UCLA
UCLA Electronic Theses and Dissertations

## Title

Individual and couple-level risk factors associated with HIV transmission, family planning, and ART initiation in an open cohort of heterosexual HIV-1 serodiscordant couples in Rwanda

## Permalink

https://escholarship.org/uc/item/6924v8kt

## Author

Dillavou, Megan Claire

## Publication Date

2016

Peer reviewed|Thesis/dissertation

eScholarship.org Powered by the California Digital Library
University of California

UNIVERSITY OF CALIFORNIA

Los Angeles

# Individual and couple-level risk factors associated with HIV transmission, family planning, and ART initiation in an open cohort of heterosexual HIV-1 serodiscordant couples in Rwanda

A dissertation submitted in partial satisfaction of the

requirements for the degree of Doctor of Philosophy

in Epidemiology

by

Megan Claire Dillavou

2016

ABSTRACT OF THE DISSERTATION

Individual and couple-level risk factors associated with HIV transmission, family planning, and ART initiation in an open cohort of heterosexual HIV-1 serodiscordant couples in Rwanda

by

Megan Claire Dillavou

Doctor of Philosophy in Epidemiology

University of California, Los Angeles, 2016

Professor Pamina M. Gorbach, Chair

Understanding the factors of heterosexual HIV-1 serodiscordant couples that lead to risky behaviors for HIV transmission are essential in controlling the HIV epidemic in sub-Saharan Africa. Predictors of HIV transmission within stable discordant couples and trends in family planning over time, as well as predictors of ART initiation provide important information for future studies and prevention and treatment program development. This dissertation evaluates these aspects of HIV-1 serodiscordant couple transmission in an ART naive 10-year observational cohort in Kigali, Rwanda.

The first study evaluated the incident HIV-1 infections and the predictors of HIV-1 transmission in ART naïve HIV-1 heterosexual serodiscordant couples. Eighty-three partner linked incident HIV-1 infections occurred in the cohort with 37 in women (IR=2.2/100 CY; 95%CI: 1.53.299) and 46 in men (IR=2.49/100 CY; 95%CI: 1.83-3.33). In the adjusted final

model of linked HIV infection in females, baseline VL (aHR 2.33; 95%CI: 1.28-4.24), female genital inflammation (cHR 4.77; 95%CI: 1.72-13.21), and having unprotected sex with study partner since last visit (cHR 3.29; 95%CI: 1.27-8.51) were significant predictors. Predictors of linked incident HIV-1 infection in males included baseline VL (aHR 2.14; 95%CI: 1.50-3.07), female genital inflammation (aHR 3.91; 95%CI: 1.71-8.94), any unprotected sex with study partner since previous visit (aHR 3.56; 95%CI: 1.48-8.56), and presence of sperm on a wet prep (aHR 3.35; 95%CI: 0.99-11.36). These findings support the need to include sexual partners in the assessment of risk and target risk reduction strategies.

The second study described pregnancy and analyzed predictors of women ever using hormonal contraception (HC) by HIV status. Overall pregnancy incidence rate was 12.7/100 PY (95%CI: 11.3-14.1) while in M-F+ couples it was 13.2/100 PY (95%CI: 11.3-15.3) and 12.1/100 PY (95%CI: 10.2-14.1) in M+F- couples. 34% of HIV positive women, 26% of HIV- women who did not seroconvert, and 25% of HIV- women who seroconverted had used hormonal contraception at point during the study. In adjusted analyses, being younger (aRR 0.97; 95%CI: 0.95-0.99), ability to read Kinyarwandan easily (aRR 1.28; 95%CI: 1.06-1.55), and no STI in the past year (aRR 0.80; 95%CI: 0.67-0.95) was associated with ever HC use in HIV+ women. Among HIV negative women who did not seroconvert, HC ever use was associated with younger age (aRR 0.98; 95%CI: 0.96-1.0) and not being pregnant at baseline (aRR 0.72; 95%CI: 0.55-0.94). Across HIV groups, injectable methods were the most frequently used type of hormonal contraception at last visit and during most of study follow-up. The overall low uptake of hormonal contraception and high pregnancy rates in both HIV + and HIV- women suggest the need for more effective and widely accessible safer conception methods.

The third study evaluated predictors of time to ART initiation, stratified by gender of seropositive partner. Of the 1837 couples (882 M+F- / 955 M-F+), 30% had an HIV positive partner initiate ART. Of those, 39% had a seropositive male partner (M+F-) and 61% had a seropositive female partner (M-F+). Shorter time to ART initiation in M+F- couples was predicted by baseline viral load (aHR1.54; 95%CI:1.01-2.34), while both baseline viral load (aHR1.43; 95%CI:1.02-2.02) and baseline WHO stage IV (aHR 4.85; 95%CI:1.45-16.26) predicted earlier time to ART initiation in M-F+ couples. As expected, clinical values were the main predictors of time to ART initiation.

