because of inability to demonstrate efficacy in HIV prevention [86]. Qualitative and biomarkers demonstrated that the lack of efficacy in the FEM-PrEP and VOICE trials was likely due to insufficient PrEP adherence [85, 86]. Unfortunately, PrEP is currently unavailable in Zambia as a potential risk reduction strategy for serodiscordant couples. However, a cost-benefit analysis should be modeled to help facilitate policy decisions for targeted prevention activities such as PrEP among serodiscordant couples in which the HIV-negative woman wants to get pregnant.

1.2 Focus of Dissertation

1.2.1 Impact of alcohol on HIV seroconversion

Alcohol is the most commonly used psychoactive substance in the world and its use is among the most prevalent behaviors associated with sexual risk for HIV and other STIs including

HIV. Chronic alcohol abuse is a widespread problem among those at risk for and living with HIV and it can affect transmission, ART adherence and disease progression [28-31]. Alcohol elevates sexual risks through multiple channels including its interaction with personality characteristics and drinking environments, and its effects on decision making. In addition, a recent study of simian immunodeficiency virus (SIV) in macaques showed that chronic alcohol treatment negatively affects the genital micro-environment prior to and over the course of SIV infection and may increase the risk of genital virus shedding and transmission [32-33].

A previous study among the same Zambian cohort by Coldiron, et al. showed that after controlling for demographic and socioeconomic covariates, alcohol use by male partners of serodiscordant couples were associated with self-reported condomless sex at follow up in Zambia [47]. However, other measures of alcohol consumption were not associated with condomless sex. Further, Coldiron et al. did not have the power to detect differences between HIV and STI incidence among those who reported high alcohol use compared with those who did not. A 2011 Ugandan study on extramarital sex, condom use, and alcohol use found associations between alcohol use, increased extra marital sex, condomless sex and HIV transmission [48]. In addition, a literature review conducted on alcohol use and high-risk sexual behavior in sub-Saharan Africa highlighted the need for continued research on the impact of alcohol on HIV and STI transmission, especially among women, who have rarely been a focus of such studies [46]. Finally, a 2014 study from South Africa suggested that interventions inadequately address alcohol use among couples. The South African study recommends that interventions must include messaging around alcohol use in order to bring the heterosexual HIV transmission in South Africa under control [70]. Our first study evaluated the impact of heavy alcohol use by males and females on seroconversion in a cohort of serodiscordant couples.

1.2.2 Non-partnership based HIV infection

Little is known about the behaviors and risk in uninfected partners of serodiscordant couples [61]. Learning one is in a HIV serodiscordant relationship may turn the HIV uninfected partner to seek outside partners, potentially of unknown HIV serostatus who might be perceived at lower risk of HIV infection than their primary HIV infected partner. Understanding the behaviors with primary and outside partners, and the association with HIV acquisition from outside partners will help inform HIV risk reduction messages for serodiscordant couples and be important for the design of HIV prevention strategies for uninfected partners.

1.2.3 Fertility desires among HIV discordant couples

Of the estimated 33 million people living with HIV worldwide, 16 million are women [54]. Since the advent of ART, pregnancy rates and the desire to conceive have been increasing among women living with HIV, including among serodiscordant couples, due in part to expectations of improved quality of life, increased life expectancy and reduced rates of mother to child transmission [34]. However, attempts to conceive may increase the risk of HIV transmission or reinfection [34, 35]. Further, some studies have found pregnancy in the HIV-infected or uninfected female to be associated with two-fold increased risk of male to female and female to male HIV transmission [89]. Pregnancy is complex in that it causes both biological and behavioral changes in a woman and consequently in the sexual dynamics of the partnership. As a result, pregnancy may confound and/or mediate the risk of HIV infection depending on the biological mechanism relating the exposure to the outcome of interest [86-89].

Despite this risk of HIV acquisition and transmission, the reproductive health concerns of serodiscordant couples has received little attention. This research gap is of concern because most

serodiscordant couples are of child-bearing age, and with the widespread use of ART, life expectancy and sexual activity continue to increase [35]. Further, preventing unintended pregnancies among HIV-positive women through family planning reduces pregnancy-related morbidity and mortality, decreases the number of pediatric HIV infections, and has also proven to be a cost-effective way to prevent mother-to-child HIV transmission [36]. In order to minimize periconception HIV transmission among serodiscordant couples, conception attempts should be delayed until the infected partner is on ART, and viral load is undetectable.

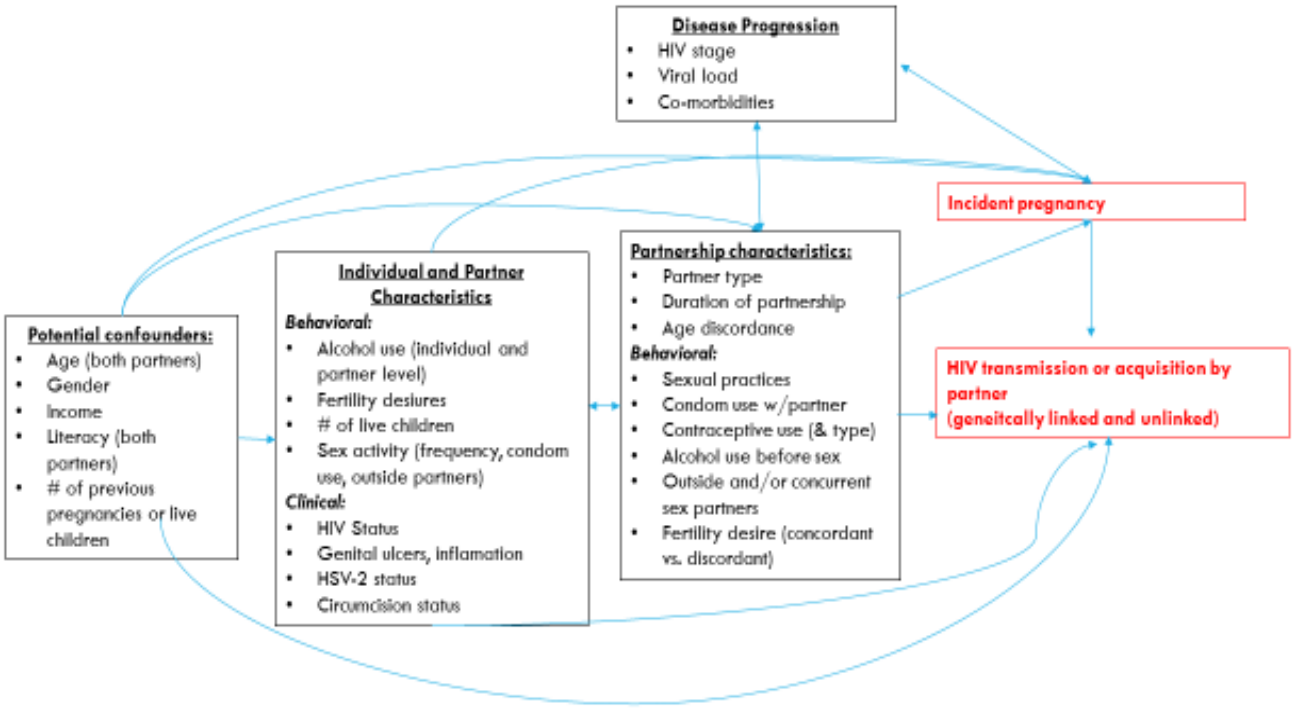
Previous research on the determinants of fertility desires has yielded mixed results [56, 57]. Men and women of younger age and those without children are more likely to desire (more) children [56, 57]. However, desire for fertility may also be influenced by gender, HIV status, ART, knowledge of prevention of mother to child transmission of HIV programs (PMTCT), stigma and social support as well as family, culture and gender norms [56]. A recent study in Zambia by Cook et al. [57] found that desire for children was often shared by couple members, and among serodiscordant couples or concordant HIV-positive couples the strongest predictor of a partner's desire for children was having a partner who wanted children. This study will examine the role of individual and couple level fertility intentions as a risk factor for HIV acquisition among men and women in HIV serodiscordant couples.

1.3 Conceptual Model

The conceptual model shown in Figure 1 was adapted from a model developed by Gorbach et al. [64] to explain how individual and couple level dynamics may affect sexual risk behaviors and the transmission and acquisition of HIV. I have expanded this model to contain key elements of the Social Action Theory as individual, partner and partnership characteristics interact to affect the self-protective behavior of condom use, sex with other partners, and alcohol use among partners.

This model was further adapted to include both behaviors and clinical factors that could affect transmission of HIV within serodiscordant couples. This model guided my analyses for each of the specific aims.

Figure 1: Conceptual model of individual, partner and couple level factors that affect HIV transmission and acquisition among HIV serodiscordant couples



Chapter II. Study 1: Risky sex and HIV acquisition among HIV serodiscordant couples in Zambia: What does alcohol have to do with it?

2.1 Abstract

Objective: This study evaluates the effects of alcohol consumption on high-risk sexual behavior and HIV acquisition among Zambian serodiscordant couples.

Design: Prospective longitudinal cohort of 3,049 serodiscordant couples in Lusaka, Zambia.

Methods: We evaluated factors associated with alcohol consumption at baseline, and its effect on having outside sexual partnerships and condomless sex with primary partner using multivariable logistic regression. We analyzed the effect of alcohol consumption on HIV acquisition using mutivariable Cox models stratified by gender of HIV-infected partner.

Results: Over one-half of couples were female HIV-positive (n=1656; 54.3%). About 4.8% of women reported drinking alcohol =>2 times a week or more. Factors associated with women's alcohol use included older age (aOR=1.03; 95%CI=1.01, 1.05), partner heavy drinking (aOR=4.13; 95%CI=3.29, 5.19), and HIV-positive status at baseline (aOR=1.62; 95%CI=1.27, 2.05). Among men, 17% reported drinking >=4 times per week. Heavy drinking among men was associated with younger age (aOR=0.98 per year; 95%CI=0.96, 0.99), greater age disparity with partner (aOR=1.04 per year increase; 95%CI=1.02, 1.06), self-reported STI in last year (at baseline) (aOR=1.21; 95%CI=1.06, 1.38), and partner heavy drinking (aOR=2.83; 95%CI=2.00, 4.04). Men who reported being drunk daily/almost daily had twice the odds of having an outside

partnership (aOR=2.02; 95%CI=1.58, 2.57) and condomless sex with main partner in past 3-months (aOR=1.61; 95%CI=1.28, 2.04) compared to men who did not report being drunk in the past year. Women who reported being drunk in the past year had almost double the odds of having 1 or more outside partners (aOR=1.89; 95%CI=1.35, 2.64), and condomless sex with main partner in the past 3-months (aOR=1.54; 95%CI=1.31, 1.82). Women's who reported being drunk daily/almost daily had an almost 4-fold increased risk of HIV acquisition (aHR=3.71; 95%CI=0.90, 15.25) compared to reporting never being drunk in the past year. Men who reported being drunk weekly or more frequently had an increased risk of HIV acquisition (aHR=1.72; 95%CI=1.01, 2.93), and transmission (aHR=1.42; 95%CI=0.97, 2.10) compared to those who reported never being drunk in the past year.

Conclusion: Alcohol consumption is a risk factor for outside sex partnerships, condomless sex and HIV acquisition and transmission (from men to women) in HIV-serodiscordant couples. Serodiscordant couples should be screened for and counseled the risk of heavy alcohol use and HIV transmission and acquisition.

2.2 INTRODUCTION

Since 2001, incident HIV infections among adults in sub-Saharan Africa have declined by 34%; however, the region remains disproportionately affected with 70% of all incident HIV infections in 2012 [54]. HIV prevalence within sub-Saharan Africa varies by region, as does the prevalence of HIV serodiscordant couples, in which one partner is HIV-positive, and the other HIV-negative. Up to two-thirds of HIV-infected adults in a cohabitating relationship in sub-Saharan Africa have an HIV uninfected partner [2, 3], and women are as likely as men to be the HIV-infected member in a serodiscordant couple [1]. HIV incidence in serodiscordant couples ranges from 2.0 to 11.8 per 100 person-years depending on the type of study, and accompanying services made available to the couples such as couples' HIV counseling and testing, ongoing counseling, antiretroviral therapy (ART), male circumcision, and free male or female condom provision [2, 3].

Though joint HIV counseling and testing reduces risk behavior and HIV incidence, HIV continues to occur in the context of stable HIV serodiscordant heterosexual relationships [1, 4, 16, 90]. Cohabiting couples account for an estimated two-thirds of incident HIV infections [4]. The high prevalence of serodiscordant couples [2], combined with the high risk of HIV acquisition and transmission within those relationships (with a median of 11.1 HIV transmissions per 100 person-years in couples who do not know their joint serostatus [3]) makes serodiscordant couples an important target for HIV prevention efforts across Zambia and other East and Southern African countries.

Heavy alcohol use is a widespread problem among those at risk for and living with HIV, and its use by at least one partner can affect transmission risks to both partners [22, 47, 55]. Alcohol elevates sexual risks through its effect on personality characteristics and drinking environments,

and its influence on decision-making, which can influence HIV risk in both members of a sexual partnership [22, 47, 55]. Further, excess alcohol consumption may lead to immune deficiency, causing increased susceptibility to opportunistic infections [33]. In addition, a recent study of simian immune-deficiency virus (SIV) in macaques showed that chronic alcohol treatment negatively affects the genital micro-environment prior to and over the course of SIV infection, and may increase the risk of genital virus shedding and transmission [32, 33].

A number of epidemiologic studies have consistently shown an association between alcohol use, sexual risk behaviors, and HIV transmission. A 2011 Ugandan study on extramarital sex, condom use, and alcohol use found associations between alcohol use, increased extramarital sex, unprotected sex, and HIV transmission [22]. A recent meta-analysis conducted on alcohol use and high-risk sexual behavior in sub-Saharan Africa highlighted the need for continued research on the impact of alcohol on HIV and STI transmission, especially among women, who have rarely been a focus of such studies [55]. A previous study among this same Zambian cohort [47] showed that, after controlling for demographic and socioeconomic factors, alcohol use by male partners of serodiscordant couples was associated with unprotected sex with their primary partner during study follow-up. However, the initial analyses lacked the power to detect differences between alcohol consumption reported by men and women, and was limited to early cohort enrollment from 1995 to 2007, which included fewer than 1,000 couples.

There is a paucity of evidence about the relationship between heavy alcohol use and HIV risk factors and transmission in women, and within couples in particular [47, 55]. This study evaluated factors associated with heavy alcohol consumption in men and women in cohabiting heterosexual discordant couples in Lusaka, Zambia. We then examined the role of heavy alcohol consumption on high-risk sexual behavior and HIV transmission and acquisition.

2.3 METHODS

The Heterosexual Transmission of HIV Study conducted by the Rwanda Zambia HIV Research Group was an open prospective cohort, which enrolled 3,049 adult heterosexual serodiscordant couples recruited from couples' voluntary counseling and testing (CVCT) centers in Lusaka, Zambia. Data collection began January 1995 and ended December 2012. This study was approved by the Office for Human Research Protections-registered Institutional Review Boards at Emory University, University of California, Los Angeles, and the University of Zambia. Written informed consent was obtained from all participating couples.

Study participants. Married or cohabiting couples in Lusaka, Zambia, attended CVCT services spontaneously, or after receiving an invitation from a community promoter [92, 93]. CVCT services included a group counseling session followed by rapid HIV testing, and joint post-test couples' counseling [91, 96]. Eligible HIV serodiscordant couples were then invited to enroll in a longitudinal open cohort follow-up study between 1995 and 2012. The primary analyses included a total of 3,049 couples.

Study eligibility criteria included: (1) confirmed HIV-1 serodiscordance (one partner confirmed serologically HIV-positive and one partner confirmed serologically HIV-negative) (2) being married or cohabitating for at least 3-months, and (3) planning on staying in the Lusaka region for the next year. Couples were ineligible if the HIV-infected partner initiated ART (which became available in government clinics in 2007). Couples were censored if either partner died, the couple separated, the HIV-positive partner started ART, or if either partner was lost to follow-up.

Data collection. At baseline, study participants completed behavioral (including alcohol assessment) and medical history questionnaires, and had a full physical examination including pelvic genital exams, as well as HIV and STI testing (visual inspection for ulcers and discharge,

RPR serology for syphilis and HSV-2 serology, vaginal wet mount for trichomoniasis). Quarterly follow-up visits included a physical exam (including STI testing if symptoms were present, and treatment according to national guidelines), a blood sample for HIV testing (HIV-negative partner), a vaginal swab wet mount (to test for the presence of sperm and trichomonas, as well as BV and candida in women with vaginal discharge), and completion of study questionnaires, comprising questions on demographic, psychosocial, behavioral, medical history, and health services data. Questionnaire data was collected through face-to-face interview between counselors/nurses and participants and had been translated into the local languages Nyanja and Bemba.

HIV Testing. HIV testing of HIV-negative partners was conducted at three-monthly intervals using Elisa and Western Blot until 1995, then using a rapid serologic test [94]. To determine time of incident infection, beginning in 2003, plasma from the last antibody negative sample was tested with p24 antigen ELISA and RNA polymerase chain reaction (PCR). The date of HIV infection was defined based on available laboratory results as the minimum of: the midpoint between the last negative and first positive antibody date; two weeks prior to the first antigen positive test date; or two weeks prior to the first positive viral load positive/HIV test negative test date.

Alcohol exposure. Data on alcohol consumption was collected at baseline using an adapted version of the AUDIT tool (Alcohol Use Disorders Identification Test) [95], which includes questions on frequency and quantity of alcohol drinking, feelings of guilt associated with drinking, and impaired control due to drinking. Three measures of alcohol were used in this analysis. The first measure was binary "ever/never" drank during the year prior to enrollment. The second measure was the question, "In the last year, how often did you drink alcohol?" Responses ranged from never, once a month, 2-4 times per month, 2-3 times per week, 4+ times per week. The last

measure was: “In the last year, how often did you get drunk?” Responses included: never, less than monthly, monthly, weekly, daily or almost daily. In addition, every 3-months, if the woman responded that they had unprotected sex, the women was asked if this was because of the men’s alcohol consumption.

Analyses. Descriptive statistics express baseline demographic and behavioral data by alcohol consumption (drinking $\geq$2 times/week for women, and $\geq$4 times/week for men vs. moderate/non-drinkers) with counts and percentages (for categorical variables), or means and standard deviations (for continuous variables). The threshold for defining men vs. women as heavy drinkers was lower for women than men based on recommendation from the AUDIT. [95]

Firstly, we analyzed factors associated with heavy drinking in the past year, stratified by gender using multivariate logistic regression with heavy drinking as the outcome. Next, we analyze the effect of heavy alcohol consumption on sexual behaviors that resulted from either partner drinking heavily at baseline, including condomless sex with partner and outside sex partners during the study. We developed binary behavioral outcomes for alcohol consumption: heavy drinking vs. moderate/no drinking. We then specified logistic regression models for factors associated with heavy drinking and alcohol’s effect on outside sex partners and condomless sex with study partner in the past 3-months, controlling for potential confounders. Lastly, we analyzed the effect of heavy alcohol consumption on HIV acquisition stratified by gender of seroconverter. We developed multivariable Cox survival models to calculate time to seroconversion by gender of seroconverter. The proportional hazards assumption was tested for time-independent covariates using Kaplan-Meier curves. *A priori* confounders (man or woman’s age, literacy, and years cohabiting) and covariates that substantially (>10%) changed the effect estimates in analyses were considered as potential confounders in the multivariable models.

Effect-measure modification was assessed for gender of HIV-positive partner in the Cox survival models by creating a model for gender of seroconverter. Multi-collinearity was assessed by analyzing the variance inflation factor, and tolerance to check the degree of collinearity between variables. All analyses were conducted with SAS v9.4 (Cary, NC).

2.4 RESULTS:

The overall cohort makeup has been previously described; therefore, it will not be reported here [28-32]. There were a total of 478 seroconversions, of which 47% (n=226) were in initially male HIV-negative and female HIV-positive couples (M-F+) and 53% (n=252) were in male HIV-positive and female HIV-negative couples (M+F-). Frequencies of demographic, clinical, and behavioral variables by alcohol use (heavy vs. moderate/non-drinkers) in the year prior to testing and enrollment are presented in Table 2.1, stratified by gender (Table 2.1: women, Table 2.2: men). Descriptive analysis demonstrates that there were differences in follow up time when stratified by heavy vs. moderate/non-drinkers with heavy drinkers having longer follow up times compared to moderate/nondrinkers among both men and women.

Men reported heavy drinking more frequently than women. About 69% of women reported drinking alcohol in the year before couples testing and study enrollment, compared to 79% of men. Approximately 17% of men reported drinking 4+ times per week, compared to 1.5% of women. As such, we broadened our definition for heavy drinking as $\geq$2 times/week for women (4.5% of our sample). We defined men's heavy drinking as $\geq$4 times/week. Among men, 8.3% reported getting drunk daily or almost daily (n=251), whereas only 0.6% of women reported the same (n=19). However, 78 women (2.6%) reported getting drunk weekly. Therefore, we broadened the

definition of getting drunk for women to include those who reported getting drunk weekly and daily.

Factors independently associated with women reporting heavy alcohol use: Women's older age (adjusted odds ratio [aOR] =1.03, 95% CI=1.01, 1.05), greater age disparity with partner (aOR=1.05, 95% CI=1.03, 1.07), more years cohabiting (aOR=1.04, 95% CI=1.01, 1.06), partner who reported heavy drinking (aOR=4.13, 95% CI=3.29, 5.19), HIV status (aOR woman HIV-positive at baseline=1.62, 95% CI=1.27, 2.05), and reporting having a STI in past 12-months (aOR=1.76; 95% CI=1.34, 2.22) were associated with heavy alcohol consumption adjusting for literacy, family income, and number of previous pregnancies, compared to moderate/non-drinkers in multivariable analysis (Table 2.3)

Factors independently associated with men reporting heavy alcohol use: Men's lower age (aOR=0.98, 95% CI=0.96, 0.99), greater age disparity with partner (aOR=01.04, 95% CI=1.02, 1.06), and having a partner who reported heavy drinking (aOR=2.83, 95% CI=2.00, 4.04), and self-reporting a STI diagnosis in the past 12-months (aOR=1.21, 95% CI=1.06, 1.38) were associated with men's heavy drinking adjusting for couple income, years cohabiting, HIV status, and literacy. (Table 2.3)

Alcohol effect on outside sex partners Men who reported being drunk resulted in a higher odds of reporting $\geq$ 1 outside sex partner in the past 3-months (aOR for daily drunk=2.02, 95% CI=1.58, 2.57; aOR for monthly drunk =2.04, 95% CI=1.58, 2.63 compared to never being drunk at baseline), adjusting for age, literacy, years cohabiting, and HIV status. (Figure 2.1). Fewer years cohabiting and HIV status were also associated with men having outside partners in the past 3-months (aOR for years cohabiting =0.97, 95% CI=0.96, 0.99; aOR man as HIV-negative partner =1.40, 95% CI=1.15, 1.70).

Women who reported being drunk in the past year was associated with increased reporting of having $\geq$ 1 outside sex partner(s) in the past 3-months (aOR=1.89, 95% CI=1.35, 2.64), adjusted for women's age, literacy, HIV status, and years cohabiting, compared to women who reported never being drunk in the past year. (Figure 2.1).

Alcohol effect on condomless sex with partner: Controlling for age, literacy, years cohabiting, and HIV status, men who reported being drunk daily or almost daily had the highest odds of having condomless sex with their study partner (aOR=1.61, 95% CI=1.28, 2.04), followed by reporting drunk weekly (aOR=1.52, 95% CI=1.21, 1.91) compared to men who reported never being drunk in the past year. Men being were HIV-negative at baseline (aOR=1.51, 95% CI=1.38, 1.64) was also associated with condomless sex in past 3-months vs. HIV-positive men at baseline (Figure 2.1).

Women who reported getting drunk in the past year also had a higher odds of reporting condomless sex with study partner (aOR drunk monthly or less than monthly=1.57, 95% CI=1.29, 1.82; aOR drunk weekly or daily/almost daily=1.54, 95% CI=1.31, 1.82) compared to women who reported never being drunk in the past year adjusting for woman's age, HIV status and years cohabiting. (Figure 2.1) Woman being the HIV-negative partner was protective against condomless sex in last 3-months (aOR=0.66, 95% CI=0.60, 0.72) controlling for woman's age, HIV status and years cohabiting.

Alcohol effect on initiating ART: After restricting analyses to those who were followed after 2007 when ART became available in Zambia, women who were HIV-positive and reported being drunk in the past year were less likely to initiate ART during the study (aOR=0.85, 95% CI= 0.72, 1.00) compared to women who reported never being drunk, adjusting for women's age, women's

literacy and follow up time (data not tabled). For men there was no association between delayed ART initiation and alcohol use.

Alcohol effect on women's HIV acquisition (n=1,393 M+F- couples): After controlling for woman's age, literacy, years cohabiting and previous pregnancies, men's heavy drinking was associated with trends of increased HIV transmission to their partners (adjusted hazard ratio [aHR] = aHR drinking ≥4/week= 1.07, 95% CI=0.99, 1.16; aHR drunk weekly or more frequently=1.42, 95% CI=0.97, 2.10) compared to moderate/non-drinkers. Heavy drinking in women was also associated with increased HIV acquisition (aHR=3.71, 95% CI=0.90, 15.25), adjusting for age, literacy, years cohabiting and number of previous pregnancies compared to women who never reported being drunk in the past year. (Table 2.4)

Alcohol on men's HIV acquisition (n=1,656 M-F+ couples): Men reporting heavy drinking was associated with increased HIV acquisition (aHR=1.72, 95% CI=1.01, 2.93) adjusting for men's age, literacy, years cohabiting, and male circumcision. Women's drinking had no effect on men's HIV acquisition. (Table 2.4)

2.5 DISCUSSION

This is one of the first studies among couples who have received joint HIV counseling and testing in Southern Africa to show how men's and women's alcohol consumption affects high-risk sexual behaviors and HIV acquisition and transmission among HIV serodiscordant couples. Our findings point to the role alcohol plays in enhancing individual risk, enhancing risk of HIV acquisition within the partnership, and compromising use of HIV treatment and care among women, suggesting that alcohol use requires great attention in the region.

An important finding was the effect of alcohol use on HIV care and treatment for women. Our study demonstrated that HIV-infected women at baseline were more likely to be heavy

drinkers. Because alcohol in general is associated with negative health condition (including impaired inflammatory response) as well as poor adherence [20], this raises concern about maintaining the wellbeing of HIV-infected adults in Africa. What is unknown is whether their alcohol consumption increased because they were infected, or whether they were infected because of their heavy alcohol consumption. Women may initiate alcohol use post-seroconversion because of the stress of HIV or because of a social environment which led to HIV exposure. Moreover, heavy alcohol use can result in general personal dysfunction and has been linked to mental health problems as well, which may produce an inability to access needed care – suggested special services may be required to link alcohol users to HIV care (including PMTCT). Further, HIV-infected women in our study who were heavy drinkers were less likely to start ART. HIV care providers must also consider how heavy alcohol use by female patients may affect their general health as well as HIV progression and encourage alcohol treatment and control if patients are to achieve better health.

Our study supports previous studies in which an individual's alcohol use is associated with their and their partner's practice of sexual risk behaviors including concurrent or multiple sex partners and condomless vaginal or anal sex [22, 47, 55]. Previous research has similarly demonstrated that HIV acquisition has been shown to increase when alcohol is consumed before sex [22]. Our results are important because the female partners were at increased risk of HIV acquisition due to their drinking, as well as their partner's drinking. Men who were heavy drinkers were also more likely to acquire HIV in our cohort. Women who acquire HIV are also at-risk of transmitting HIV to their infants (especially if they seroconvert while pregnant or trying to conceive). Men and women who are chronic drinkers, or who binge drunk, are also at risk of rapid HIV progression [27, 29, 32, 33, 55]. Finally, intoxication is known to lead to disinhibition around

sex (i.e. lower condom use, multiple or concurrent sex partners), but may also contribute to a minimization of perceived risk of infection and may result in continuing transmission of HIV from those who are positive and heavy drinkers to other partners as well as perinatally.

Our findings suggest the use of alcohol may act as a counter-factor in secondary HIV prevention – offsetting the counseling that women, men and couples may receive by care providers. For example, over 50% of couples reported condomless sex with their partner in the past 3-months at some point during the study, though this is lower than the 59% of couples who reported condomless sex in the past 3-months at baseline, before they knew they were HIV serodiscordant. Over 7.5% of men reported having an outside partner at some point during the study, but only 1.4% of women reported the same. We suspect the different rates of reporting outside partnerships between men and women is due in part to under-reporting by women.

Limitations. Our cohort was a highly select group attending clinic-based couple's HIV testing and counseling, and follow-up over time. Because these couples self-selected into the cohort after couples' counseling and testing, they have inherent differences from the general population and other African serodiscordant couples, including their behavior to seek HIV testing as a couple. Further, there may be a tendency to under-report unsafe sexual behaviors (other sex partners, unprotected sex) because participants may have been interviewed by study staff who were also encouraging them to use condoms and remain faithful to their study partner. Data collection methods in this study were completed with a nurse or counselor who was familiar with the couple, which could cause social desirability bias. In addition, alcohol was only collected at baseline, which did not enable us to analyze alcohol consumption trends over time. Finally, there was poor response to components of the AUDIT tool resulting in the inability to analyze it by score and we used as individual items only. AUDIT may require further adaptation for use in Southern Africa.

As a result of these limitations, information bias due to exposure variables captured as self-reported is possible. Selection bias may also have affected the study as couples who stayed in the cohort may differ in terms of their health-seeking behavior, or other demographic or behavioral data, from similar couples who were not enrolled or were lost to follow up, or separated [91]. At the beginning of this study, ART was not commonly available in Zambia. However, after 2007, ART became available in government clinics, and those on ART were not eligible for this study. Therefore, there is an important difference between couples in this study and those not eligible. Those on ART may have had more advanced virus, with lower CD-4 cell counts, and with better access to health care, which will also affect the generalizability of results.

2.6 CONCLUSIONS

HIV-negative partners in serodiscordant couples who consume alcohol frequently, or who have partners who do, enhance their risk of HIV acquisition. Given the strong association between heavy alcohol use and condomless sex, multiple sex partnerships, HIV acquisition and adoption of HIV care, serodiscordant couples should be screened for and counseled about alcohol consumption for HIV-positive and HIV-negative partners. The risk of heavy alcohol consumption needs to be managed by their health care providers who need to ensure that those who are heavy drinkers access treatment and care for potential alcoholism and recognize the challenge of this for those living with other drinkers. By counseling couples together, and making them more aware that they may be at increased risk of transmitting or acquiring HIV when either partner consumes alcohol, the rate of HIV transmission among serodiscordant couples may decrease and general well-being of this population increase. More research is needed on how to address the risk of

alcohol use, both chronic heavy alcohol drinking, and binge drinking, in African settings with high HIV prevalence.

2.7 Tables and figures

Figure 2.1. Frequency of serodiscordant couples reporting drinking alcohol in past year at baseline by gender in Zambia

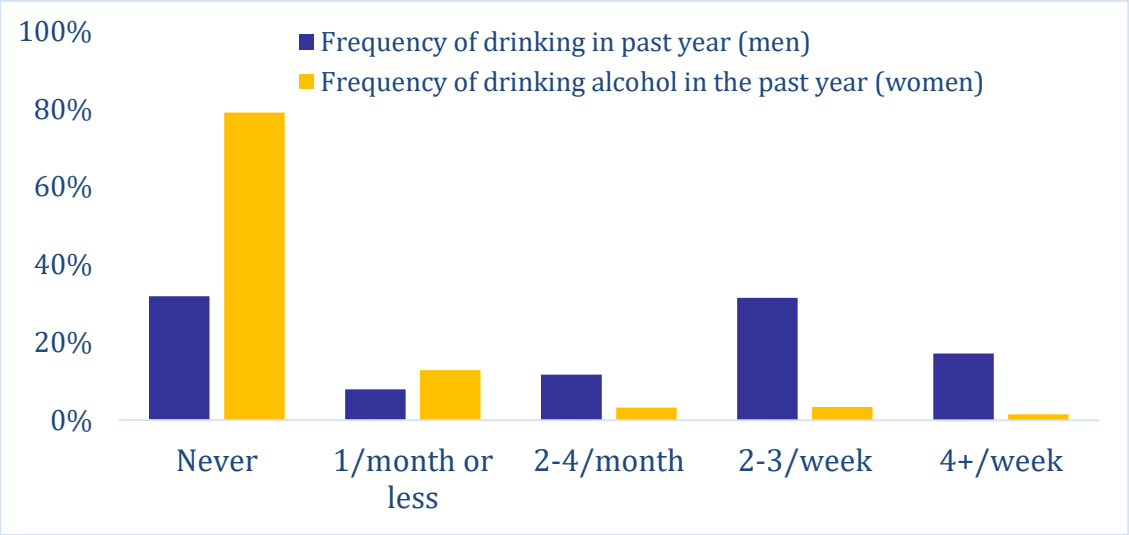


Figure 2.2. Frequency of serodiscordant couples reporting getting drunk in past year at baseline by gender in Zambia

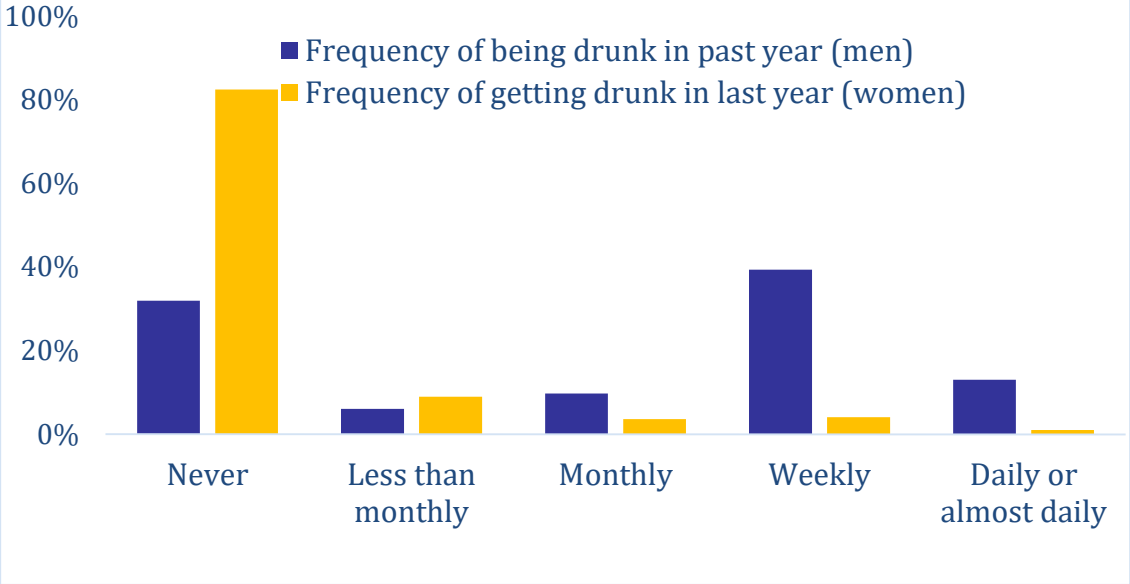


Table 2.1. Women's Demographic and behavioral characteristics by alcohol use (N = 3014 couples)

	Total	**Heavy drinkers (reported drinking =>2 times/week)**		**Moderate/no drinkers (reported drinking <2 times/week)**	
Total	**3014**	**146**	**4.8%**	**2868**	**95.2%**
Demographics					
Study follow up time in days (mean, SD)**	744.5 (841.9)	910.5	1000.9	732.7	831.6
Women's age (mean, SD) **	28.5 (6.8)	31.0	7.4	28.4	6.8
Age difference in years (mean, SD)[1]	6.8 (4.8)	6.2	5.2	6.9	4.8
Years cohabiting (mean, SD)	6.8 (6.3)	7.4	7.6	6.8	6.2
Monthly family income (USD, mean, SD)	90.2 (113.7)	88.9	131.0	90.3	112.9
Women read Nyanja					
Yes, easily	740 (25%)	31	21%	709	25%
With difficulty/not at all	2267 (75%)	114	79%	2153	75%
Partner reported heavy drinking **	516 (17%)	47	32%	469	16%
Sexual history					
Women # of sex partners in last year (mean, SD) *	1.1 (0.9)	1.3	0.8	1.1	0.9
# of previous pregnancies**	3.4 (2.3)	4.1	2.6	3.3	2.3
Women self-report STI last year **	988 (33%)	65	45%	923	32%
Clinical					
Woman HIV-positive partner**	1641 (55%)	101	69%	1540	54%
Log viral load of positive partner, log10 copies	4.6 (0.9)	4.6	1.2	4.6	0.9
Behavioral (time-varying)					
Condomless sex with partner in last 3-months	9115 (33%)	475	35%	8640	33%
Sex with another partner **	49 (2%)	7	5%	42	2%

**= $p<0.05$ (in row comparison)

*= $P<0.10$ (in row comparison)

n= 35 couples missing

[1] Age difference calculated as any age difference, positive or negative in couple.

Table 2.2. Men's Demographic and behavioral characteristics by alcohol use (N = 3018 couples)

	Total	**Heavy drinkers (reported drinking =>4/week)**		**Moderate/no drinkers (reported drinking <4/week)**	
Total	**3018**	**516**	**17%**	**2502**	**83%**
Demographics					
Study follow up time (days, mean, SD) **	745 (841.9)	819.2	892.67	725.7	830.3
Men's age (mean, SD)	35.1 (8.1)	35.5	7.6	35.1	8.2
Age difference in years (mean, SD)	6.8 (4.8)	6.6	4.3	6.9	4.9
Years cohabiting (mean, SD)	6.8 (6.3)	7.1	6.1	6.8	6.3
Monthly family income (USD, mean, SD)	90.2 (113.7)	92.5	114.7	89.9	113.5
Men read Nyanja					
Yes, easily	1341 (45%)	224	43%	1117	45%
With difficulty/not at all	1670 (55%)	291	57%	1379	55%
Partner reported heavy drinking**	146 (5%)	47	9%	99	4%
Sexual history					
Men # of sex partners last year (mean, SD)	1.7 (1.7)	1.8	1.4	1.7	1.8
Men history of STI last year*					
Yes	1019 (34%)	191	37%	828	33%
No	1998 (66%)	325	63%	1673	67%
Clinical					
Man HIV-positive	1375 (46%)	229	44%	1146	46%
Log viral load of positive partner, log10 copies	4.6 (0.9)	4.5	0.9	4.6	0.9
Behavioral (time-varying)					
Condomless sex with partner in past 3-months**	9267 (33%)	2136	38%	7131	32%
Sex with another partner **	259 (9%)	49	19%	210	9%

**= p<0.05 (in row comparison)
*= P<0.10 (in row comparison)
n=31 couples missing

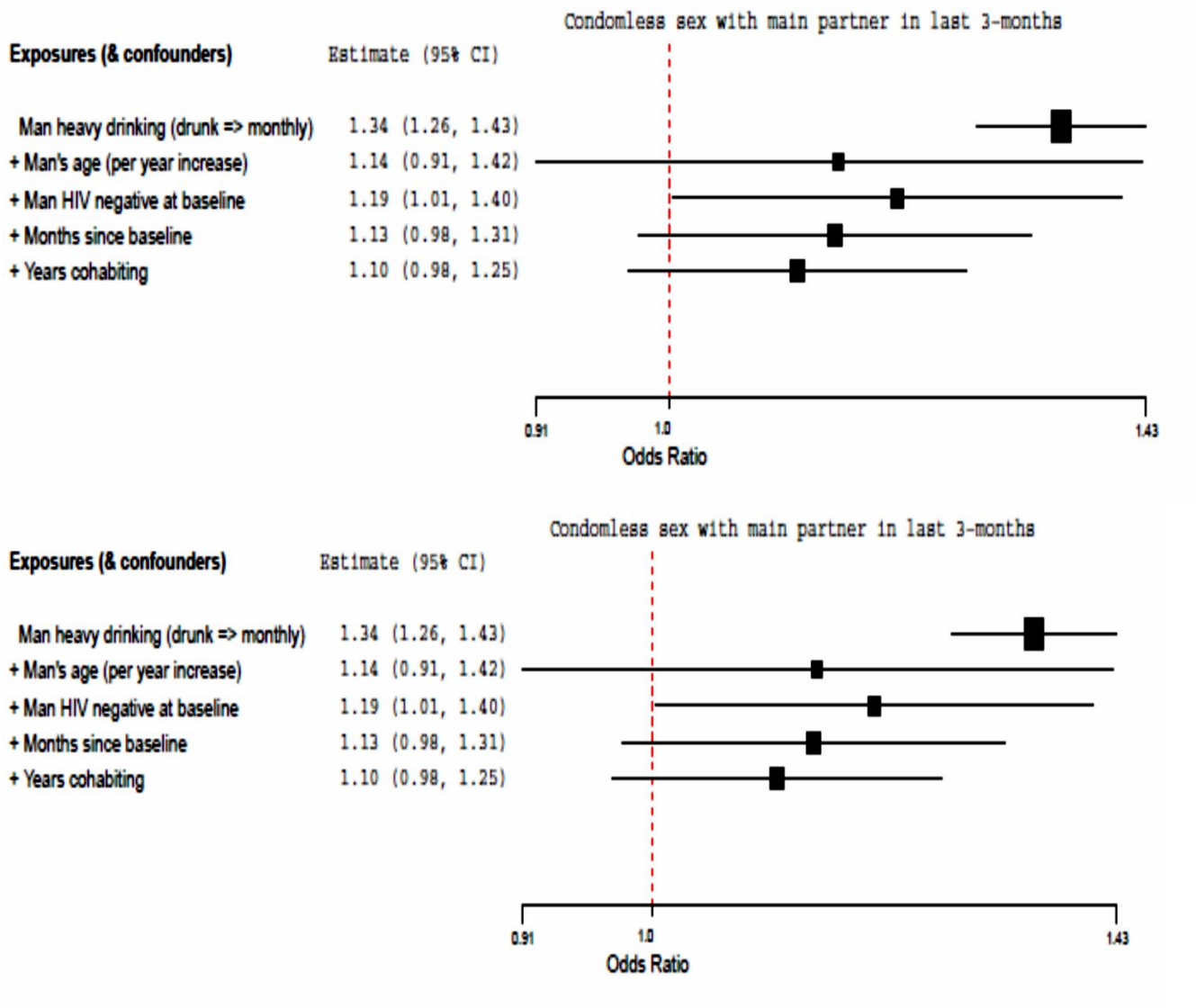
Table 2.3. Factors associated with women's and men's heavy drinking (reporting being drunk weekly or daily/almost daily at baseline) in the Zambia cohort study (n=3049 couples).