In conclusion, partner and partnership characteristics play an important in risk of HIV-1 acquisition and transmission in heterosexual serodiscordant couples. Family planning and fertility desires are particularly complex and important risk factors that may change over time for serodiscordant couples. These findings can help improve the targeted HIV prevention, safer conception and family planning services, and ART treatment programs focusing on sustained viral load suppression among heterosexual serodiscordant couples in Africa.

The dissertation of Megan Claire Dillavou is approved.

Marjan Javanbakht

Sung-Jae Lee

Christina M. Kitchen

Pamina M. Gorbach

University of California, Los Angeles

2016

## Contents

## Abbreviations

Acquired Immunodeficiency Syndrome (AIDS)

Adjusted Hazard Ratio (aHR)

Adjusted Odds Ratio (aOR)

Adjusted Risk Ratio (aRR)

Antiretroviral therapy (ART)

Confidence Interval (CI)

Bacterial Vaginosis (BV)

Couples HIV Testing and Counseling (CHTC)

Couple Years (CY)

Hazard Ratio (HR)

Herpes Simplex Virus 2 (HSV-2)

HIV Prevention Trials Network (HPTN)

Human Immunodeficiency Virus (HIV)

Institutional Review Boards (IRB)

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

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

Men who have Sex with Men (MSM)

Odds Ratio (OR)

Partners in Prevention (PiP)

Polymerase Chain Reaction (PCR)

Pre-exposure Prophylaxis (PrEP)

Prevention of mother to child transmission (PMTCT)

Rapid Plasma Reagin (RPR)

Risk Ratios (RR)

Rwanda Zambia HIV Research Group (RZHRG)

Sexually Transmitted Infection (STI)

Standard Deviation (SD)

Total Fertility Rate (TFR)

Viral Load (VL)

## Acknowledgements

I dedicate this dissertation to my husband, Daniel Jarashow, who endlessly gave of his time during this process. He is a wonderful husband, an exemplary father to our two children, and my biggest supporter. I also want to thank my children, Mac and Luc, who have arguably made this much more difficult but also made it that much more important to complete. Thank you for your patience and love during my hours and days of sub-optimal parenting during this process.

To Pamina Gorbach, my dissertation advisor, who has not only been an academic advisor but an employer and trusted mentor as well. I thank you for helping me navigate the Epidemiology doctoral program and the UCLA system, providing professional advice and guidance, and being equally supportive of my personal goals. I could not have had two children during this academic program without your support and insights in how best to manage it all and remain focused on skill acquisition. I learned many invaluable lessons that will make me a better epidemiologist as well as better public health professional.

My deep appreciation also extends to my Doctoral Committee for their guidance and time in reviewing, commenting and helping make my dissertation better. A particular thank you to Marjan Javanbakht for also being a mentor throughout my time in the Epidemiology program, giving generously of her time and sage advice when it was most needed.

This dissertation would not have been possible without the generosity and support of Dr. Susan Allen and her research team at the RZHRG and Emory University. I worked closely with Dr. Allen and she was helpful, insightful, and generous and member of her team, Dr. Ilene Brill and Naw Htee Khu provided technical assistance and support. I also want to thank Dr. Kristin Wall, part of the RZHRG and a committee member who gave incredibly timely and very informative feedback on both my assumptions and my analyses based on her extensive knowledge of the topics and experience with the studies.

Lastly, a thank you to the UCLA AIDS Institute, UCLA Center for AIDS Research and the UCLA Pediatric AIDS Coalition for their seed grant that partially supported my research.

# Biographical Sketch

2000 BS, Cognitive Science and English Literature

Vanderbilt University

Nashville, TN

2006 MPH, Population and Family Health

Columbia University

New York, NY

2016 PhD Candidate, Epidemiology

Fielding School of Public Health, University of California

Los Angeles, California

SELECT AWARDS

2013 Grand Explorations Awardee: Bill & Melinda Gates Foundation
2014 Graduate Student Mentorship Award: UCLA Graduate School
2015 Global HIV Fellowship: UCLA CHIPTS/AIDS Institute

SELECT PUBLICATIONS

Javanbakht M, Gorbach, P, Dillavou C, Rigg RW, Pacheco S, & Kerndt PR (10 June 2014). Adult Film Performers: Transmission Behaviors and STI Prevalence. Poster session presented at the CDC STD Prevention Conference, Atlanta, GA.