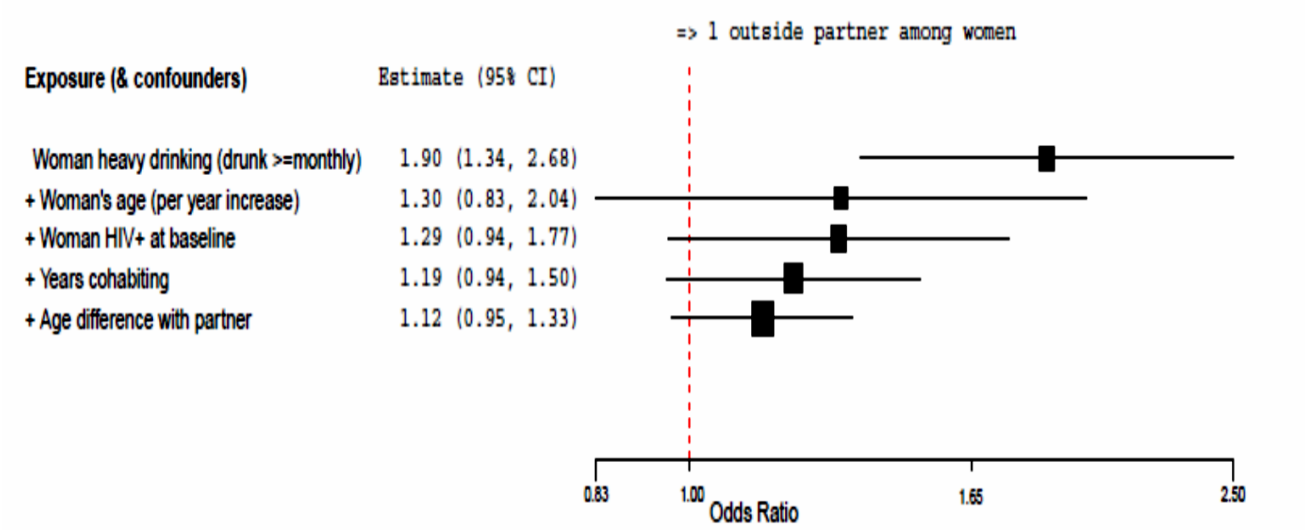
Models for Women: Outcome Women's heavy drinking		
	Crude OR (95% CI)	**Adjusted OR (95% CI)**
Women age	**1.04 (1.02, 1.05)**	**1.03 (1.01, 1.05)**
Years cohabiting	**1.03 (1.02, 1.05)**	**1.04 (1.01, 1.06)**
Women illiterate	**1.23 (1.05, 1.44)**	1.18 (0.89, 1.57)
Monthly family income (in USD)	1.00 (0.99, 1.00)	1.00 (0.99, 1.00)
Age disparity with male partner	**1.03 (1.01, 1.05)**	**1.05 (1.03, 1.07)**
Woman is HIV-positive partner	**1.90 (1.53, 2.36)**	**1.62 (1.27, 2.05)**
Men reported heavy drinking	**3.56 (2.89, 4.37)**	**4.13 (3.29, 5.19)**
Number of previous pregnancies	**1.05 (1.01, 1.10)**	0.95 (0.89, 1.01)
Women self-reported STI in last year	**1.70 (1.38, 2.08)**	**1.76 (1.34, 2.22)**
Models for Men: Outcome Men's heavy drinking		
Men age	0.99 (0.98, 1.00)	**0.98 (0.96, 0.99)**
Years cohabiting	**0.99 (0.98, 0.99)**	1.01 (0.99, 1.03)
Men illiterate	1.24 (1.17, 1.32)	1.26 (0.84, 1.80)
Monthly family income (in USD)	1.00 (0.99, 1.00)	1.00 (0.99, 1.00)
Age disparity with female partner	**0.99 (0.98, 0.99)**	**1.04 (1.02, 1.06)**
Man is HIV-positive partner	**0.80 (0.76, 0.85)**	0.92 (0.80, 1.05)
Women reported heavy drinking	**3.56 (2.89, 4.37)**	**2.83 (2.00, 4.04)**
Number of live children*	**0.93 (0.90, 0.97)**	**0.93 (0.88, 0.99)**
Men self-reported STI in last year	1.04 (0.98, 1.11)	**1.21 (1.06, 1.38)**

Bold= p<0.05

*In model for men we included live children instead of pregnancies for men

Figure 2.3. Forest plots of effect of heavy alcohol use on condomless sex with main partner in last 3-months, and outside partner(s) during study by gender




=> 1 outside partner among women
Exposure (& confounders)
Estimate (95% CI)
Woman heavy drinking (drunk >=monthly) 1.90 (1.34, 2.68)
+ Woman's age (per year increase) 1.30 (0.83, 2.04)
+ Woman HIV+ at baseline 1.29 (0.94, 1.77)
+ Years cohabiting 1.19 (0.94, 1.50)
+ Age difference with partner 1.12 (0.95, 1.33)
0.83
1.00
1.65
2.50
Odds Ratio

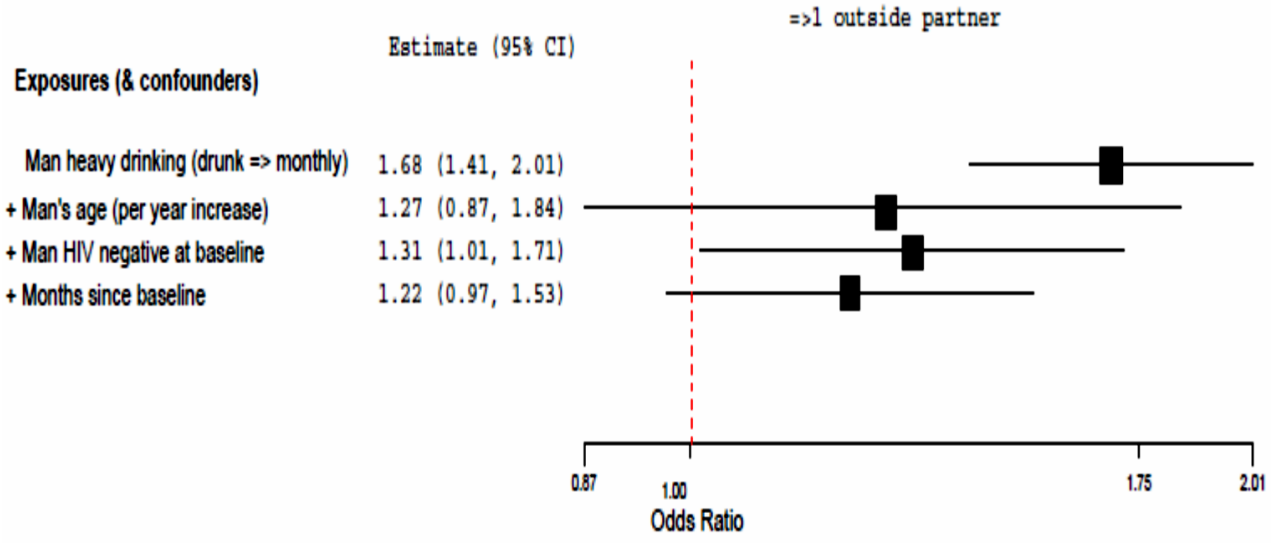

=>1 outside partner
Estimate (95% CI)
Exposures (& confounders)
Man heavy drinking (drunk => monthly) 1.68 (1.41, 2.01)
+ Man's age (per year increase) 1.27 (0.87, 1.84)
+ Man HIV negative at baseline 1.31 (1.01, 1.71)
+ Months since baseline 1.22 (0.97, 1.53)
0.87
1.00
1.75
2.01
Odds Ratio

Table 2.4. Time to seroconversion stratified by gender of seroconverter as estimated by Cox regression models		
	Crude HR (95% CI)	**Adjusted HR (95% CI)**
Model 1: Men heavy drinking and women's HIV acquisition (n=1393 M+F- couples)		
Men reported never drinking in past year	Ref	Ref
Men reported drinking =>4/week	**1.08 (1.00, 1.14) ***	**1.07 (0.99, 1.16) ***
Men reported being drunk weekly or greater	1.36 (0.94, 1.97)	**1.42 (0.97, 2.10) ***
Women's age	**0.96 (0.93, 0.99) ***	**0.96 (0.93, 0.99) ****
Woman illiterate	0.84 (0.57, 1.23)	0.66 (0.39, 1.14)
Years cohabiting	**0.94 (0.90, 0.97) ***	1.00 (0.97, 1.00)
Number of previous pregnancies	**0.89 (0.84, 0.95) ****	0.95 (0.88, 1.03)
Model 2: Women heavy drinking and HIV acquisition (n=1393 M+F- couples)		
Women never reported being drunk in past year	Ref	Ref
Women reported being drunk less than monthly	1.43 (0.78, 2.61)	1.47 (0.80, 2.70)
Women reported being drunk daily or almost daily	3.63 (0.88, 14.91)	**3.71 (0.90, 15.25) ***
Women's age	**0.95 (0.92, 0.98) ****	0.98 (0.95, 1.01)
Women illiterate	**1.76 (1.03, 2.99) ****	**1.38 (0.97, 1.95) ***
Years cohabiting	**0.94 (0.90, 0.97) ****	0.99 (0.96, 1.03)
Number of previous pregnancies	**0.81 (0.73, 0.90) ****	0.95 (0.87, 1.03)
Model 3: Men heavy drinking and HIV acquisition (n=1656 M-F+ couples)		
Men never reported being drunk in past year	Ref	Ref
Men reported being drunk monthly or less than monthly	1.51 (0.73, 3.13)	1.23 (0.78, 1.93)
Men reported being drunk weekly or daily/almost daily	1.62 (0.89, 2.96)	**1.72 (1.01, 2.93) ****
Men's age	**0.97 (0.94, 0.99) ****	**0.98 (0.96, 1.01) ***
Men illiterate	1.13 (0.78, 1.62)	1.04 (0.72, 1.51)
Male circumcised	**0.23 (0.09, 0.56) ****	**0.23 (0.09, 0.57) ****
Years cohabiting	**0.95 (0.91, 0.99) ****	**0.96 (0.92, 1.00) ***
Model 4: Women heavy drinking and men's HIV acquisition (n=1656 M-F+ couples)		
Women never reported being drunk in past year	Ref	Ref
Women reported being drunk monthly or less than monthly	0.68 (0.33, 1.40)	0.71 (0.34, 1.46)
Women reported being drunk weekly, daily or almost daily	1.23 (0.30, 5.01)	1.70 (0.42, 6.93)
Men's age	**0.98 (0.95, 1.01) ***	**0.98 (0.96, 1.00) ***
Men illiterate	1.53 (0.96, 2.46)	0.95 (0.72, 1.24)
Male circumcised	**0.23 (0.09, 0.56) ****	**0.46 (0.28, 0.76) ****
Years cohabiting	**0.95 (0.91, 0.99) ****	**0.97 (0.94, 1.00) ***

****=p<0.05**
***=p<0.10**

Chapter III. Study 2: HIV incidence and predictors of HIV acquisition from an outside partner in serodiscordant couples in Lusaka, Zambia, 1994-2012

3.1 Abstract

Objectives: To evaluate the incidence and predictors of seroconversion from an outside partner in serodiscordant couples in Zambia.

Methods: Serodiscordant couples were enrolled from couples-counseling and testing sites in Lusaka. Demographic, behavioral, and clinical exposures were measured. HIV-uninfected partners were re-tested every three-months. Genetic analysis classified incident infections as those acquired from the study partner (linked), or acquired from an outside partner (unlinked). Factors associated with time to unlinked HIV infection were evaluated using multivariable Cox models. We did not analyze HIV linked infection for this analysis.

Results: Forty-five unlinked HIV infections occurred among women (1.85/100-couple-years [CY]; 95% CI: 1.35, 2.47). Risk of female unlinked infection (relative to non-seroconverting couples) was associated with baseline female alcohol consumption (aHR=5.44; 95% CI: 1.03, 28.73), recent genital ulcers and/or genital inflammation (aHR=6.09; 95% CI: 2.72, 13.64 and aHR=11.92; 95% CI: 5.60, 25.37, respectively) adjusting for age, age disparity with partner, literacy, previous pregnancies, income, contraceptive method use, and sperm present on wet prep (bio-marker for condomless sex). Reporting outside partner(s) was not associated with unlinked infection among women. Fifty-five unlinked HIV infections occurred among men (1.82/100 CY; 95% CI: 1.37, 2.37). Risk of male unlinked HIV infection (relative to non-seroconverting couples) was associated with recent genital inflammation (aHR=8.52; 95% CI: 3.82, 19.03), genital

ulceration (aHR=4.27; 95% CI: 2.05, 8.89), self-reporting =>1 outside partner (aHR=3.36; 95% CI: 1.53, 7.37) versus none, and reporting being drunk weekly/daily at baseline (aHR=3.52; 95% CI= 1.19, 10.46) vs. never, controlling for age, literacy, couple income, and male circumcision status.

Conclusions: The strongest predictors of unlinked infection in serodiscordant relationships were alcohol use, genital inflammation and ulceration prior to infection. Genital symptoms should be screened for and treated, or treated prophylactically for HIV-negative partners in serodiscordant couples. The risks of alcohol use should be included for both partners in risk-reduction counseling.

3.2 Background

HIV incidence in studies of HIV-serodiscordant couples ranges from 2.0 to 11.8 per 100 person-years depending on the type of study, study location, and the accompanying services made available to the couples [4, 5]. Among couples with HIV infections acquired from a partner outside the primary serodiscordant partnership, as indicated by viral sequencing, incidence rates ranged from 0.51 per 100 person years among women reporting no outside sex partners, to 3.46 per 100 person years among women reporting outside sex partners [18]. Though uptake of ART among serodiscordant couples has increased, it does not remove the need for behavioral interventions to reduce HIV acquisition from outside partners [26, 27]. As a result of continued risk of HIV infection in couples, several African countries are adopting couples-based HIV counseling and testing as an HIV-prevention and care intervention [42]. Thus, more couples are becoming aware of each other's HIV status, and many find out that they are HIV-serodiscordant. Despite this increased knowledge of HIV-serodiscordance among couples, there is a limited focus on effective messages and interventions to prevent HIV acquisition from outside partners. Appropriate and effective interventions for HIV prevention in serodiscordant couples depend on accurate assessments of the sexual risk behaviors among the HIV-positive and HIV-negative partners, as well as understanding their own sexual behaviors with other men and women.

Improved evidence around factors that increase the risk of HIV infection in cohabiting relationships is needed in order to develop and improve evidence-based interventions [60]. Specifically, more research is needed on factors with a disputed and unclear influence on HIV acquisition and transmission at the individual- and couple-level, including hormonal contraception [6-8], concurrent sexual partnerships [13-18], alcohol use [19-21], and age discordancy [48, 49]. Greater understanding of HIV-negative partners' behaviors with primary and outside or concurrent

sex partners, and these behaviors' associations with HIV acquisition, will inform HIV risk-reduction messages for serodiscordant couples, and will be important for the design of HIV-prevention strategies for uninfected partners.

Learning that one is in an HIV-serodiscordant relationship may cause the HIV-uninfected partner to seek outside partners, potentially of unknown HIV serostatus, who might be perceived to create a lower risk of HIV infection than the primary, HIV-infected, partner. Understanding an individual's behaviors with primary and outside partners, and these behaviors' associations with HIV acquisition ('unlinked infections'), will inform HIV risk-reduction messages for serodiscordant couples and will be important for the design of HIV-prevention strategies for uninfected partners.

A recent study of serodiscordant couples found that the proportion of subjects reporting sex with an outside partner increased from 3.1% to 13.9% ($p<0.001$) after serodiscordant testing. Condomless sex was more common with outside partners than with the primary HIV-infected partners, and a small portion (<5%) reported concurrent sexual partnership with their infected partner and an outside partner within the same month [42]. Another study showed that sex with infected partners decreases during follow-up and increases with outside partners over time, likely reflecting relationship dissolution [60]. However, in our Zambian cohort, we did not see the same rates of partner dissolution [110]. This study examined the incidence of unlinked infections, and risk factors associated with unlinked seroconversion among cohabiting heterosexual couples in a long-running cohort in urban Zambia.

3.3 Methods

The Heterosexual Transmission of HIV Study, conducted by the Rwanda Zambia HIV Research Group, was an open prospective cohort which enrolled 3,049 adult heterosexual serodiscordant couples recruited from a couples-counseling and testing site in Lusaka, Zambia. Data collection began in January 1995 and ended in December 2012. All participants were informed of the risks and benefits of participating in the study, and signed informed consent forms. This study was approved by the Office for Human Research Protections-registered Institutional Review Boards (IRB) at Emory University, University of California, Los Angeles, and the University of Zambia.

Study participants. Married or cohabiting couples in Lusaka, Zambia, attended voluntary couples' HIV counseling and testing (CVCT) services, either spontaneously or after receiving an invitation from a community promoter [93]. CVCT services include group counseling, rapid HIV testing, and post-test couples' counseling [93]. HIV-serodiscordant couples were invited to enroll in a longitudinal open cohort follow-up study between 1995 and 2012. The primary analyses compared a total of 3,049 couples. In these couples, there were 478 seroconversions: 100 determined to be acquired from an outside partner (i.e. their virus was not genetically linked to the partner's virus: 45 women, 55 men), the remaining 378 individuals had a linked seroconversion (i.e. their virus was genetically linked to the study partner's virus). There were no seroconversions among the remaining 2,571 couples.

Study eligibility. The cohort's inclusion criteria were: (1) confirmed HIV-1 serodiscordance after attending couples' HIV counseling and testing services (2) the participants had been married or cohabitating for at least three-months, and (3) the participants planned on staying in the Lusaka region for the next year. Couples were ineligible if either partner had a CD4 count of <200 or was

on ART. Couples were censored when either partner died, the couple separated, the HIV-positive partner started ART, or either partner was lost to follow-up.

Data collection. The study participants completed behavioral and medical history questionnaires and underwent a full physical examination, including pelvic/genital exams, as well as HIV and STI screening at baseline. Follow-up visits included a physical exam (including STI testing if symptoms were present), a blood sample for HIV testing (of the HIV-negative partner), vaginal swab wet prep, and completion of study questionnaires that collected demographic, psychosocial, behavioral, medical history, and health-services data. The data were collected by participant completion of written questionnaires and face-to-face interviews if literacy was a problem. All data-collection instruments were in English and translated into local dialects.

Data collection varied over the 18 years of follow-up. Plasma banking for VL testing began in 1999 and p24 ELISA screening began in 2003. From 1994-2002, both partners were seen quarterly, had routine physical and genital exams, and received laboratory screening for trichomonas and syphilis. Beginning in 2003, physical exams and STI screenings for were performed at baseline, annually, and when signs or symptoms were reported. From 2002-2011, fertility intentions were recorded. From 2007-2008, HIV-negative partners were seen at months 0,1,2,3, and quarterly thereafter at which time they completed a sexual exposure risk assessment including self-reported unprotected sex, sperm or trichomonas on a wet mount, incident pregnancy, or incident STI at quarterly visits; couples with at least one exposure received monthly HIV testing until the next quarterly visit, at which time the risk assessment was repeated. From 2008-2012, all HIV-negative partners were tested for HIV monthly.

Genital ulceration and inflammation. Measures of chronic/recurrent or acute genital or perianal ulcers were assessed via physical exam at baseline and quarterly visits. They were considered

positive if reported by the client or if diagnosed on exam at the research clinic, ulceration was positive if noted on physical examination (including erosion or friability of the cervix or vagina in women), or treated for chancroid, or newly positive RPR serology for syphilis. Genital inflammation included cervical or vaginal inflammation in women, genital discharge (urethral discharge in men, vaginal or cervical discharge in women), inguinal adenopathy, or laboratory diagnosis of or sign/symptom-based treatment for trichomoniasis, gonorrhea, chlamydia, or bacterial vaginosis [25]. Time-varying indicators for genital ulceration and genital inflammation were created as composites from the above measures.

HIV testing. HIV testing of HIV-negative partners was conducted at three-month intervals using a rapid serologic test. To define the time of incident infection, when available, plasma from the last antibody-negative sample was tested with p24 ELISA and RNA polymerase chain reaction (PCR). The date of HIV infection was defined based on available laboratory results as the minimum of: the midpoint between the last negative and first positive antibody date; two weeks prior to the first antigen positive test date; or two weeks prior to the first positive viral load positive/ HIV test negative test date.

The molecular epidemiology of the incident transmission events that occurred during the study follow-up were reported by examining the genetic characterization of HIV viral strains. Comparison of conserved PCR-amplified nucleotide sequences from each member of the couple classified incident infections as linked to the study partner or unlinked (acquired from outside the study couple). Blood samples were collected from both members of the discordant couples in whom seroconversion had been documented.

To establish suitable linkage criteria for HIV-1 viral strains infecting these Zambian couples, amplified viral sequences were first subjected to preliminary phylogenetic tree analyses to identify

all circulating HIV-1 Group-M subtypes. Full-length and non-recombinant reference sequences representing these subtypes were then obtained from the Los Alamos HIV Sequence Database and subjected to pairwise sequence comparisons in the genomic regions corresponding to the PCR amplification products. Eight partially overlapping regions in gag, three in gp120, one in gp41, and one in the LTR were used for analysis. Uncorrected nucleotide sequence distances were then calculated for each transmission pair and compared to the mean sequence distances calculated for the reference sequence set in the corresponding genomic region. The latter minus two standard deviations were arbitrarily assigned as the cutoff value for epidemiologically linked sequence pairs. Transmission pairs were tentatively classified as having epidemiologically linked viruses when their pairwise sequence distances fell below this limit, and unlinked viruses when their pairwise distances exceeded this limit [76, 113].

Analyses. HIV incidence rates were calculated as the number of unlinked incident infections per 100 couple-year (CY) of follow-up, removing those with a linked infection. CYs of follow-up were calculated from enrollment until either an outcome of interest occurred or the couple was censored. Rates were also calculated by calendar time. Baseline exposures were stratified by gender of seroconverter and by whether the couple had an unlinked seroconversion or no seroconversion. Baseline and time-varying exposures were described using counts and percentages (for categorical variables) or means and standard deviations (for continuous variables). Bivariate hazard ratios, p-values, and 95% confidence intervals between covariates and unlinked infections are based on single-predictor Cox models.

Four multivariable Cox models were constructed to evaluate predictors of time to acquisition of genetically unlinked HIV infection among (1) female and (2) male partners, comparing to acquisition of a linked infection, and to no infection. *A priori* confounders (e.g. age, age disparity

between partners, literacy, income, and previous pregnancies), and covariates that substantially (>10%) changed the effect estimates in univariate analyses were considered potential confounders in the multivariable models. The proportional hazards assumption was confirmed for time-independent covariates. Because we identified effect-measure modification for gender of seroconverter, stratified analysis was used for all subsequent models. Multi-collinearity was assessed by analyzing the variance inflation factor, and tolerance to check the degree of collinearity between variables. For collinear variables, we selected one variable, depending on the relationship between the exposure and unlinked seroconversion of each model. All analyses were conducted with SAS v9.4 (Cary, NC).

3.4 Results

Women who acquired an HIV infection unlinked to their partner's virus. Forty-five genetically unlinked seroconversions were observed in women over 2432.79 CY of follow-up, for a seroconversion rate of 1.85 seroconversions/100 CY (95% CI: 1.35, 2.47) (Table 3.1). Women who had an incident HIV infection from another partner were younger (mean age 25.5 vs. 28.8, respectively) and lived with partners fewer years than those who did not seroconvert (mean of 5.6 years vs. mean of 8.3 years, respectively). Monthly family income was lower in couples in which the woman had an unlinked seroconversion ($50.9 vs. $94.0/month, respectively). Women who acquired HIV from another partner had fewer pregnancies that women who did not seroconvert (mean of 2.7 previous pregnancies vs. 3.7 respectively). Women who read Nyanja (a proxy for education) was lower among those with an unlinked infection compared with those who did not seroconvert (11% vs. 24%). Only 1% (n=157) of non-convertors reported having an outside sex

partner in the past 3-months compared to 4% (n=2) of women who acquired HIV from an outside partner. Women with an unlinked infection were more likely to report a history of STI in the year prior to enrollment (42% vs 26%), to have genital inflammation (53% vs 15%), and ulceration (27% vs 5%) than women who remained HIV-negative. (Table 3.2).

Controlling for woman's age, age disparity with male partner, couple monthly income, previous pregnancies, contraceptive method use, and sperm presence on wet prep, female literacy, women's risk of acquiring HIV from another partner, when compared with non-seroconverters, was associated with the woman reporting drinking =>4 times/week at baseline (vs. moderate/non-drinkers in past year) (adjusted hazard ratio [aHR]=5.44; 95%CI:1.03, 28.73), female genital ulcers in the past 3-months (aHR=6.09; 95% CI=2.72, 13.64), female genital inflammation in the last 3-months (aHR=11.92; 95% CI=5.60, 25.37). However self-reporting =>1 outside partner in the past 3-months was not associated with incident unlinked infection among women (aHR=2.07, CI=0.42, 10.28). (Table 3.4)

Men who acquired an HIV infection unlinked to their partner's virus. Fifty-five genetically unlinked incident seroconversions were observed in men over 3025.40 CY of follow-up, for a seroconversion rate of 1.82 seroconversions/100 CY (95% CI: 1.37, 2.37) (Table 3.1). In descriptive analyses, men who had an unlinked seroconversion tended to be younger than men who did not seroconvert (mean age of 32.8 vs. 35.4). Similar to women who seroconverted, men who reported a lower monthly family income tended to have higher rates of unlinked seroconversion ($68.3 for unlinked seroconversion vs. $95.6 for non-seroconverting couples). Further, men who acquired HIV from an outside partner were more likely to self-report having a STI in the past year (42% vs. 25% of men who did not seroconvert), and to have genital inflammation (35% vs. 12%) and ulceration (26% vs. 6%). Men who had an unlinked infection

were more likely to report having outside sex partner(s) in the past 3-months (21% vs. 8%, respectively). (Table 3.3)

Controlling for man's age, monthly family income, male literacy, and male circumcision before or during the study, the risk of unlinked incident HIV infection among men was associated with male alcohol use (reporting being drunk weekly or daily vs. never in the year before enrollment) (aHR=3.52, 95% CI=1.19, 10.46), male genital ulcers in the past 3-months (aHR=4.27, 95% CI=2.05, 8.89), male genital inflammation in the past 3-months (aHR=8.52, 95% CI=3.82, 19.03), and reporting =>1 outside sex partner in the past 3-months (aHR=3.36, 95% CI=1.53, 7.37). (Table 3.4)

Finally, in male HIV-positive/female HIV-negative couples, most women who reported outside sex partner(s) did not report sex with their main partner in the past 3-months (74%, or 31 of 42 women). In male HIV-negative/female HIV-positive couples, 61% of men who reported outside partner(s) did not report sex with their main partner.

3.5 Discussion

This study contributes to the understanding of acquisition of HIV from outside sex partners in Southern Africa [15, 16]. We observed a genetically unlinked HIV incidence rate of 1.85/100 CY among women in serodiscordant couples, and of 1.82/100 CY among men in serodiscordant couples in this large 18-year prospective study. This incidence rate is significantly lower than the 7.27/100 CY found for linked infections among females and 5.08/100 CY linked infections among

men for the entire study (Wall et al, In Press). Overall, 20% of seroconversions were genetically unlinked during the 17-year study follow up.

Our unlinked infection incidence rate is similar to other studies in the region [4, 18], demonstrating the importance of this as a risk for HIV acquisition in generalized HIV epidemics. Our study also demonstrated the role of male and female alcohol use on acquisition of HIV from an outside partner. We confirmed that genital ulceration and inflammation [19, 26, 42], heavy alcohol use [46, 47, 55], and reporting more than one sex partner during the study (among seroconverting men in our study) [19] were important predictors of unlinked seroconversion among male and female partners. Since genital tract infections and ulcerations have been significantly associated with HIV genital tract shedding in a systematic review and meta-analysis [114], diagnosing infections and symptoms in the HIV-positive partner, as well as in the HIV-negative partner, is critical. Compared to linked infections, unlinked seroconversion was more associated with higher rates of genital inflammation and ulcers and number of outside partners (among women). This finding demonstrates that these couples have very similar behavioral and clinical characteristics, and that it may be difficult to distinguish couples who are having sex with outside partners. Clinical characteristics of the HIV-infected partner, including viral load, HIV stage, and CD-4 cell count were not associated with HIV acquisition among these couples who acquired HIV from an outside partner. Similar to the larger cohort [61], after controlling for potential confounders, we did not find that pregnancy (prevalent or incident) or contraceptive use were risk factors for unlinked seroconversion.

Further, more men acquired an unlinked vs. linked HIV infection compared to women (24% vs. 18% respectively), indicating a higher risk of infections due to concurrent or outside partnerships among discordant couples in which the man is HIV-negative. The lack of sexual

activity with the HIV-positive partner may reflect the dissolution of the sexual partnership among both men and women. It may also reflect an increased social or cultural acceptance of men and women having outside sexual partnerships once they know their main partner is HIV-positive. Despite the relatively high incidence of unlinked infections among women, the self-reporting of outside partnerships was low and not associated with seroconversion among female partners, likely due to being underreporting about outside partners. This highlights the challenges of self-reports collected by face-to-face interviews, especially among women, when aiming to identify individuals at risk in outside partnerships.

Between 55-60% of Zambian adults requiring treatment were on ARV therapy in 2013 [45]. Zambia has adopted the WHO-recommended approach to couples in whom one person is HIV-positive by providing treatment regardless of their CD4 cell count [45]. However, despite this policy change, the high rate of seroconversion among serodiscordant couples is only partially mitigated by ART in real-world settings [26, 27], partly because of ongoing sex with outside, HIV-infected, partners. Therefore, primary prevention strategies are still needed for HIV-negative partners in serodiscordant couples, especially considering that within this cohort, over 1.83 incident infections from an outside sex partner occurred over 100 couple years.

Limitations. Because these couples self-selected into both the couples-counseling/testing and the cohort study, they had inherent differences from the general population and other African serodiscordant couples, including in their behavior of seeking HIV testing as a couple. There may be a self-selection bias in which those continuing in CVCT may have higher health-motivation and may be more likely to commit to safer sexual practices. Another limitation is the use of self-reported information on important exposure and confounder information, which has low sensitivity. Information bias due to exposure variables captured as self-reports is possible. Couples

were followed-up every 3-months; however, that amount of time could lead to recall bias on certain measures that may not be salient to the participants. In addition, we did not collect data on condom use with outside partners. As a result, there may be residual confounding in our analysis. Our ability to analyze the true effect of genital ulcers or inflammation is limited by a lack of systematic testing in the study, which could result in a misclassification of those with ulcers and inflammation as having STIs, because many (up to potentially 50% in women) actual STI infections are asymptomatic. Interpretation of findings should be made with caution due to small sample size and limited power. The analysis by gender of seroconverter further segmented the data to 45 female seroconversions, and 55 male seroconversions, which resulted in wide confidence intervals due to sparse data.

3.6 CONCLUSIONS

Our study found that the incidence of HIV acquisition from outside partners of serodiscordant couples is high. Factors associated with unlinked infection among men and women included alcohol use, genital inflammation and ulceration, and reporting having outside sex partners among men. Compared to linked infections, unlinked seroconversion was more associated with higher rates of genital inflammation and ulcers and number of outside partners (among women). Greater understanding of associations between HIV-negative partners' behaviors with primary and outside partners and HIV acquisition will be important for the design of HIV-prevention strategies for uninfected partners. Our findings provide further evidence HIV-negative partners in serodiscordant couples should be regularly screened for symptomatic STIs, and other non-sexually transmitted causes of genital inflammation in women, such as bacterial vaginosis or candida. Screening and prophylactic treatment will help reduce inflammation and ulceration, which will

reduce the risk of HIV acquisition and secondary transmission. We also bring attention to the role of heavy alcohol use by males and females in acquisition of HIV, especially acquisition of HIV from an outside partner.

3.8 Tables

Figure 3.1.

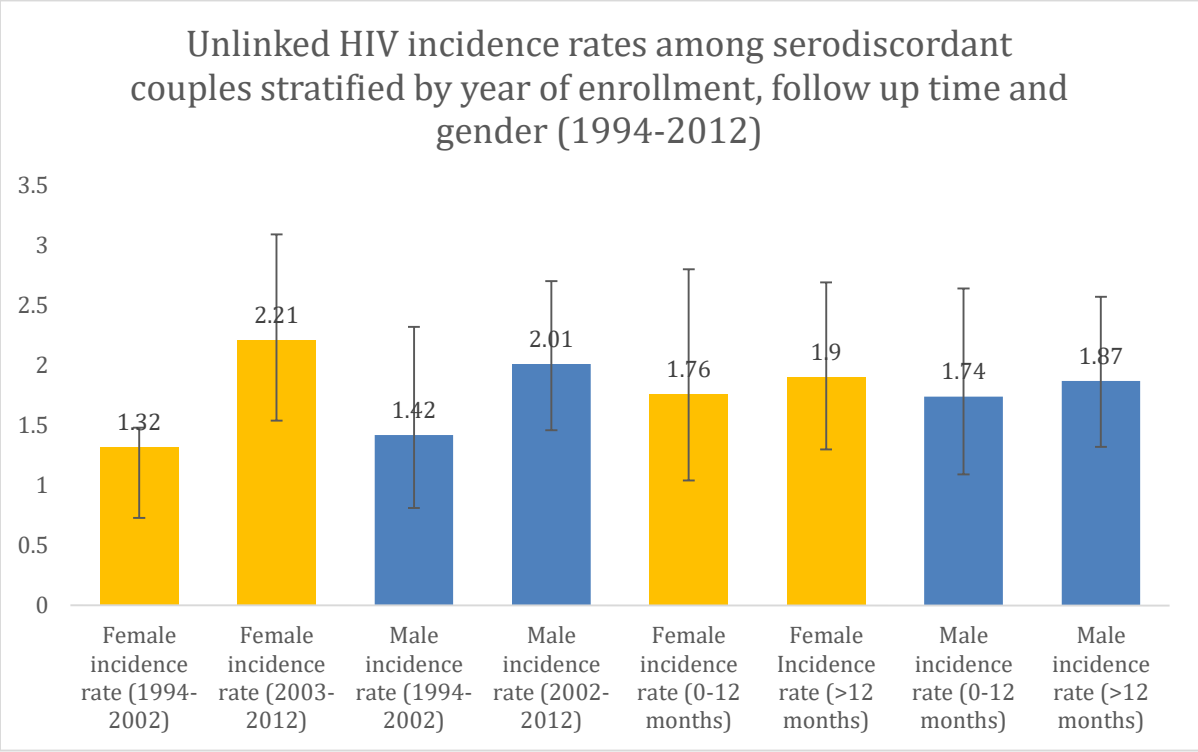


Table 3.1. Demographic and behavioral characteristics of serodiscordant couples by seroconversion outcome (N = 1186 M+F- couples)

	Non-seroconverting couples		**Unlinked infections in women**	
Total	**1141**	**96%**	**45**	**4%**
	N/mean	**%/SD**	**N/mean**	**%/SD**
Demographics				
Woman age (mean, SD) **	28.8	7.0	25.5	6.3
Age disparity (mean, SD)	6.9	4.4	6.8	3.9
Years cohabiting (mean, SD) **	8.3	6.8	5.6	4.9
Family income/month (mean, SD) **	94.0	118.8	50.9	41.6
Woman reads Nyanja *	272	24%	5	11%
Woman alcohol use last year*	192	17%	11	27%
Family planning and sexual history				
# previous pregnancies (mean, SD) **	3.7	2.5	2.7	1.7
Pregnant at baseline	155	14%	6	13%
Fertility intentions of woman				
Yes, next year	87	8%	4	9%
Yes, but not next year	88	8%	5	11%
Woman lifetime sex partners (mean, SD)	2.8	3.4	3.0	2.5
Woman last year sex partners (mean, SD)	1.1	0.36	1.3	0.9
Woman history of STI last year (self-reported) **	292	26%	19	42%
Time-varying variables	**N interval/ mean**	**% or SD**	**N interval/ mean**	**% or SD**
Clinical characteristics				
Genital inflammation in past 3-months **	1799	15%	24	53%
Genital ulcer in past 3-months **	593	5%	12	27%
Sexual behaviors				
# of times sex with partner in project with a condom in the last 3-months reported by woman (mean, SD) **	18.6	18.9	21.3	24.1
# of times sex with partner in project without a condom in the last 3-months (mean, SD) **	2.3	8.2	5.0	13.9
Sex with study partner with a condom in past 3-months	10,036	85%	39	87%
Sex with study partner without a condom in past 3-months	3299	28%	12	27%
Sperm present on wet prep **	831	6%	26	11%
Sex with =>1 outside partner during study	157	1%	2	4%
Family planning characteristics				
Contraceptive method at follow-up visit **				
Non-hormonal	9060	65%	20	44%
Implant	1000	7%	3	7%
Injectables	1899	14%	8	18%
OCPs	1921	14%	14	31%
Pregnant during interval	1130	9%	2	5%

*=p<0.10; ** p<0.05

Table 3.2. Demographic and behavioral characteristics of serodiscordant couples by seroconversion outcome (N = 1485 M-F+ couples)

	Non-seroconverting couples		**Unlinked infections in men**	
Total	**1430**	**97%**	**55**	**3%**
	N/mean	**%/SD**	**N/mean**	**%/SD**
Demographics				
Man age (mean, SD) **	35.4	8.5	32.8	6.8
Age disparity (mean, SD) *	6.8	5.1	6.9	5.2
Years cohabiting (mean, SD)	6.0	6.0	5.5	4.8
Monthly family income (USD) (mean, SD)	95.6	117.5	68.3	50.9
Man reads Nyanja	630	44%	20	38%
Man alcohol use last year	1023	73%	42	79%
Family planning and sexual history				
# of previous pregnancies (mean, SD) *	3.2	2.2	3.0	2.2
Pregnant at baseline (N, %)	155	14%	6	13%
Fertility intentions of man				
Yes, next year	92	19%	3	5%
Yes, but not next year *	169	35%	11	19%
Don't know/No	226	46%	44	76%
Man lifetime sex partners (mean, SD)	11.2	17.8	10.1	11.5
Man last year sex partners (mean, SD)	1.6	1.8	2.1	1.9
Man history of STI last year**	358	25%	23	42%
Circumcised male partner	267	19%	5	9%
Time-varying variables	**N interval/ mean**	**% or SD**	**N interval/ mean**	**% or SD**
Clinical characteristics				
Genital inflammation in past 3 months (man)**	1589	12%	19	35%
Genital ulcer in past 3 months (man)**	851	6%	14	26%
Sexual behaviors				
# of times sex with partner in project with a condom in the last 3 months reported by woman (mean, SD)	21.7	22.9	20.4	20.1
# of times sex with partner in project without a condom in the last 3-months (mean, SD)	3.4	11.8	3.9	11.0
Sex with study partner with a condom in past 3-months	10,037	85%	49	89%
Sex with study partner without a condom in past 3-months	4056	34%	19	35%
Sex with =>1 partner during study**	866	8%	11	21%

*=p<0.10; ** p<0.05

Table 3.3. Multivariable Cox survival models of predictors of time to unlinked HIV infection among women (Model 1) and men (Model 2).	
	Adjusted Hazard Ratio (95% CI)
MODEL 1: Female unlinked seroconversion* (M+F- couples, n=1186 couples)	
Baseline	
Woman's alcohol use at baseline (>=4 times/week)	5.44 (1.03-28.73)
Time-varying	
Genital inflammation in past 3-months (woman)	11.92 (5.60, 25.37)
Genital ulcer in past 3-months (woman)	6.09 (2.72, 13.64)
≥1 outside partner during study (woman)	2.07 (0.42, 10.28)
MODEL 2: Male unlinked seroconversion (M-F+ couples, n=1485 couples)**	
Baseline	
Alcohol use in past year	
Man reported being drunk weekly or daily (vs. never)	3.52 (1.19, 10.46)
Man reported being drunk monthly or less (vs. never)	2.24 (0.50, 10.12)
Time-varying	
Genital inflammation in past 3-months (man)	8.52 (3.82, 19.03)
Genital ulcer in past 3-months (man)	4.27 (2.05, 8.89)
≥1 outside sex partner during study (man)	3.36 (1.53, 7.37)

* Adjusting for woman's age, age difference, woman's literacy, number of previous pregnancies, couple income (in USD), contraceptive method use, and sperm present on wet-prep (proxy for recent condomless sex)

** Adjusting for man's age, woman's literacy, couple income (in USD), and male circumcision status (before or during study)

Chapter IV. Study 3: Difficult Decisions: Evaluating individual and couple-level fertility desires and HIV acquisition among HIV serodiscordant couples in Zambia

4.1 Abstract

Background: This study evaluates the effect of fertility desires on HIV acquisition among HIV serodiscordant couples in Zambia.

Methods: We collected demographic, behavioral, clinical exposures, and data on fertility desires in a prospective cohort of HIV serodiscordant couples. We evaluated factors associated with fertility desires stratified by gender using multivariable logistic regression. Data on fertility desire was collected from 2007 to 2012 (n=1.029 couples). To estimate the overall effect of fertility desire on risk of HIV infection, we used inverse-probability-of-treatment-weighted estimation of a marginal structural model to adjust for time-varying confounders affected by prior exposure.

Results: Among 1,029 serodiscordant couples, 311 agreed that they wanted a child in the future (30.4%), 368 agreed they did not want a child (36.0%), and 344 couples disagreed about having more children (33.6%). Women's fertility desire was associated with younger age (aOR=0.95; 95% CI=0.91, 0.99), not being pregnant at baseline (aOR=0.21; 95% CI=0.12, 0.37), fewer living children (aOR=0.75; 95% CI=0.62, 0.90), fewer previous pregnancies (aOR=0.87; 95% CI=0.61, 0.98), and partner wanting a child (aOR=2.79; 95% CI=1.97, 3.95). Men's fertility desire was associated with younger age (aOR=0.88; 95% CI=0.80, 0.97), and partner wanting a child (aOR=2.83; 95% CI=2.00, 4.01). In marginal structural models for the results of fertility intentions

on HIV acquisition, the adjusted risk ratio for woman's HIV acquisition was 2.06 (95% CI=1.40, 3.03) among women wanting a child, and 1.75 (95% CI=1.07, 2.87) for men wanting a child, and 2.55 (95% CI=1.32, 4.93) when the couple agreed on wanting a child compared to couples who agreed they didn't want a child, adjusting for age, literacy, previous pregnancies. Male seroconversion was not associated with fertility desire.