Yeganeh N, Simon M, Dillavou C, Varella I, Santos BR, Fonseca R, Rocha M, Lira R, Gorbach P and Nielsen-Saines K. HIV Testing of Male Partners of Pregnant Women in Porto Alegre, Brazil: A Strategy for Reduction of Seroconversion during Pregnancy. AIDS Care: Psychological and Socio-medical Aspects of AIDS/HIV. 2013 Nov 8.

N Yeganeh, C Dillavou, M Simon, P Gorbach, B Santos, R Fonseca, J Saraiva, M Melo and K Nielsen-Saines. 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 24:279.

# Chapter I. Introduction and background

Current declining rates of HIV-1 incidence in sub-Saharan Africa and widespread scale-up of Antiretroviral Therapy (ART) may imply waning importance of HIV prevention, yet a large proportion of incident HIV infections still occur in stable HIV-1 serodiscordant heterosexual relationships. Within sub-Saharan Africa, the relatively high prevalence of HIV-1 serodiscordant couples is a cause of concern given this high transmission risk within those couples, but also provides a well-defined target population for specific HIV prevention interventions. With an increased understanding of predictive factors of HIV-1 transmission specific to partner linked infections and factors associated with ART initiation among these serodiscordant couples, we can improve targeted programs through integrating prevention and treatment efforts.

This dissertation focuses on individual and partnership level factors associated with heterosexual HIV-1 transmission, hormonal contraception use and pregnancy, and ART initiation in HIV-1 serodiscordant couples in Rwanda over 10 years. The first study examines HIV-1 incident infections and predictors of partner linked HIV-1 transmission and acquisition among men and women in serodiscordant partnerships. The second study describes hormonal contraception use and pregnancy among HIV-1 serodiscordant couples. The third study analyzes predictors of HIV-1 serodiscordant couples that initiate ART compared to those who do not, and identifies factors associated with early ART initiation. These studies use data from the Heterosexual Transmission of HIV Study conducted by the Rwanda Zambia HIV Research Group (RZHRG) at Emory University. The Heterosexual Transmission of HIV Study was an open prospective cohort that enrolled 1837 adult heterosexual HIV-1 serodiscordant couples

recruited from couples counseling and testing sites in Kigali, Rwanda from January 2002 - October 2011.

## 1.1 Epidemiology of Human Immunodeficiency Virus (HIV)

### 1.1.1 HIV worldwide and in Africa

In 2012, an estimated 35.3 million people were living with HIV worldwide and 2.3 million people became newly infected. [1] However, the annual number of new HIV infections among adults in sub-Saharan Africa has declined by 34% since 2001, with the most dramatic decline in new infections occurring in the Caribbean (49%). [1]

Trends in new adult infections differ among regions. The epidemic continues to disproportionately affect sub-Saharan Africa, where 70% of all new HIV infections occurred in 2012. [2] HIV-1 prevalence within sub-Saharan Africa varies by region, as does the prevalence of HIV-1 serodiscordant couples. As the primary route of heterosexual transmission of HIV-1 is through sexual contact and most new infections occur in individuals of reproductive age [3,4], the high prevalence of HIV-1 serodiscordant couples is concerning. Up to 50% of HIV-positive people in on-going relationships have HIV-negative partners. [3-5] Of those HIV-positive individuals who know their status and are in serodiscordant relationships, many have not disclosed their status nor do they know their partners' HIV status. [2] Consequently, a significant number of new infections occur within these HIV discordant partnerships. [5]

Half to two-thirds of HIV-1-infected adults in a cohabitating relationship in Africa have an HIV-1-uninfected partner, [4-8] and either sex is equally likely to be the HIV-1-infected member in a serodiscordant couple. [1] A community cohort study in Uganda found 18% of new HIV-1 infections occurring in serodiscordant couples were attributable to ART naïve HIV-1 serodiscordant couples. [9] HIV-1 incidence in studies with HIV-1 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. [10] Fewer studies have genetically sequences HIV strains to quantify transmission within versus outside of couple, but in the HPTN 052 randomized clinical trial, viral linkage indicated 25% of the HIV-1 transmission within serodiscordant couples was attributable to outside partners. [11] Although many gains have been made in HIV-1 prevention, particularly in reducing mother to child transmission, there have been concerning recent signs of an increase in risky sexual behaviors such as an increase in number of sexual partners, and decline in condom use in population-based studies in several countries. [1]

### 1.1.2 Epidemiology of HIV in Rwanda

Rwanda is the most densely populated country in Africa with a population of 11 million in 2012, residing in a country the approximate size of the US state of Maryland. [1] Approximately 20% of the population lives in urban areas, mainly Kigali, and 43% of the total population are under the age of 15; likely due in part to the 1994 genocide and the conflicts between 1996 and 2000. [12] Rwanda's population is predominately Christian and Catholic denominations, with 5% of the population reported as Muslim. [12]