Conclusion: Women had increased risk of seroconverting if they or their partner wanted a child. Safer conception interventions are needed to protect HIV uninfected women from HIV acquisition in HIV serodiscordant couples who want children.

4.2 Background

Of the estimated 33 million people living with HIV worldwide, 16 million are women [54]. Since the introduction of ART, pregnancy rates and the desire to conceive have increased among women and men living with HIV, due in part to improved quality of life, increased life expectancy, and reduced rates of mother to child transmission [86, 98]. In Zambia, 86% of pregnant women living with HIV received ARVs for PMTCT in 2014, and 42% of children living with HIV were on treatment [131]. Despite improved access to treatment for PMTCT, attempts to conceive, and pregnancy may increase HIV transmission to sex partners in HIV serodiscordant couples (in which one partner is HIV-infected and the other HIV-uninfected) [86, 98]. Further, a recent analysis of population-based data on maternal deaths in sub-Saharan Africa estimated that roughly a quarter of pregnancy related deaths are related to HIV [99].

Previous research on the determinants of fertility desires in HIV-infected women, and serodiscordant couples has yielded mixed results [55, 56]. Men and women of younger age and those without children are more likely to desire (more) children [55, 56]. However, desire to have children may also be influenced by gender, access and adherence to ART, knowledge of prevention of mother to child transmission of HIV programs (PMTCT), stigma, and social support as well as family, culture and gender norms [12, 55, 100]. A recent study in Zambia by Cook et al. found that desire to have children was often shared by couple members, and among serodiscordant couples or concordant HIV-positive couples the strongest predictor of a partner's desire for children was having a partner who wanted children [56]. Addressing the varied needs of women of reproductive age who are living with HIV, or are at risk of acquiring HIV, as in the case of serodiscordant couples, is a complicated task that requires improved access to, utilization and quality of comprehensive women's healthcare.

Despite the risk of infection and mortality, safer conception interventions for serodiscordant couples are not readily available in sub-Saharan Africa [98, 99, 126, 127] where the vast majority of serodiscordant couples reside. Safer conception interventions include timed condomless intercourse (with ovulation) [42], self-insemination [125, 128, 129], sperm washing [123, 126, 128] ART for HIV-positive partners [120, 126], and pre-exposure prophylaxis (PrEP) for HIV-negative partners [126, 129]. This research gap is of concern because most serodiscordant couples are of child-bearing age, and with the widespread use of ART, life expectancy and sexual activity continue to increase [11, 99]. Further, preventing unintended pregnancies among HIV-positive women and serodiscordant couples who do not want children, through family planning, especially longer-term methods, reduces pregnancy-related morbidity and mortality, decreases the number of pediatric HIV infections, and has also proven to be a cost-effective way to prevent mother-to-child HIV transmission [100, 124].

This study examined the role of fertility desire as a risk factor for HIV acquisition and transmission among serodiscordant couples. We evaluated factors associated with fertility desire among men and women in HIV serodiscordant couples. We then evaluated the effect of the female partner, male partner and both partners wanting more children on HIV acquisition over time.

4.3 Methods

The Heterosexual Transmission of HIV Study conducted by the Rwanda Zambia HIV Research Group (RZHRG) was an open prospective cohort, which enrolled 3,049 adult heterosexual serodiscordant couples recruited from couples' voluntary counseling and testing (CVCT) centers in Lusaka, Zambia. Data collection began January 1995 and ended December 2012. This study was approved by the Office for Human Research Protections-registered Institutional Review

Boards (IRB) at Emory University, University of California, Los Angeles, and the University of Zambia. Written informed consent was obtained from all participating individuals in the couple.

Study participants. Married or cohabiting couples in Lusaka, Zambia, attended CVCT services spontaneously, or after receiving an invitation from a community promoter [93, 96, 118]. CVCT services included a group counseling session followed by rapid HIV testing, and joint post-test couples' counseling [91, 97]. Eligible HIV serodiscordant couples were then invited to enroll in a longitudinal open cohort follow-up study between 1995 and 2012 [91]. The primary analyses for this paper includes couples enrolled from 2007 through 2012, when data on fertility intentions was collected, for a total of 1,023 couples.

Study eligibility criteria included: (1) confirmed HIV-1 serodiscordance (one partner confirmed HIV-positive and one partner confirmed HIV-negative) (2) participants were married or cohabitating for at least three months, and (3) the couple planned on staying in the Lusaka region for the next 12-months. Couples were ineligible if the HIV-infected partner was on ART, which became available in government clinics in 2007 with CD-4 cell count eligibility criteria changing over time. Couples were censored if either partner died, the couple separated, the HIV-positive partner started ART, or if either partner was lost to follow-up.

Data collection. At baseline, study participants completed behavioral assessments and medical history questionnaires, and had a full physical examination including pelvic/genital exams, as well as HIV and STI testing (RPR serology for syphilis, wet mount for trichomonas, documentation of ulcers and discharge on genital exam) and treatment. Quarterly follow-up visits included a physical exam (including STI testing if symptoms were present, and treatment according to national guidelines), a blood sample for HIV testing (HIV-negative partner), a vaginal swab wet prep (trichomonas and sperm), and completion of study questionnaires, comprising questions on

demographic, psychosocial, behavioral, medical history, and health services data. Couples were asked to complete a coital diary to record number of sex acts, with a condom and without daily. The data was collected through face-to-face interviews by a study counselor or nurse. All data collection instruments were in English and were translated into local languages Nyanja and Bemba.

HIV Testing. HIV testing of HIV-negative partners was conducted at three-monthly intervals using a rapid serologic test. To determine time of incident infection, when available, plasma from the last antibody negative sample was tested with p24 ELISA and RNA polymerase chain reaction (PCR). The date of HIV infection was defined using available laboratory results as the minimum of: the midpoint between the last negative and first positive antibody date; two weeks prior to the first antigen positive test date; or two-weeks prior to the first positive viral load positive/rapid HIV test negative test date.

Fertility intention exposure measurement. Data on fertility intention was collected at baseline from 2007 to 2012, when the study ended. The first measure was "Do you want to have a child?" If the respondent answered "yes", then they were asked whether they "want a child in the coming year", or "yes, but not in the next year".

Analyses. Descriptive statistics express baseline demographic and behavioral data by fertility intention. We developed binary outcomes combining (1) Yes, in the coming year, and (2) Yes, but not in the coming year vs. no/don't know with counts and percentages (for categorical variables), or means and standard deviations (for continuous variables).

We first analyzed factors associated with wanting a child by gender. We then specified logistic regression models for factors associated with wanting a child by gender, controlling for potential confounders within each exposure group. Finally, we analyzed seroconversion stratified

by gender of seroconverter as a result of the woman, man, or couple wanting a child in the coming 12-months, vs. those not wanting a child. We also compared concordant desire in wanting a child in the couple, vs. disagreement in the couple (man wants more children/woman does not or woman wants more children/man does not). We developed Inverse-Probability-of-Treatment weighted estimates of a marginal structural model to estimate the causal effect of fertility desire on HIV acquisition, by creating weights to remove the relationship between fertility desire and condomless sex. A priori confounders (woman's age, woman's literacy, couple's income, and number of previous pregnancies for women, or number of live children at baseline for men) and covariates that substantially (>10%) changed the effect estimates in univariate analyses were considered as potential confounders in the multivariable models.

Marginal structural models. We used a weighted pooled logistic regression model to approximate the parameters of a marginal structural Cox model, as described by Hernan et al. [71] Pooled logistic regression approximates the Cox model well when the risk of events is less than 10%. In the absence of unmeasured confounding, unmeasured informative censoring and model misspecification, weighting creates a pseudo-population in which the probability of treatment (couples counseling and testing) and censoring are not a function of time-varying covariates but the effect of the fertility desires on HIV acquisition in the original population. Thus, the inverse probability of treatment weight effectively removes any association between prior confounding variables and fertility desires, but preserves the relationship between fertility desires and HIV acquisition. To increase the efficiency of our estimator, we stabilized the weights [71].

Effect-measure modification was assessed for gender of HIV-positive partner. Multi-collinearity was assessed by analyzing the variance inflation factor and tolerance to check the degree of collinearity between variables. When variables were collinear in a model, we selected

one variable depending on the exposure and outcome of the model. All analyses were conducted with SAS v9.4 (Cary, NC).

4.4 Results

The overall cohort makeup has been previously described; therefore, it will not be reported here [11, 16, 29]. Of 1,029 men, 168 (16.3%) wanted to have a child in the next 12-months, and 358 (34.8%) wanted to have a child in the future, but not the next 12-months. Almost 45% of men (n=461) did not want a child in the future, and 4% did not know. Of 1,283 women, 318 (24.8%) wanted to have a child in the 12-months, and 283 (22.1%) wanted to have a child, but not in the next 12-months. Over 47% (n=682) did not want to have a child, and 6% did not know. Men who were HIV-negative with HIV-positive partners wanted children more than when they were HIV-positive (56% vs. 45%). Women who were HIV-positive with HIV-negative partners wanted children more than if they were HIV negative (51% vs. 42%).

For couple level analyses, of 1,029 couples, 311 (30.4%) couples agreed that they wanted to have a child in the future, and 368 (36.0%) agreed that they did not want to have a child in the future. Almost 34% (n=344) disagreed about whether or not to have a child, the majority of which were couples in which the man wanted a child but the woman did not (n=212 couples, 20.6%). Couple-level disagreement was greatest among couples in which the man was HIV-negative and woman was HIV-positive, in which the man wanted a child whereas the woman did not (22% of M-F+ couples). In couples in which the man was HIV-positive and the woman was HIV-negative, 19% of men wanted a child when their partner did not. About 13% of M+F- or M-F+ couples had disagreement in which the woman wanted a child but a man did not. (Figure 1)

Women who wanted to have a child tended to be slightly younger (mean age of 27.2 vs. 28.0), and live with their partners fewer years (5.2 vs. 8.6 years) compared with women who did

not want to have children. Women who wanted to have a child had fewer mean pregnancies (2.6 vs. 3.9) and live children (1.1 vs. 2.2 mean live children) compared with women who did not want a child. Half of the women who did not want to get pregnant were using a method of contraception compared to one-third of women who wanted a child at some point in the future. Women who wanted a child reported greater number of condomless sex acts in the past 3-months (2.4 vs. 2.0) compared with women who did not want a child. Ten percent of women who wanted to get pregnant got pregnant during the study interval, compared with 6% of women who did not want children. Age disparity between partners, monthly family income, man's literacy, women's alcohol use, and women's genital ulcers were not associated with wanting a child. (Table 2)

We also compared the baseline covariates between man HIV-positive/woman HIV-negative (M+F-) and man HIV-negative/woman HIV-positive couples (M-F+). Over 50% of couples in which the woman was HIV-infected wanted a child, compared to 41.7% of couples in which the woman was HIV-negative, and 46.8% couples overall. Women who were HIV-infected had fewer live children (0.9) and fewer pregnancies (2.5) compared to HIV infected women who did not want children. Fewer women who wanted a child and were HIV-positive were using a method of hormonal contraception (29.1% vs. 51.3% of women who were HIV-negative). Condomless sex was higher in couples in which the woman was HIV-infected (36.4% vs. 30.5%). (Table 4.1)

In this cohort, men who wanted a child tended to be younger (33.0 vs. 38.1), and have greater age disparity with their partner (7.1 vs. 6.4 years' difference) when compared to men who did not want a child. Men who wanted a child lived with their partners for 5.2 vs. 9.3 years among men who did not want a child. Men who did not want a child had a partner with greater number of pregnancies (4.1 vs. 2.7 mean pregnancies) compared with men who did want a child. Condomless

sex was reported more frequently in follow-up intervals with men who wanted a child (33% vs 27% for men who did not want a child). Correspondingly, 4% of intervals for men who wanted a child included partners who had sperm present on a vaginal wet mount, compared to 2% of intervals for men who did not want a child. Men's alcohol use, genital inflammation, and sex with a condom were not associated with men's fertility desire. (Table 4.2)

Among M+F- couples, 45.2% wanted a child, compared with 55.8% of M-F+ couples. Condomless sex was higher in couples in M-F+ follow-up intervals (37.4% vs. 27.2% for M+F- intervals). HIV-negative men who wanted a child were more likely to have outside partners than their HIV-positive counterparts (8.2% vs. 4.4%) (Table 4.2).

Factors associated with woman wanting a child: Adjusting for man's age, literacy, years cohabiting, and woman's HIV status, woman's younger age (aOR=0.95, 95% CI=0.91, 0.99), partner wanting children (aOR=2.79, 95% CI=1.97, 3.95), not being pregnant at baseline (aOR=0.21, 95% CI=0.12, 0.37), fewer live children (aOR= 0.75, 95% CI=0.62, 0.90) and fewer previous pregnancies (aOR=0.87, 95% CI=0.61, 0.98) were associated with wanting a child in the future compared to women who did not want a child. (Table 4.3)

Factors associated with man wanting a child: Adjusting for woman's age, literacy, number of live children, and man's HIV status, men's younger age (aOR=0.88, 95% CI=0.80, 0.97), fewer years cohabiting (aOR=0.95, 95% CI=0.90, 1.00), and partner wanting to have children (aOR=2.83, 95% CI=2.00, 4.01), were also associated with wanting a child in the future. Unlike the women's model, having a partner who was pregnant at baseline was not associated with decreased odds of wanting to have a child in the future. (Table 4.3)

Effect of fertility desire on HIV acquisition for women: In marginal structural models, using inverse probability of treatment weights, we found that risk of HIV acquisition among women in

male HIV-infected/woman HIV-uninfected couples (n=540 M+F- couples, and 87 incident infections in women) was increased compared to couples that did not want a child in the future. Women's HIV acquisition was associated with the woman wanting a child in the next 12-months (aRR=2.06, 95% CI= 1.40, 3.03), and in the future, beyond 12-months (aRR=1.64, 95% CI=1.12, 2.40). When the man wanted a child risk of HIV acquisition was almost double that of couples in which the man did not want a child (aRR=1.75, 95% CI=1.07, 2.87). Couple agreement about wanting to have a child increased the risk of female HIV acquisition (aRR=2.55, 95% CI=1.32, 4.93) adjusting for age, literacy, and previous pregnancies compared to couples who agreed that they did not want children. (Figure 4.2).

Effect of fertility desire on HIV acquisition for men: In this cohort of 743 M-F+ couples in which 95 men seroconverted during this analysis period, neither men's, nor women's, nor couple agreement in fertility desire were associated with increased risk of HIV acquisition among men. (Figure 4.2)

4.5 Discussion

This is one of the first studies in Southern Africa to show the effects of fertility desires on HIV acquisition within cohabiting serodiscordant couples. Our analysis demonstrated that fertility intentions were highest among men, especially HIV-negative men, but couple agreement on wanting a child was highest when the woman was HIV-negative. Further, women in serodiscordant couples were particularly vulnerable to HIV acquisition when either they, or their partner wanted to have a child, especially in the next 12-months. When both partners agreed that they wanted to have a child, the woman's risk of acquiring HIV was almost double that of women in couples who agreed they did not want a child. Men's risk of HIV acquisition was unchanged whether or not the couple agreed or not about having a child. Our study's effect estimates are stronger than previous

results because of the use of marginal structural models and inverse probability weights which estimate the overall effect of fertility desire on risk of HIV infection, and adequately adjust for time-varying confounders affected by prior exposure of couples counseling and testing over time.

The predictors of wanting to have a child among men and women were similar to previous studies in the region, including woman's age [112], number of live children [101, 111, 112], and being pregnant or having a pregnant partner [112]. Unlike a recent study in Ethiopia, we did not find that serostatus was associated with fertility desire in men or women [111]. Our study demonstrated that men and women who believed their partner wanted a child was a major determinant of also wanting to have a child, which has been demonstrated in several other African studies [101, 103, 107, 111, 112]. A Kenyan qualitative study of serodiscordant couples found that values and preferences of the couple as a unit may mediate fertility decision making in serodiscordant couples [112]. Similarly, we found that there was a higher risk of HIV acquisition among women in which her and her partner agreed about having a child in the future.

Our study did not evaluate knowledge of contraception, other family expectations of having a child, nor the influence of health care providers on contraceptive uptake. However, other researchers demonstrated that relatives' expectations were associated with wanting a child among Ugandan serodiscordant couples [103]. In the same Ugandan study, knowledge of contraception was associated with decreased fertility desire, while knowledge of ART effectiveness was associated with increased fertility desire [103]. In addition, research on health care providers found that providers may perceive that a HIV-positive woman who gets pregnant is 'wrong' or 'irresponsible' [102]. In a recent study of HIV-infected women in South Africa, only 41% of women had communicated with providers about future pregnancy options [109]. Further, high rates of unintended pregnancy have been reported among HIV-infected women [210]. These

studies, along with our findings, highlight the urgent need for integrating family planning, and interventions for safer conception into HIV care and treatment [127].

Access to ART during pregnancy increased during the cohort when PMTCT interventions were introduced in Lusaka in 2001, and by 2009, 69% of HIV infected pregnant women received ART compared to 86% in 2014 [131], which may be an important determinant of fertility intentions among HIV-infected women in serodiscordant couples. However, our cohort included serodiscordant couples who were ART-naïve. As such we could not compare couples on ART vs. not on ART. However, a recent study in South Africa demonstrated that women who recently initiated ART were more likely to have incident pregnancies [109]. Prior research has shown that ART use is also associated with higher incidence of pregnancy [98]. However, women on ART may choose to conceive at times that are sub-optimal, such as after recent ART initiation, for infant, maternal, and partner health outcomes. In addition, some ART regimens may also increase contraceptive failure rates [130].

Limitations. Because these couples self-selected into the cohort after couples' counseling and testing, they have inherent differences from the general population and other African serodiscordant couples who may not know their serostatus. Another important limitation is that fertility desire was only reported at baseline, and fertility desire may change over time, as HIV-infected individuals get sicker. Questionnaire data was interviewer collected by a nurse or counselor who were familiar with the couple, which could cause social desirability bias. As a result of these limitations, information bias due to exposure variables captured as self-reported is possible. Selection bias may also have affected the study as couples who stayed in the cohort may differ in terms of their health-seeking behavior, or other demographic or behavioral data, from similar couples who were not enrolled or were lost to follow up, or separated. At the beginning of

this study, ART was not commonly available in Zambia. However, after 2002, ART became more widely available, and those on ART were not eligible for this study. Therefore, there is an important difference between couples in this study and those not eligible. Those on ART may have had more advanced virus, with lower CD-4 cell counts, and with better access to health care, which may affect the generalizability of results.

4.6 Conclusions

This is one of the first studies in Southern Africa to demonstrate the effects of fertility intentions among men, women and couple-level on HIV acquisition within cohabiting, heterosexual HIV serodiscordant couples. Our analysis demonstrated that women were particularly vulnerable to HIV acquisition when either they, or their partner wanted to have a child in the next 12-months. Optimizing delivery of HIV risk reduction strategies during peri-conception periods (i.e. safer conception) requires understanding how HIV serodiscordant couples approach fertility decisions. Further work is needed on the impact of provider's attitudes and training on safer conception and contraception intervention. Our study highlights the importance of pregnancy planning and safer conception services as a critical, but oftentimes neglected, component of HIV care and treatment. It is important that couples receive HIV counseling and testing together to identify when they are serodiscordant. Our study also demonstrated that the partner has an important say on whether or when couples try to get pregnant. Safer conception programs should therefore include both partners, and consider the relationship during risk reduction counseling and when recommending risk reduction interventions.

4.6 Tables and Figures

Figure 4.1:

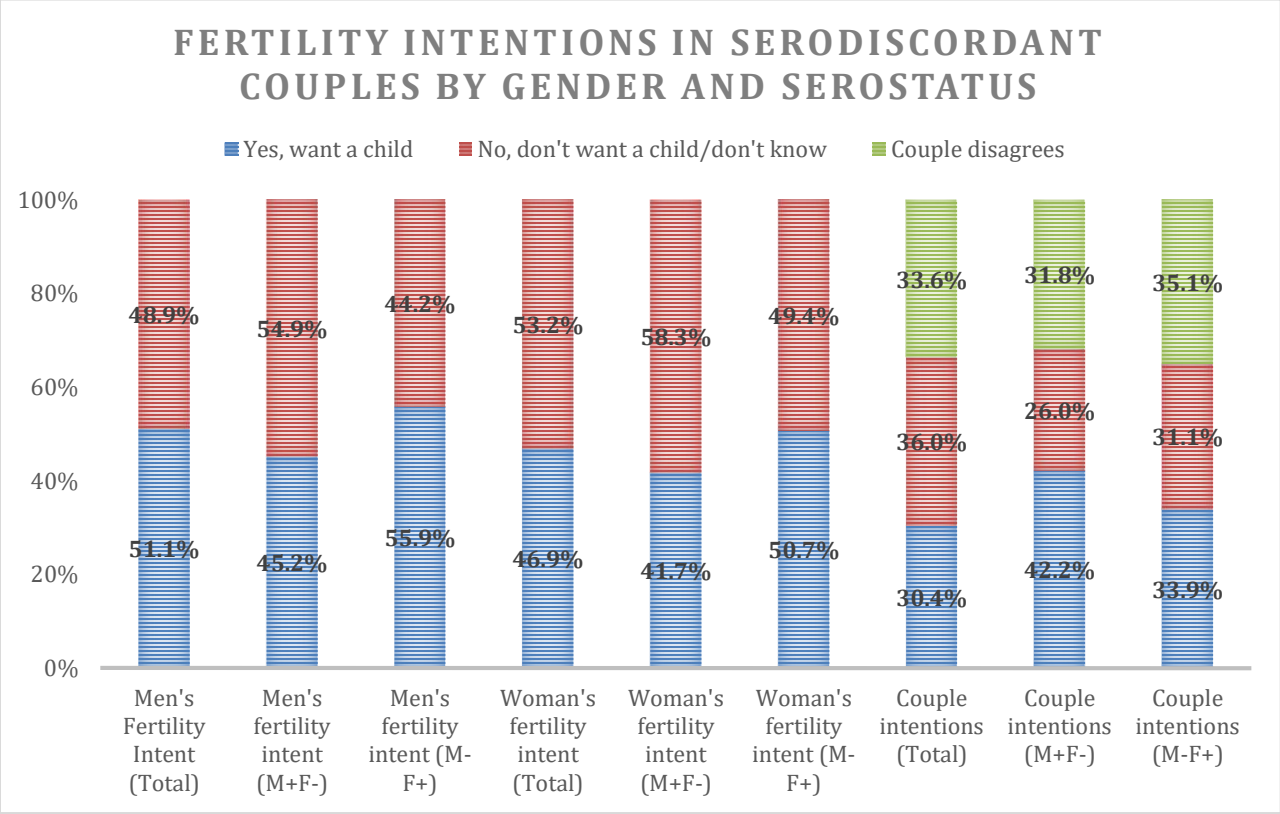


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Table 4.1 Descriptive analyses of baseline covariates by fertility intention of women (n=1283)

	Total	Woman doesn't want child		Woman wants child in next 12-months or longer		Woman wants child (Man HIV+/Woman HIV-)	Woman wants child (Man HIV-/Woman HIV+)
Total	**1283**	**682**	**53.2%**	**601****	**46.8%**	**225 (41.7%)****	**376 (50.6%)**
Demographics							
Woman age (mean, SD)	28.5	28.0	6.0	27.2	5.9	26.4 (5.8) **	27.7 (5.9) **
Age disparity (mean, SD)	6.8	6.7	4.7	6.6	4.3	7.2 (4.3) **	6.2 (4.3)
Years cohabiting (mean, SD)	6.8	8.6	6.9	5.2**	4.5	5.5 (4.5) **	5.1 (4.6)
Monthly family income (USD) (mean, SD)	90.2	93.2	115.4	98.2	119.1	92.9 (109.1)	101.4 (124.7)
Woman reads Nyanja	301 (23%)	178	26%	123**	20%	36 (16.0%)**	87 (23.2%)*
Man reads Nyanja	567 (44%)	299	44%	268	45%	92 (41.1%)	176 (46.9%)
Woman heavy alcohol use last year	53 (4%)	24	4%	29	5%	7 (3.1%)	17 (4.5%)
Family planning and sexual history							
Number of previous pregnancies (mean, SD)	3.4	3.9	2.3	2.6**	1.8	2.5 (1.7) **	2.7 (1.9) **
Number of live children (mean, SD)	1.7	2.2	1.8	1.1**	1.2	1.4 (1.3) **	0.9 (1.1) **
Pregnant at baseline	125 (10%)	31	4.6%	94**	15.6%	45 (20.0%)**	49 (13.0%)**
Contraceptive method at baseline							
None	2265 (74.5)	338	49.7%	392**	66.7%	132 (58.7%)**	260 (69.9%)**
Implant	92 (3.0)	40	5.9%	18**	3.0%	11 (4.9%)**	7 (1.9%)**
Injectables	298 (9.8)	151	22.2%	88**	14.7%	39 (17.3%)**	49 (13.2%)**
OCPs	316 (10.4)	125	18.4%	93	15.6%	40 (17.8%)**	53 (14.3%)**
Tubal ligation/vasectomy	23 (0.8)	13	1.9%	0	0.0%	0 (0.0%)	0 (0.0%)
Time varying: Clinical characteristics & sexual behaviors (n=intervals)							
Genital inflammation in past 3 months (woman)	875	405	8%	470	9%**	122 (5.8%)**	348 (11.0%)**
Genital ulcer in past 3 months (woman)	613	293	6%	320	6%	67 (3.2%)*	203 (6.2%)
# of times sex with partner in project <u>with</u> a condom in the last 3-months (mean, SD)	20.2	20.5	20.4	21.0**	21.2	20.2 (21.2)	24.1 (25.3) **
# of times sex with partner in project <u>without</u> a condom in the last 3-months (mean, SD)	3.0	2.0	7.3	3.9**	13.1	2.5 (8.1) **	4.8 (15.6) **
Sex with study partner <u>with</u> a condom in past 3-months	10,253	5,839	88.2%	4414	87.5%	1803 (88.7%)	2611 (86.6%)
Sex with study partner <u>without</u> a condom in past 3-months	3,583	1867	52.1%	1716	47.9%	619 (30.5%)**	1097 (36.4%)**
Sperm present on wet prep	336	158	2%	178	3.7**	67 (3.3%)**	111 (4.0%)
Outside sex partner (woman)	129	52	1%	77	1.7%**	33 (1.7%)**	44 (1.7%)
Pregnant during interval	770	330	6%	440	10.1%**	223 (12.0%)**	217 (8.7%)**

**p<0.01, *p=<0.05

Note: p-values compare (1) couples in which the woman doesn't want a child with wanting a child for all women, (2) HIV negative women in M+/F- couples who want a child vs. women who do not, and (3) HIV positive women in M-/F+ couples in which women want a child vs. women who do not want a child

Table 4.2 Descriptive analyses of baseline and time-varying covariates by fertility intention of men (n=1029)

	Total	Man doesn't want child		Man wants child in coming 12-months or beyond		Man wants child (male HIV+/Female HIV-)	Man wants child (male HIV-/Female HIV+)
Total	**1029**	**503**	**52.6%**	**526**	**51.0%**	**205 (45.2)**	**321 (55.8)**
Demographics							
Man age (mean, SD)	35.1	38.1	8.2	33.0**	6.7	33.4 (6.6) **	32.7 (6.6) **
Age disparity (mean, SD)	6.8	7.1	4.9	6.4*	4.3	7.2 (4.3)	6.0 (4.2) *
Years cohabiting (mean, SD)	6.8	9.3	7.2	5.2**	4.4	5.5 (4.5) **	4.9 (4.1) **
Monthly family income (USD) (mean, SD)	90.2	85.3	102.3	80.8	97.9	92.9 (109.1)	76.5 (101.2)
Man reads Nyanja	443 (43%)	236	47%	207**	39%	76 (37.1%)**	131 (40.9%)
Man alcohol use last year	185 (18%)	94	19%	91	17%	38 (18.5%)	53 (16.5%)
Family planning and sexual history							
# previous pregnancies (mean, SD)	3.4	4.1	2.4	2.7**	1.9	2.5 (1.7) **	2.7 (1.9) **
# live children (mean, SD)	1.7	2.2	1.8	1.2**	1.2	1.4 (1.3) **	0.9 (1.0) **
Pregnant at baseline	168 (16%)	122	12%	46**	5%	35 (17.1%)**	41 (12.8%)
Time varying: Clinical characteristics & sexual behaviors (n=intervals)							
Genital inflammation in past 3 months (man)	412	98	4%	314	4%	100 (6.5%)	96 (2.9%)*
Genital ulcer in past 3 months (man)	781	125	6.0%	656	8%	211 (13.7%)*	253 (8.01%)
# of times sex with partner in project <u>with</u> a condom in the last 3-months (mean, SD)	20.2	22.6	24.5	21.0**	21.2	20.1 (21.2) *	24.2 (25.1) **
# of times sex with partner in project <u>without</u> a condom in the last 3-months (mean, SD)	3.0	1.9	7.4	3.6**	11.5	2.5 (8.1) **	4.5 (13.6) **
Sex with study partner <u>with</u> a condom in past 3-months	8927	4333	88%	4594	87%	1851 (87.6%)	2886 (86.9%)
Sex with study partner <u>without</u> a condom in past 3-months	3087	1332	27%	1755**	33%	574 (27.2%)*	1241 (37.4%)**
Outside sex partner (man)	554	225	5%	329**	7%	61 (4.4%)	268 (8.2%)**

**p<0.01, *p=<0.05

Note: p-values compare (1) couples in which the man doesn't want a child with wanting a child for all men, (2) HIV negative men in M+/F- couples who want a child vs. men who do not, and (3) HIV positive men in M-/F+ couples in which men want a child vs. men who do not

Table 4.3 Clinical and behavioral predictors of fertility intentions among HIV serodiscordant men and women (n=730 couples in women's model, n=732 couples in men's model)

	Crude OR (95% CI)	aOR (95% CI)
Model 1: Women who want (more) children		
Woman age (per year increase)	0.91 (0.90, 0.93)	0.95 (0.91, 0.99)
Man age (per year increase)	0.93 (0.92, 0.95)	1.02 (0.98, 1.06)
Woman can read Nyanja	0.73 (0.56, 0.94)	0.85 (0.57, 1.28)
Years cohabiting	0.90 (0.88, 0.92)	1.03 (0.98, 1.08)
Man wants (more) children	2.81 (2.66, 2.97)	2.79 (1.97, 3.95)
Pregnant at baseline	0.72 (0.68, 0.77)	0.21 (0.12, 0.37)
Number of live children	0.63 (0.52, 0.71)	0.75 (0.62, 0.90)
HIV status (woman HIV+)	0.70 (0.56 (0.87)	0.84 (0.59, 1.19)
Number of previous pregnancies	0.72 (0.68, 0.77)	0.87 (0.61, 0.98)
Model 2: Men who want (more) children)		
Man age (per year increase)	0.91 (0.90, 0.93)	0.88 (0.80, 0.97)
Woman age (per year increase)	0.90 (0.88, 0.92)	1.00 (0.95, 1.04)
Man can read Nyanja	0.73 (0.57, 0.94)	0.87 (0.62, 1.21)
Years cohabiting	0.89 (0.87, 0.91)	0.95 (0.90, 1.00)
Woman wants (more) children	2.54 (2.42, 2.67)	2.83 (2.00, 4.01)
Partner pregnant at baseline	0.72 (0.67, 0.77)	0.91 (0.56, 1.48)
Number of live children	0.62 (0.56, 0.70)	0.84 (0.70, 1.01)
HIV status (man HIV+)	0.65 (0.51, 0.83)	0.93 (0.66, 1.31)
Number of previous pregnancies	0.72 (0.67, 0.77)	0.95 (0.85, 1.05)

Figure 4.2a. Forest plot of marginal structural model results of effect of fertility intentions on HIV acquisition in women (n=540 M+F- couples, 87 female incident infections) adjusting for age, literacy, and # of previous pregnancies

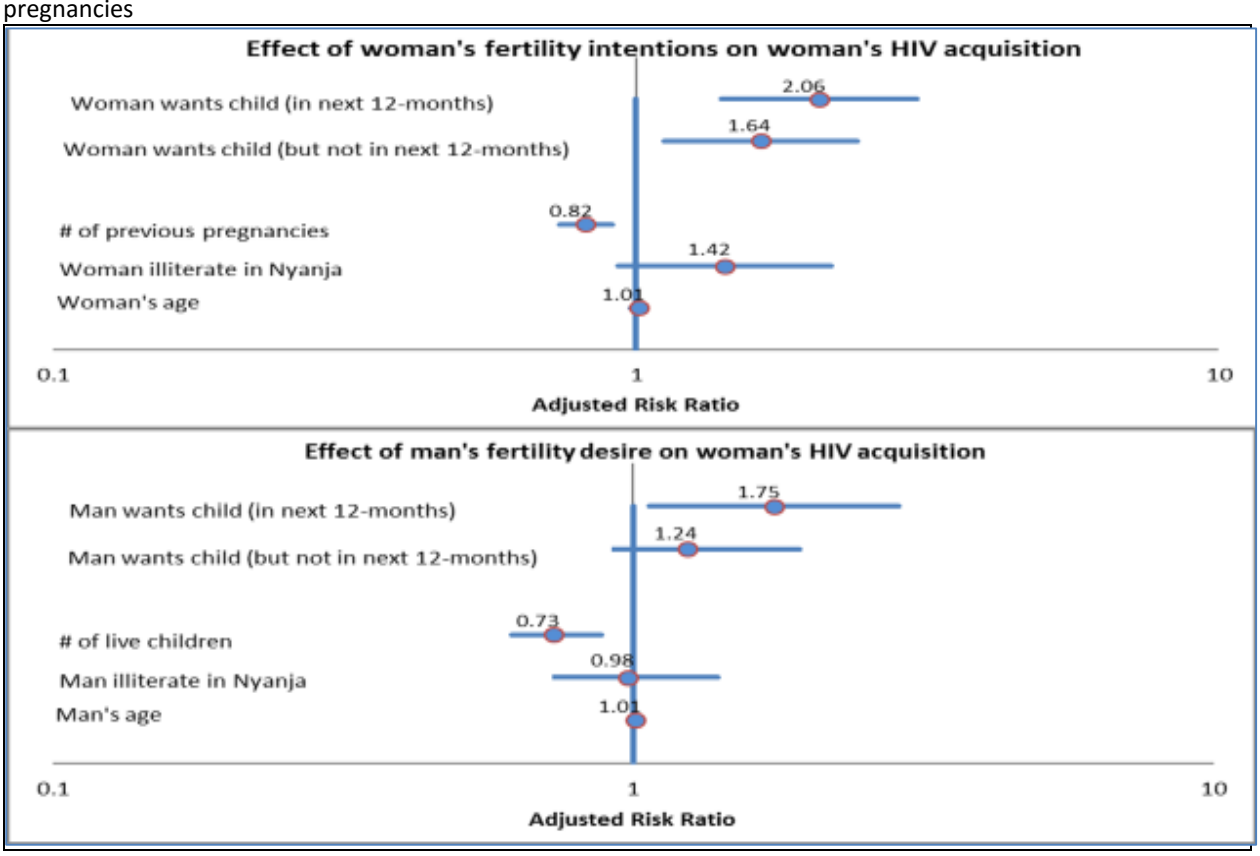


Figure 4.2b. Forest plot of marginal structural model result of the effect of fertility intentions on HIV acquisition in men (n=743 M-F+ couples, 95 male incident infections) adjusting for age, literacy, income and # of previous pregnancy/live children, and male circumcision status

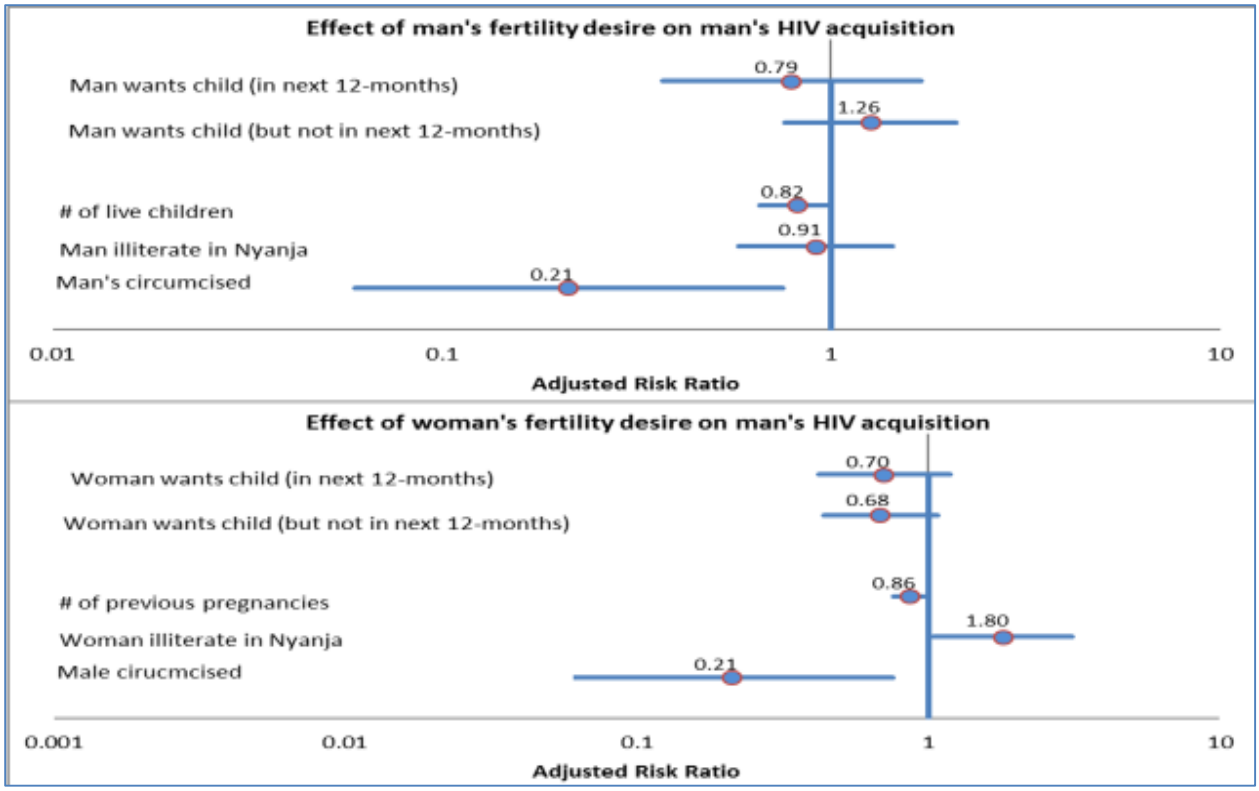
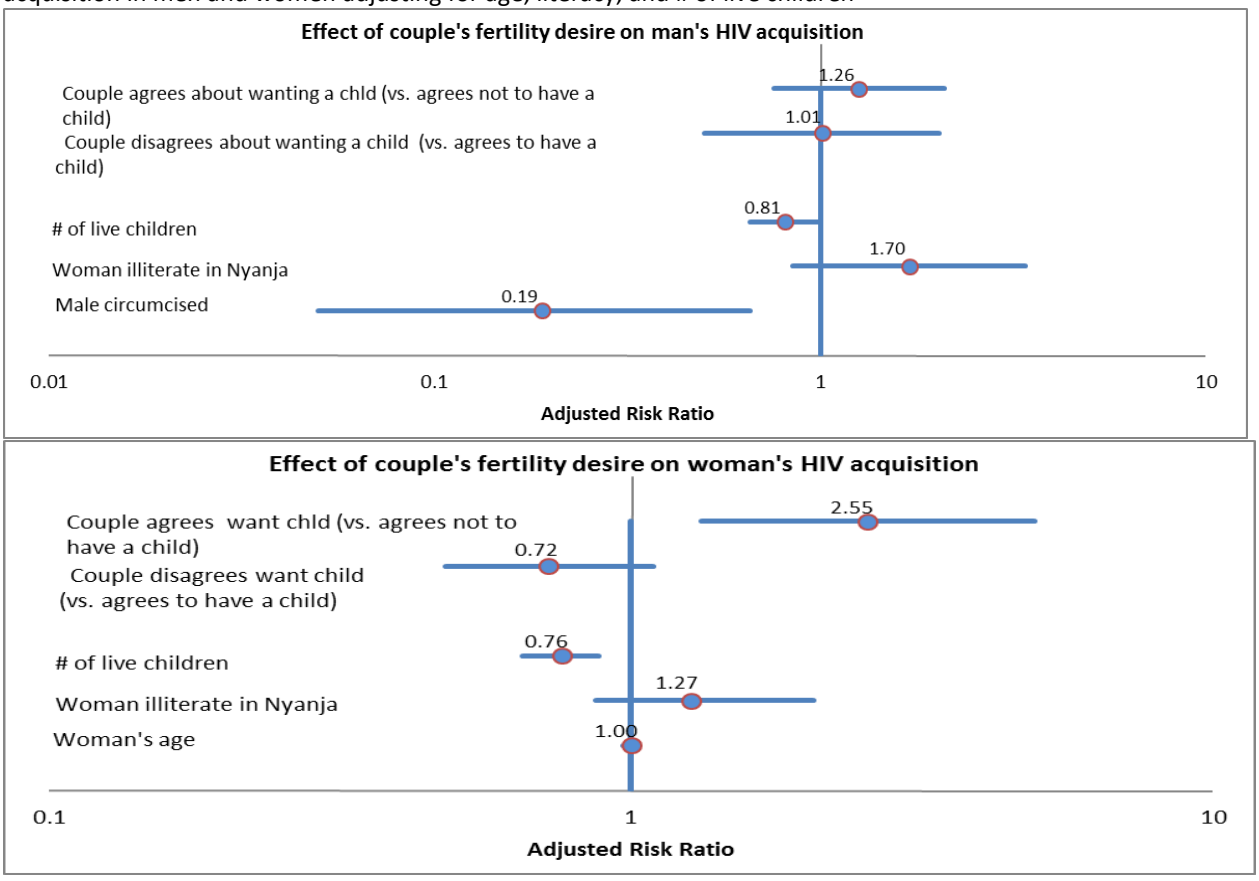


Figure 4.2c. Forest plot of marginal structural model result of the effect of couple's fertility intentions on HIV acquisition in men and women adjusting for age, literacy, and # of live children



Chapter V. Public health importance

Despite increased access to and uptake of ART, HIV incidence among HIV serodiscordant couples remains high in sub-Saharan Africa [4, 5]. Together, our studies found that heavy alcohol use, fertility desire, and having outside sex partners were important risk factors for HIV acquisition and transmission among serodiscordant couples in Zambia.

Importantly, women had increased risk of HIV acquisition when she or her partner was a heavy drinker. Our findings suggest the use of alcohol may act as a counter-factor in secondary HIV prevention – offsetting the counseling that women, men and couples may receive by care providers. The risk of heavy alcohol consumption needs to be managed by health care providers to help ensure that those who are heavy drinkers access treatment and care for potential alcoholism and recognize the challenge of this for those living with heavy drinkers. More research is needed on how to address the risk of alcohol use, both chronic heavy alcohol drinking, and binge drinking, in African settings with high HIV prevalence.

Secondly, greater understanding of associations between HIV-negative partners' behaviors with primary and outside partners and HIV acquisition will be important for the design of HIV-prevention strategies for uninfected partners. Our findings provide further evidence HIV-negative partners in serodiscordant couples may seek outside partners who they perceive to be at lower risk of HIV. HIV-negative partners in discordant couples should also be regularly screened for STIs, and other non-sexually transmitted causes of genital inflammation in women, such as bacterial vaginosis or candida. Screening and prophylactic treatment will help reduce inflammation and ulceration, which will reduce the risk of HIV acquisition and secondary transmission. We also bring attention to the role of heavy alcohol use by males and females in acquisition of HIV, especially acquisition of HIV from an outside partner.

Thirdly, optimizing delivery of HIV risk reduction strategies during peri-conception periods (i.e. safer conception) requires understanding how HIV serodiscordant couples approach fertility decisions. These data highlight the importance of pregnancy planning and safer conception services as a critical, but oftentimes neglected, component of HIV care and treatment. It is important that couples receive HIV counseling and testing together to identify when they are serodiscordant. Then our study demonstrated that the partner has an important say on whether or when couples try to get pregnant. Safer conception programs should therefore include both partners, and consider the relationship during risk reduction counseling and when recommending risk reduction interventions.

Finally, despite several advantages to bringing couples together to learn how to protect themselves, their sex partners, and newborn children from the risk of HIV infection, most interventions are designed for individuals or groups, not for dyads. A recent meta-analysis evaluated the effect of couples vs. individual level interventions, and found that couple-level interventions were more effective at increasing protective sex, HIV testing, and Nevirapine uptake [117]. The evidence demonstrated the usefulness of couple-based interventions in protecting individuals, partners, and new-born children from the risk of HIV transmission and infection. By counseling couples together, and making them more aware that they may be at increased risk of transmitting or acquiring HIV when either partner consumes alcohol, the rate of HIV transmission among serodiscordant couples may decrease and general well-being of this population increase. Further, ART adherence may also improve by ensuring that HIV-positive partners disclose their status, and gaining support to curb or stop heavy alcohol use. We strongly advocate for improved interventions to bring couples – heterosexual, homosexual, young, and old—together for HIV counseling, testing, and treatment to ensure that they receive support to get diagnosed, but also

supported in making decisions about how to have children safely, how to prevent HIV acquisition in a couple in which one or both partners drink heavily, and how best to protect themselves, their partners, and newborn children from the risk of HIV infection.

Chapter VI. References

1. Dunkle KL, Stephenson R, Karita E, Chomba E, Kayitenkore K, Vwalika C, Greenberg L, Allen S. New heterosexually transmitted HIV infections in married or cohabiting couples in urban Zambia and Rwanda: an analysis of survey and clinical data. Lancet, 2008. 371(9631): p. 2183-91.
2. Allen S, Meinzen-Derr J, Kautzman M, Zulu I, Trask S, Fideli U, Musonda R, Kasolo F, Gao F, Haworth A. Sexual behavior of HIV discordant couples after HIV counseling and testing. AIDS, 2003. 17(5): p. 733-40.
3. Matovu, J.K., Preventing HIV transmission in married and cohabiting HIV-discordant couples in sub-Saharan Africa through combination prevention. Curr HIV Res, 2010. 8(6): p. 430-40
4. Chemaitelly H, Awad SF, Shelton JD, Abu-Raddad LJ Sources of HIV incidence among stable couples in sub-Saharan Africa. J Int AIDS Soc, 2014. 17: p. 18765.
5. Chemaitelly, H., S.F. Awad, and L.J. Abu-Raddad, The risk of HIV transmission within HIV sero-discordant couples appears to vary across sub-Saharan Africa. Epidemics, 2014. 6: p. 1-9.
6. Polis, C.B. and K.M. Curtis, Use of hormonal contraceptives and HIV acquisition in women: a systematic review of the epidemiological evidence. Lancet Infect Dis, 2013. 13(9): p. 797-808.
7. Polis, C.B., S.J. Phillips, and K.M. Curtis, Hormonal contraceptive use and female-to-male HIV transmission: a systematic review of the epidemiologic evidence. Aids, 2013. 27(4): p. 493-505.
8. Haddad LB, Polis CB, Sheth AN, Brown J, Kourtis AP, King C, Chakraborty R, Ofotokun I. Contraceptive Methods and Risk of HIV Acquisition or Female-to-Male Transmission. Curr HIV/AIDS Rep, 2014.
9. Kinuthia J, Kiarie JN, Farquhar C, Richardson B, Nduati R, Mbori-Ngacha D, John-Stewart G. Cofactors for HIV incidence during pregnancy and postpartum period. Curr HIV Res, 2010. 8(7): p. 510-4.
10. Mugo NR, Heffron R, Donnell D, Wald A, Were EO, Rees H, Celum C, Kiarie JN, Cohen CR, Kayintekore K, Baeten JM; Partners in Prevention HSV/HIV Transmission Study Team. Increased risk of HIV transmission in pregnancy: a prospective study among African HIV-serodiscordant couples. Aids, 2011. 25(15): p. 1887-95.
11. Marston M, Newell ML, Crampin A, Lutalo T, Musoke R, Gregson S, Nyamukapa C, Nakiyingi-Miiro J, Urassa M, Isingo R, Zaba B. Is the risk of HIV acquisition increased during and immediately after pregnancy? A secondary analysis of pooled HIV community-based studies from the ALPHA network. PLoS One, 2013. 8(12): p. e82219.
12. Keating MA, Hamela G, Miller WC, Moses A, Hoffman IF, Hosseinipour MC High HIV incidence and sexual behavior change among pregnant women in Lilongwe, Malawi: implications for the risk of HIV acquisition. PLoS One, 2012. 7(6): p. e39109.
13. Tanser F, Bärnighausen T, Hund L, Garnett GP, McGrath N, Newell ML. Effect of concurrent sexual partnerships on rate of new HIV infections in a high-prevalence, rural South African population: a cohort study. Lancet, 2011. 378(9787): p. 247-55.
14. Shelton, J.D., A tale of two-component generalised HIV epidemics. Lancet, 2010. 375(9719): p. 964-6.
15. Mah, T.L. and J.D. Shelton, Concurrency revisited: increasing and compelling epidemiological evidence. J Int AIDS Soc, 2011. 14: p. 33.
16. Mah, T.L. and D.T. Halperin, Concurrent sexual partnerships and the HIV epidemics in Africa: evidence to move forward. AIDS Behav, 2010. 14(1): p. 11-6; dicussion 34-7.
17. Halperin, D.T. and H. Epstein, Concurrent sexual partnerships help to explain Africa's high HIV prevalence: implications for prevention. Lancet, 2004. 364(9428): p. 4-6.
18. Ndase P, Celum C, Thomas K, Donnell D, Fife KH, Bukusi E, Delany-Moretlwe S, Baeten JM; Partners in Prevention HSVHIV Transmission Study Team. Outside sexual partnerships and risk of HIV acquisition for HIV uninfected partners in African HIV serodiscordant partnerships. J Acquir Immune Defic Syndr, 2012. 59(1): p. 65-71.

19. Seeley J, Nakiyingi-Miiro J, Kamali A, Mpendo J, Asiki G, Abaasa A, De Bont J, Nielsen L, Kaleebu P; CHIVTUM Study Team. High HIV incidence and socio-behavioral risk patterns in fishing communities on the shores of Lake Victoria, Uganda. Sex Transm Dis, 2012. 39(6): p. 433-9.
20. Hahn, J.A., S.E. Woolf-King, and W. Muyindike, Adding fuel to the fire: alcohol's effect on the HIV epidemic in Sub-Saharan Africa. Curr HIV/AIDS Rep, 2011. 8(3): p. 172-80.
21. Coldiron ME[1], Stephenson R, Chomba E, Vwalika C, Karita E, Kayitenkore K, Tichacek A, Isanhart L, Allen S, Haworth A. The relationship between alcohol consumption and unprotected sex among known HIV-discordant couples in Rwanda and Zambia. AIDS Behav, 2008. 12(4): p. 594-603.
22. Loganantharaj N E, Wandiembe S, Abaasa A, Bwanika AN, Bahemuka U, Amornkul P, Price MA, Grosskurth H, Kamali A. HIV incidence and risk factors for acquisition in HIV discordant couples in Masaka, Uganda: an HIV vaccine preparedness study. PLoS One, 2011. 6(8): p. e24037.
23. Harling G, Newell ML, Tanser F, Kawachi I, Subramanian SV, Bärnighausen T Do age-disparate relationships drive HIV incidence in young women? Evidence from a population cohort in rural KwaZulu-Natal, South Africa. J Acquir Immune Defic Syndr, 2014. 66(4): p. 443-51.
24. WHO, National AIDS Programmes: A Guide to Indicators for Monitoring and Evaluating National HIV/AIDS Prevention Programmes for Young People. , 2004, World Health Organization: Geneva, Switzerland.
25. Biraro S, Ruzagira E, Kamali A, Whitworth J, Grosskurth H, Weiss HA. HIV transmission within marriage in rural Uganda: a longitudinal study. PLoS One, 2013. 8(2): p. e55060.
26. Anglemyer A, Horvath T, Rutherford G. Antiretroviral therapy for prevention of HIV transmission in HIV-discordant couples. Cochrane Database Syst Rev, 2011(5): p. Cd009153.
27. Jia Z, Mao Y, Zhang F, Ruan Y, Ma Y, Li J, Guo W, Liu E, Dou Z, Zhao Y, Wang L, Li Q, Xie P, Tang H, Han J, Jin X, Xu J, Xiong R, Zhao D, Li P, Wang X, Wang L, Qing Q, Ding Z, Chen RY, Liu Z, Shao Y. Antiretroviral therapy to prevent HIV transmission in serodiscordant couples in China (2003-11): a national observational cohort study. Lancet, 2013. 382(9899): p. 1195-203.
28. Lefevre F, O'Leary B, Moran M, Mossar M, Yarnold PR, Martin GJ, Glassroth J. Alcohol consumption among HIV-infected patients. Journal of General Internal Medicine 10(8):458–460, 1995. PMID: 7472704
29. Pandrea, I.; Happel, K.I.; Amedee, A.M.; Bagby, G.J.; and Nelson, S. Alcohol's role in HIV transmission and disease progression. Alcohol Research & Health 33(3):203–218, 2010.
30. Needle RH, Coyle SL, Normand J, Lambert E, Cesari H. HIV prevention with drug-using populations — current status and future prospects: Introduction and overview. Public Health Reports 113(Suppl 1):4–18, 1998. PMID: 9722806
31. Braithwaite, R.S., and Bryant, K. Influence of alcohol consumption on adherence to and toxicity of antiretroviral therapy and survival. Alcohol Research & Health 33(3):280–287. 2010.
32. Bagby GJ, Stoltz DA, Zhang P, Kolls JK, Brown J, Bohm RP Jr, Rockar R, Purcell J, Murphey-Corb M, Nelson S. The effect of chronic binge ethanol consumption on the primary stage of SIV infection in rhesus macaques. Alcoholism: Clinical and Experimental Research 27:495–502, 2003. PMID: 12658116
33. Loganantharaj N[1], Nichols WA, Bagby GJ, Volaufova J, Dufour J, Martin DH, Nelson S, Amedee AM. "The effects of chronic binge alcohol on the genital microenvironment of SIV-infected female rhesus macaques" AIDS Res Hum Retroviruses, 2014 Jun 6. PMID: 24902876
34. Mugo NR, Heffron R, Donnell D, Wald A, Were EO, Rees H, Celum C, Kiarie JN, Cohen CR, Kayintekore K, Baeten JM; Partners in Prevention HSV/HIV Transmission Study Team. Increased risk of HIV transmission in pregnancy: a prospective study among African HIV-serodiscordant couples. Aids, 2011. 25(15): p. 1887-95.
35. Marston M[1], Newell ML[2], Crampin A[3], Lutalo T[4], Musoke R[4], Gregson S[5], Nyamukapa C[5], Nakiyingi-Miiro J[6], Urassa M[7], Isingo R[7], Zaba B[1]. Is the risk of HIV acquisition increased during and immediately after pregnancy? A secondary analysis of pooled HIV community-based studies from the ALPHA network. PLoS One, 2013. 8(12): p. e82219.

36. Keating MA[1], Hamela G, Miller WC, Moses A, Hoffman IF, Hosseinipour MC. High HIV incidence and sexual behavior change among pregnant women in Lilongwe, Malawi: implications for the risk of HIV acquisition. PLoS One, 2012. 7(6): p. e39109.
37. Tanser F[1], Bärnighausen T, Hund L, Garnett GP, McGrath N, Newell ML. Effect of concurrent sexual partnerships on rate of new HIV infections in a high-prevalence, rural South African population: a cohort study. Lancet, 2011. 378(9787): p. 247-55.
38. Shelton, J.D., A tale of two-component generalised HIV epidemics. Lancet, 2010. 375(9719): p. 964-6.
39. Mah, T.L. and J.D. Shelton, Concurrency revisited: increasing and compelling epidemiological evidence. J Int AIDS Soc, 2011. 14: p. 33.
40. Mah, T.L. and D.T. Halperin, Concurrent sexual partnerships and the HIV epidemics in Africa: evidence to move forward. AIDS Behav, 2010. 14(1): p. 11-6; dicussion 34-7.
41. Halperin, D.T. and H. Epstein, Concurrent sexual partnerships help to explain Africa's high HIV prevalence: implications for prevention. Lancet, 2004. 364(9428): p. 4-6.
42. Ndase P, Celum C, Thomas K, Donnell D, Fife KH, Bukusi E, Delany-Moretlwe S, Baeten JM; Partners in Prevention HSVHIV Transmission Study Team. Outside sexual partnerships and risk of HIV acquisition for HIV uninfected partners in African HIV serodiscordant partnerships. J Acquir Immune Defic Syndr, 2012. 59(1): p. 65-71.
43. http://www.aidsinfoonline.org/devinfo/libraries/aspx/Home.aspx
44. Campbell MS, Mullins JI, Hughes JP, Celum C, Wong KG, Raugi DN, Sorensen S, Stoddard JN, Zhao H, Deng W, Kahle E, Panteleeff D, Baeten JM, McCutchan FE, Albert J, Leitner T, Wald A, Corey L, Lingappa JR; Partners in Prevention HSV/HIV Transmission Study Team. Viral linkage in HIV-1 seroconverters and their partners in an HIV-1 prevention clinical trial. PLoS One. 2011 Mar 2;6(3):e16986. doi: 10.1371/journal.pone.0016986. PubMed PMID: 21399681; PubMed Central PMCID: PMC3047537.
45. Zambian Guidelines on Antiertroviral Therapy, Ministry of Health, Zambia, 2013.
46. Hahn, J.A., S.E. Woolf-King, and W. Muyindike, Adding fuel to the fire: alcohol's effect on the HIV epidemic in Sub-Saharan Africa. Curr HIV/AIDS Rep, 2011. 8(3): p. 172-80.
47. Coldiron ME[1], Stephenson R, Chomba E, Vwalika C, Karita E, Kayitenkore K, Tichacek A, Isanhart L, Allen S, Haworth A. The relationship between alcohol consumption and unprotected sex among known HIV-discordant couples in Rwanda and Zambia. AIDS Behav, 2008. 12(4): p. 594-603.
48. Ruzagira E, Wandiembe S, Abaasa A, Bwanika AN, Bahemuka U, Amornkul P, Price MA, Grosskurth H, Kamali A. HIV incidence and risk factors for acquisition in HIV discordant couples in Masaka, Uganda: an HIV vaccine preparedness study. PLoS One, 2011. 6(8): p. e24037.
49. Harling G[1], Newell ML, Tanser F, Kawachi I, Subramanian SV, Bärnighausen T. Do age-disparate relationships drive HIV incidence in young women? Evidence from a population cohort in rural KwaZulu-Natal, South Africa. J Acquir Immune Defic Syndr, 2014. 66(4): p. 443-51.
50. WHO, National AIDS Programmes: A Guide to Indicators for Monitoring and Evaluating National HIV/AIDS Prevention Programmes for Young People. , 2004, World Health Organization: Geneva, Switzerland.
51. Biraro S[1], Ruzagira E, Kamali A, Whitworth J, Grosskurth H, Weiss HA., HIV transmission within marriage in rural Uganda: a longitudinal study. PLoS One, 2013. 8(2): p. e55060
52. Anglemyer A[1], Horvath T, Rutherford G. Antiretroviral therapy for prevention of HIV transmission in HIV-discordant couples. Cochrane Database Syst Rev, 2011(5): p. Cd009153.
53. Jia Z[1], Mao Y, Zhang F, Ruan Y, Ma Y, Li J, Guo W, Liu E, Dou Z, Zhao Y, Wang L, Li Q, Xie P, Tang H, Han J, Jin X, Xu J, Xiong R, Zhao D, Li P, Wang X, Wang L, Qing Q, Ding Z, Chen RY, Liu Z, Shao Y. Antiretroviral therapy to prevent HIV transmission in serodiscordant couples in China (2003-11): a national observational cohort study. Lancet, 2013. 382(9899): p. 1195-203.
54. UNAIDS Report on the Global AIDS Epidemic, 2013. http://www.unaids.org/sites/default/files/en/media/unaids/contentassets/documents/epidemiology/2013/gr2013/UNAIDS_Global_Report_2013_en.pdf

55. Kalichman SC, Simbayi LC, Kaufman M, Cain D, Jooste S.. Alcohol use and sexual risks for HIV/AIDS in Sub-Saharan Africa: Systematic Review of Empirical Findings. Prev Sci (2007) 8:141-151.
56. Berhan, Y. and A. Berhan. Meta analysis of fertility desires of people living with HIV. BMC Public Health 13 (1): 409.
57. Cook R, Hayden R, Weiss SM, Jones DL. Desire for fertility among HIV-seroconcordant and discordant couples in Lusaka, Zambia. Culture, Health and Sexuality. 2014. Vol. 16, No 7, 74-751.
58. Kempf MC, Allen S, Zulu I, Kancheya N, Stephenson R, Brill I, Tichacek A, Haworth A, Chomba E; Rwanda Zambia HIV Research Group. Enrollment and retention of HIV discordant couples in Lusaka, Zambia. J Acquir Immune Defic Syndr, 2008. 47(1): p. 116-25.
59. Li X, Price MA, He D, Kamali A, Karita E, Lakhi S, Sanders EJ, Anzala O, Amornkul PN, Allen S, Hunter E, Kaslow RA, Gilmour J, Tang J; IAVI Africa HIV Prevention Partnership. Host genetics and viral load in primary HIV infection: clear evidence for gene by sex interactions. Hum Genet, 2014. 133(9): p. 1187-97.
60. Prentice HA[1], Pajewski NM[2], He D[1], Zhang K[3], Brown EE[1], Kilembe W[4], Allen S[5], Hunter E[6], Kaslow RA[1], Tang J[7]. Host genetics and immune control of HIV infection: fine mapping for the extended human MHC region in an African cohort. Genes Immun, 2014. 15(5): p. 275-81.
61. Bunnell RE[1], Nassozi J, Marum E, Mubangizi J, Malamba S, Dillon B, Kalule J, Bahizi J, Musoke N, Mermin JH. Living with discordance: knowledge, challenges, and prevention strategies of HIV-discordant couples in Uganda. AIDS Care. 2005; 17:999-1012.
62. Stephenson, R., Vwalika, B., Greenberg, L., Ahmed, Y., Vwalika, C., Chomba, E., Tichacek, A., Allen, S. (2011). A Randomized-Control Trial to Promote Long-Term Contraceptive Use Among HIV Sero-Discordant and Concordant Positive Couples in Zambia. Journal of Women's Health, 20(3).
63. Merino, A., Malhotra, R., Morton, M., Mulenga, J., Allen, S., Hunter, E., Tang, J., Kaslow, R.A. (2011). Impact of a Functional KIR2DS4 Allele on Heterosexual HIV Transmission among Discordant Zambian Couples. J Infect Dis
64. Price, M.A., Wallis, C.L., Lakhi, S., Karita, E., Kamali, A., Anzala, O., Sanders, E.J., Bekker, L.G., Twesigye, R., Hunter, E., Kaleebu, P., Kayitenkore, K., Allen, S., Ruzagira, E., Mwangome, M., Mutua, G., Amornkul, P.N., Stevens, G., Pond, S.L., Schaefer, M., Papathanasopoulos, M.A., Stevens, W., Gilmour, J. (2011). Transmitted HIV Type 1 Drug Resistance Among Individuals with Recent HIV Infection in East and Southern Africa. AIDS Res Hum Retroviruses, 27(1), 5-12.
65. Conkling, M., Shutes, E.L., Karita, E., Chomba, E., Tichacek, A., Sinkala, M., Vwalika, B., Iwanowski, M., Allen, S.A. (2010). Couples' voluntary counselling and testing and nevirapine use in antenatal clinics in two African capitals: a prospective cohort study. J Int AIDS Soc, 13, 10. PMCID: 2851580.
66. Gorbach PM, Holmes KK. Transmission of STIs/HIV at the partnership level: beyond individual level analyses. J Urban Health. 2003;80(4 Suppl 3):iii15-25.
67. Dionne-Odom J[1], Karita E, Kilembe W, Henderson F, Vwalika B, Bayingana R, Li Z, Mulenga J, Chomba E, Del Rio C, Khu NH, Tichacek A, Allen S. Syphilis treatment response among HIV-discordant couples in Zambia and Rwanda. Clin Infect Dis, 2013. 56(12): p. 1829-37
68. Hsieh, F.Y. and Lavori, P.W. 2000. 'Sample-Size Calculations for the Cox Proportional Hazards Regression Model with Nonbinary Covariates', Controlled Clinical Trials, Volume 21, pages 552-560.
69. Schoenfeld, David A. 1983. 'Sample-Size Formula for the Proportional-Hazards Regression Model', Biometrics, Volume 39, pages 499-503.
70. Wechsberg WM[1], Zule WA, Ndirangu J, Kline TL, Rodman NF, Doherty IA, Novak SP, van der Horst CM. The biobehavioral Women's Health CoOp in Pretoria, South Africa: study protocol for a cluster-randomized design. BMC Public Health. 2014 Oct 15;14:1074. doi: 10.1186/1471-2458-14-1074.
71. Hernan,MA, Brumback BA, Robins JM. Estimating the causal effect of zidovudine on CD4 count with a marginal structural model for repeated measures. Statist. Med. 2002; 21:1689–1709.
72. Hintze, J. 2008. PASS 2008. NCSS, LLC. Kaysville, Utah. www.ncss.com.
73. Yeganeh N, Dillavou C, Simon M, et. al. Audio computer-assisted survey instrument versus face-to-face interviews: optimal method for detecting high-risk behaviour in pregnant women and their sexual

partners in the south of Brazil. Int J STD AIDS. 2013 Apr;24(4):279-85. doi: 10.1177/0956462412472814. Epub 2013 Jun 18.
74. Gorbach PM, Mensch BS, Husnik M, et al. Effect of computer-assisted interviewing on self-reported sexual behavior data in a microbicide clinical trial. AIDS Behav. 2013 Feb;17(2):790-800. doi: 10.1007/s10461-012-0302-2.
75. Langhaug LF, Cheung YB, Pascoe SJ, et al. How you ask really matters: randomised comparison of four sexual behaviour questionnaire delivery modes in Zimbabwean youth. Sex Transm Infect. 2011 Mar;87(2):165-73. doi: 10.1136/sti.2009.037374. Epub 2010 Oct 13.
76. Lingappa JR, Kahle E, Mugo N, Mujugira A, Magaret A, et al. (2009) Characteristics of HIV-1 Discordant Couples Enrolled in a Trial of HSV-2 Suppression to Reduce HIV-1 Transmission: The Partners Study. PLoS ONE 4(4): e5272. doi: 10.1371/journal.pone.0005272.
77. Cohen MS, Chen YQ, McCauley M, et al. Prevention of HIV-1 infection with early antiretroviral therapy. N Engl J Med. 2011;365(6):493–505.
78. Cotton D. Life expectancy in Africa: back to the future? Annals of internal medicine. 2011;155(4):265–6.
79. Donnell D, Baeten JM, Kiarie J, et al. Heterosexual HIV-1 transmission after initiation of antiretroviral therapy: a prospective cohort analysis. Lancet. 2010;375(9731):2092–8.
80. Mills EJ, Bakanda C, Birungi J, et al. Life expectancy of persons receiving combination antiretroviral therapy in low-income countries: a cohort analysis from Uganda. Annals of internal medicine. 2011;155(4): 209–16.
81. Reynolds SJ, Makumbi F, Nakigozi G, et al. HIV-1 transmission among HIV-1 discordant couples before and after the introduction of antiretroviral therapy. AIDS. 2011;25(4):473–7.
82. Sullivan P, Kayitenkore K, Chomba E, et al. Is the reduction of HIV transmission risk while prescribed antiretroviral therapy (ARVT) different for men and women? Results from discordant couples in Rwanda and Zambia. V International AIDS Society Conference on HIV Pathogenesis, Treatment and Prevention.; Cape Town, South Africa. July 19-22, 2009.
83. Baeten, J.M., Donnell, D., Ndase, P., et al. Antiretroviral Prophylaxis for HIV Prevention in Heterosexual Men and Women. N Engl J Med 2012;367:399-410. doi: 10.1056/NEJMoa1108524
84. Ware, N.C., et al. What's love got to do with it? Explaining adherence to oral antiretroviral pre-exposure prophylaxis for HIV-serodiscordant couples. J Acquir Immune Defic Syndr. 2012 Apr 15;59(5):463-8.
85. Abdool Karim Q, Abdool Karim SS, Frohlich JA, et al. Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. Science.2010;329(5996):1168–74.
86. Mugo NR, Heffron R, Donnell D, et al. Increased risk of HIV-1 transmission in pregnancy: a prospective study among African HIV-1 serodiscordant couples. AIDS. 2011;25(15):1887–95
87. Kinuthia, J., et al., Cofactors for HIV-1 incidence during pregnancy and postpartum period. Curr HIV Res, 2010. 8(7): p. 510-4.
88. Keating, M.A., et al., High HIV incidence and sexual behavior change among pregnant women in Lilongwe, Malawi: implications for the risk of HIV acquisition. PLoS One, 2012. 7(6): p. e39109.
89. Marston, M., et al., Is the risk of HIV acquisition increased during and immediately after pregnancy? A secondary analysis of pooled HIV community-based studies from the ALPHA network. PLoS One, 2013. 8(12): p. e82219.
90. Central Statistical Office (CSO) [Zambia], Ministry of Health (MOH) [Zambia], and ICF International. 2014. Zambia Demographic and Health Survey 2013-14. Rockville, Maryland, USA: Central Statistical Office, Ministry of Health, and ICF International.
91. Stephenson R, Barker J, Cramer R, Hall MA, Karita E, Chomba E, Vwalika C, Allen S. The demographic profile of sero-discordant couples enrolled in clinical research in Rwanda and Zambia. AIDS Care. 2008 Mar;20(3):395-405. doi: 10.1080/09540120701593497. PubMed PMID: 18351489.

92. Wall K, Karita E, Nizam A, Bekan B, Sardar G, Casanova D, Joseph Davey D, De Clercq F, Kestelyn E, Bayingana R, Tichacek A, Allen S. Influence network effectiveness in promoting couples' HIV voluntary counseling and testing in Kigali, Rwanda. AIDS. 2012 Jan 14;26(2):217-27. doi: 10.1097/QAD.0b013e32834dc593. PubMed PMID: 22008653; PubMed Central PMCID: PMC3679893.
93. Wall KM, Kilembe W, Nizam A, Vwalika C, Kautzman M, Chomba E, Tichacek A, Sardar G, Casanova D, Henderson F, Mulenga J, Kleinbaum D, Allen S. Promotion of couples' voluntary HIV counselling and testing in Lusaka, Zambia by influence network leaders and agents. BMJ Open. 2012 Sep 6;2(5). pii: e001171. doi: 10.1136/bmjopen-2012-001171. Print 2012. PubMed PMID: 22956641; PubMed Central PMCID: PMC3467632.
94. Boeras DI, Luisi N, Karita E, McKinney S, Sharkey T, Keeling M, Chomba E, Kraft C, Wall K, Bizimana J, Kilembe W, Tichacek A, Caliendo AM, Hunter E, Allen S. Indeterminate and discrepant rapid HIV test results in couples' HIV testing and counselling centres in Africa. J Int AIDS Soc. 2011 Apr 8;14:18. doi: 10.1186/1758-2652-14-18. PubMed PMID: 21477317; PubMed Central PMCID: PMC3086828.
95. WHO (2001) AUDIT: The alcohol use disorders identification test: Guidelines for use in primary care (2nd ed.). Geneva: World Health Organization Deparment of Mental Health and Substance Dependence; 2001.
96. Allen, S., et al., Promotion of couples' voluntary counselling and testing for HIV through influential networks in two African capital cities. BMC Public Health, 2007. 7: p. 349.
97. Chomba, E., et al., Evolution of couples' voluntary counseling and testing for HIV in Lusaka, Zambia. J Acquir Immune Defic Syndr, 2008. 47(1): p. 108-15.
98. Myer L, Carter RJ, Katyal M, Toro P, El-Sadr WM, Abrams EJ. Impact of antiretroviral therapy on incidence of pregnancy among HIV-infected women in Sub-Saharan Africa: a cohort study. PLoS Med. 2010 Feb 9;7(2):e1000229. PubMed PMID: 20161723; PubMed Central PMCID: PMC2817715.
99. Zaba B, Calvert C, Marston M, Isingo R, Nakiyingi-Miiro J, Lutalo T, Crampin A, Robertson L, Herbst K, Newell ML, Todd J, Byass P, Boerma T, Ronsmans C. Effect of HIV infection on pregnancy-related mortality in sub-Saharan Africa: secondary analyses of pooled community-based data from the network for Analysing Longitudinal Population-based HIV/AIDS data on Africa (ALPHA).Lancet. 2013 May 18;381(9879):1763-71. doi: 10.1016/S0140-6736(13)60803-X. PubMed PMID: 23683643.
100. Kendall T, Bärnighausen T, Fawzi WW, Langer A. Towards comprehensive women's healthcare in sub-Saharan Africa: addressing intersections between HIV, reproductive and maternal health. J Acquir Immune Defic Syndr. 2014 Dec 1;67 Suppl 4:S169-72. doi: 10.1097/QAI.0000000000000382. PubMed PMID: 25436814; PubMed Central PMCID: PMC4251906.
101. Mantell JE, Exner TM, Cooper D, Bai D, Leu CS, Hoffman S, Myer L, Moodley J, Kelvin EA, Constant D, Jennings K, Zweigenthal V, Stein ZA. Pregnancy intent among a sample of recently diagnosed HIV-positive women and men practicing unprotected sex in Cape Town, South Africa. J Acquir Immune Defic Syndr. 2014 Dec 1;67 Suppl 4:S202-9. PubMed PMID: 25436819; PubMed Central PMCID: PMC4251915.
102. Schaan MM, Taylor M, Puvimanasinghe J, Busang L, Keapoletswe K, Marlink R. Sexual and reproductive health needs of HIV-positive women in Botswana - a study of health care worker's views. AIDS Care. 2012;24(9):1120-5. doi: 10.1080/09540121.2012.672814. Epub 2012 May 9. PubMed PMID: 22568549.

103. Beyeza-Kashesya J, Ekstrom AM, Kaharuza F, Mirembe F, Neema S, Kulane A. My partner wants a child: a cross-sectional study of the determinants of the desire for children among mutually disclosed sero-discordant couples receiving care in Uganda. BMC Public Health. 2010 May 13;10:247. doi: 10.1186/1471-2458-10-247. PubMed PMID: 20465794; PubMed Central PMCID: PMC2877675.
104. Carpenter LM, Kamali A, Ruberantwari A, Malamba SS, Whitworth JA. Rates of HIV-1 transmission within marriage in rural Uganda in relation to the HIV sero-status of the partners. AIDS. 1999 Jun 18;13(9):1083-9. PubMed PMID: 10397539
105. Lurie MN, Williams BG, Zuma K, Mkaya-Mwamburi D, Garnett GP, Sweat MD, Gittelsohn J, Karim SS. Who infects whom? HIV-1 concordance and discordance among migrant and non-migrant couples in South Africa. AIDS. 2003 Oct 17;17(15):2245-52. PubMed PMID: 14523282.
106. Ryder RW, Kamenga C, Jingu M, Mbuyi N, Mbu L, Behets F. Pregnancy and HIV-1 incidence in 178 married couples with discordant HIV-1 serostatus: additional experience at an HIV-1 counselling centre in the Democratic Republic of the Congo. Trop Med Int Health. 2000 Jul;5(7):482-7. PubMed PMID: 10964270.
107. Lingappa JR, Lambdin B, Bukusi EA, Ngure K, Kavuma L, Inambao M, Kanweka W, et al. Regional differences in prevalence of HIV-1 discordance in Africa and enrollment of HIV-1 discordant couples into an HIV-1 prevention trial. PLoS One. 2008 Jan 9;3(1):e1411. doi: 10.1371/journal.pone.0001411. PubMed PMID: 18183292; PubMed Central PMCID: PMC2156103.
108. Pintye J, Ngure K, Curran K, Vusha S, Mugo N, Celum C, Baeten JM, Heffron R. Fertility Decision-Making Among Kenyan HIV-Serodiscordant Couples Who Recently Conceived: Implications for Safer Conception Planning. AIDS Patient Care STDS. 2015 Sep;29(9):510-6. doi: 10.1089/apc.2015.0063. PubMed PMID: 26301703; PubMed Central PMCID: PMC4553369.
109. Schwartz SR, Mehta SH, Taha TE, Rees HV, Venter F, Black V. High pregnancy intentions and missed opportunities for patient-provider communication about fertility in a South African cohort of HIV-positive women on antiretroviral therapy. AIDS Behav. 2012 Jan;16(1):69-78. doi: 10.1007/s10461-011-9981-3. PubMed PMID: 21656145
110. Wall KM, Kilembe W, Vwalika B, Htee Khu N, Brill I, Chomba E, Johnson BA, Haddad L, Tichacek A, Allen S. Hormonal contraception does not increase women's HIV acquisition risk in Zambian discordant couples, 1994-2012. Contraception. 2015 Jun;91(6):480-7. doi: 10.1016/j.contraception.2015.02.004. Epub 2015 Feb 21. PubMed PMID: 25708502; PubMed Central PMCID: PMC4442041.
111. Sufa A, Wordofa MA, Wossen BA. Determinants of fertility intention among women living with hiv in western Ethiopia: implications for service delivery. Afr J Reprod Health. 2014 Dec;18(4):54-60. PubMed PMID: 25854093
112. Demissie DB, Tebeje B, Tesfaye T. Fertility desire and associated factors among people living with HIV attending antiretroviral therapy clinic in Ethiopia. BMC Pregnancy Childbirth. 2014 Nov 20;14:382. doi: 10.1186/s12884-014-0382-2. PubMed PMID: 25410125; PubMed Central PMCID: PMC4243275
113. Song W, He D, Brill I, Malhotra R, Mulenga J, Allen S, et al. Disparate associations of HLA class I markers with HIV-1 acquisition and control of viremia in an African population. PLoS One 2011; 6(8): e23469. doi: 10.1371/journal.pone.0023469. Epub 2011 Aug 17. PubMed PMID: 21858133; PubMed Central PMCID: PMC3157381.

114. Johnson, L.F. and D.A. Lewis, The effect of genital tract infections on HIV-1 shedding in the genital tract: a systematic review and meta-analysis. Sex Transm Dis, 2008. 35(11): p. 946-59. PubMed PMID: 18685546.
115. Mujugira A, Celum C, Thomas KK, Farquhar C, Mugo N, Katabira E, Bukusi EA, Tumwesigye E, Baeten JM; Partners PrEP Study Team. Delay of antiretroviral therapy initiation is common in East African HIV-infected individuals in serodiscordant partnerships. J Acquir Immune Defic Syndr. 2014 Aug 1;66(4):436-42. doi: 10.1097/QAI.0000000000000192. PubMed PMID: 24798765; PubMed Central PMCID: PMC4077929.
116. Allison, Paul D. "Survival Analysis using SAS: A Practical Guide." Second Edition, 2010. SAS Institute, Cary, NC.
117. Crepaz N, Tungol-Ashmon MV, Vosburgh HW, Baack BN, Mullins MM. Are couple-based interventions more effective than interventions delivered to individuals in promoting HIV protective behaviors? A meta-analysis. AIDS Care. 2015 Nov;27(11):1361-6. doi: 10.1080/09540121.2015.1112353. Epub 2015 Nov 25. PubMed PMID: 26608175; PubMed Central PMCID: PMC4693597
118. Lambdin BH, Kanweka W, Inambao M, Mwananyanda L, Shah HD, Linton S, Wong F, Luisi N, Tichacek A, Kalowa J, Chomba E, Allen S. Local residents trained as 'influence agents' most effective in persuading African couples on HIV counseling and testing. Health Aff (Millwood). 2011 Aug;30(8):1488-97. doi: 10.1377/hlthaff.2009.0994. PubMed PMID: 21821565; PubMed Central PMCID: PMC3503238.
119. Boeras DI, Luisi N, Karita E, McKinney S, Sharkey T, Keeling M, Chomba E, Kraft C, Wall K, Bizimana J, Kilembe W, Tichacek A, Caliendo AM, Hunter E, Allen S. Indeterminate and discrepant rapid HIV test results in couples' HIV testing and counselling centres in Africa. J Int AIDS Soc. 2011 Apr 8;14:18. doi: 10.1186/1758-2652-14-18. PubMed PMID: 21477317; PubMed Central PMCID: PMC3086828.
120. Barreiro, P., del Romero, J., Leal, M., Hernando, V., Asencio, R., de Mendoza, C., … Soriano, V. (2006). Natural pregnancies in HIV-serodiscordant couples receiving successful antiretroviral therapy. *Journal of Acquired Immune Deficiency Syndromes (1999)*, *43*(3), 324–326. doi:10.1097/01.qai.0000243091.40490.fd
121. Bujan, L., Hollander, L., Coudert, M., Gilling-Smith, C., Vucetich, A., Guibert, J., … Semprini, A. E. (2007). Safety and efficacy of sperm washing in HIV-1-serodiscordant couples where the male is infected: results from the European CREAThE network. *AIDS (London, England)*, *21*(14), 1909–1914. doi:10.1097/QAD.0b013e3282703879
122. Chen, W.-J., & Walker, N. (2010). Fertility of HIV-infected women: insights from Demographic and Health Surveys. *Sexually Transmitted Infections*, *86 Suppl 2*(Suppl 2), ii22–i27. doi:10.1136/sti.2010.043620
123. Fiore, J. R., Lorusso, F., Vacca, M., Ladisa, N., Greco, P., & De Palo, R. (2005). The efficiency of sperm washing in removing human immunodeficiency virus type 1 varies according to the seminal viral load. *Fertility and Sterility*, *84*(1), 232–234. doi:10.1016/j.fertnstert.2004.12.060
124. Grabbe, K., Stephenson, R., Vwalika, B., Ahmed, Y., Vwalika, C., Chomba, E., … Allen, S. (2009). Knowledge, use, and concerns about contraceptive methods among sero-discordant couples in Rwanda and Zambia. *Journal of Women's Health (2002)*, *18*(9), 1449–56. doi:10.1089/jwh.2008.1160

125. Marina, S., Marina, F., Alcolea, R., Expósito, R., Huguet, J., Nadal, J., & Vergés, a. (1998). Human immunodeficiency virus type 1--serodiscordant couples can bear healthy children after undergoing intrauterine insemination. *Fertility and Sterility*, *70*(1), 35–39
126. Matthews, L. T., Baeten, J. M., Celum, C., & Bangsberg, D. R. (2010). Periconception pre-exposure prophylaxis to prevent HIV transmission: benefits, risks, and challenges to implementation. *AIDS*, *24*(13), 1975–1982. doi:10.1097/QAD.0b013e32833bedeb
127. Matthews, L. T., Milford, C., Kaida, A., Ehrlich, M. J., Ng, C., Greener, R., … Smit, J. A. (2014). Lost Opportunities to Reduce Periconception HIV Transmission. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, *67*, S210–S217. doi:10.1097/QAI.0000000000000374
128. Semprini, A. E., Macaluso, M., Hollander, L., Vucetich, A., Duerr, A., Mor, G., … Jamieson, D. J. (2013). Safe conception for HIV-discordant couples: Insemination with processed semen from the HIV-infected partner. *American Journal of Obstetrics and Gynecology*, *208*(5), 402.e1–402.e9. doi:10.1016/j.ajog.2013.02.009
129. Vitorino, R. L., Grinsztejn, B. G., De Andrade, C. a F., Hökerberg, Y. H. M., De Souza, C. T. V, Friedman, R. K., & Passos, S. R. L. (2011). Systematic review of the effectiveness and safety of assisted reproduction techniques in couples serodiscordant for human immunodeficiency virus where the man is positive. *Fertility and Sterility*, *95*(5), 1684–1690. doi:10.1016/j.fertnstert.2011.01.127
130. Patel RC, Onono M, Gandhi M, Blat C, Hagey J, Shade SB, Vittinghoff E, Bukusi EA, Newmann SJ, Cohen CR. Pregnancy rates in HIV-positive women using contraceptives and efavirenz-based or nevirapine-based antiretroviral therapy in Kenya: a retrospective cohort study. Lancet HIV. 2015 Nov;2(11):e474-82. doi: 10.1016/S2352-3018(15)00184-8. Epub 2015 Oct 22. PubMed PMID: 26520927; PubMed Central PMCID: PMC4632202.
131. Interagency Task Team on Prevention and Treatment of HIV Infection in Pregnant Women, Mothers and Children. Zambia Report. http://www.emtct-iatt.org/countries/zambia/Accessed February 17, 2016.