HIV prevalence among the general population aged 15-49 in Rwanda has remained relatively stable since 2005, with a second population-based survey in 2010 reporting the same 3% prevalence as that in 2005. [13] In 2010, the HIV prevalence was found to be higher among women than among men (3.7% vs. 2.2%), with the highest HIV prevalence among women aged 35-39 (7.9%) and among men aged 40-44 (7.3%). [13]

Regional variation in HIV prevalence within Rwanda exists in terms of magnitude of the disease and trends over time and may be associated with unique patterns of behavior, culture, and geographic influences on local epidemics. Kigali, the capital, had the highest prevalence at

7.3%, while all other provinces measured prevalence below 3%. HIV prevalence among young people aged 15-24 in 2012, was 1.1% (1% among males and 1.3% among females). [13] Urbanization, higher levels of wealth in Kigali, especially among women, and high prevalence of HIV-1 serodiscordant couples in Kigali have been identified as underlying drivers of the local epidemic. [15]

In terms of individual risk behaviors, less than 1% of women and 4% of men age 15–49 report that they had sex with two or more partners in the past 12 months. [13] There was little variation when stratified by urban vs. rural. Slightly over 25% of these women and men reported using a condom at last sexual intercourse. Among the women who had two or more partners in the past 12 months, 63% had concurrent sexual partnerships and the majority of men (80%) who had two or more partners in the past 12 months had concurrent sexual partnerships. [13] According to the DHS, 75.5% of all women and 68.6% of all men age 15-49 reported being ever HIV tested and receiving their results. Relative to the region, quite a high percentage of ever-married men and women reported being HIV tested as a couple (84% and 72% respectively). [13]

Populations most at risk for HIV infection in Rwanda are commercial sex workers, truck-drivers, men who have sex with men (MSM) and serodiscordant couples. [14] A national behavioral surveillance survey that included biomarkers was conducted in 2010 among female sex workers that measured a high HIV prevalence of 51% nationally, with a prevalence of 56% among this population in Kigali. [16]

According to a probability model that combined clinical data on couples' HIV status with population-based data on sexual behavior, more than 90% of heterosexually transmitted HIV infections were estimated to occur within marital or cohabiting relationships in urban areas of

Rwanda. [2] In other modeled estimates using only population-based data, Chemaitelly et al. estimated an annual risk of HIV transmission from the infected to the uninfected partner in stable HIV serodiscordant couples as 30 per 100 person-years in Burundi, Rwanda, and Swaziland, while 20 other sub-Saharan countries had a lower median HIV infection rate of 11.1 per 100 person-years. [17,18]

Rwanda's neighbors have varying general population HIV prevalence with Burundi (1%) and Democratic Republic of Congo (1.1%) reporting low prevalence to Tanzania (5.1%) and Uganda (7.4%) with higher prevalence than Rwanda. [1] Data gathered from the Great Lakes Initiative in AIDS (GLIA) show that indicators of individual risk in Rwanda appear to be substantially lower than those in other GLIA countries, such as Kenya, Tanzania, and Uganda [14].

### 1.1.3 Access to HIV Treatment in Rwanda

ART services in Rwanda were established in 2002, and made widely available with a large scale-up in public facilities in 2007. [19] By 2012, over 400 facilities were offering ART services and more than 100,000 people living with HIV had initiated ART. [20,21] Rwanda has achieved universal ART access, with at least 80% of the people eligible for ART receiving it [22]. Although current treatment guidelines for HIV serodiscordant couples in stable relationship are in line with current WHO recommendations to start the positive partner in such a relationship immediately [5], the many constraints of scaling-up and sustaining ART and achieving sustained viral load suppression in sub-Saharan Africa highlights the continuing need for specifically targeted, evidence-based prevention interventions that can be used in conjunction with ART to reduce HIV transmission among this high-risk group.

## 1.2 Focus of Dissertation

### 1.2.1. HIV-1 transmission in serodiscordant couples

Many risk factors for HIV transmission are well established such as lack of male circumcision [23-26]; presence of sexually transmitted infections [27-28]; high plasma viral load of the infected partner [29]; outside/concurrent partnerships in high prevalence areas [30-32]; and incorrect or inconsistent condom use [33-35]. Potential risk factors in the literature with inconsistent findings on their influence on HIV transmission that warrant further investigation include fertility desires [36-39], hormonal contraception use [39-42], pregnancy and/or postpartum periods [36,43-46], couples' age difference [47-49], and alcohol use among partners [50-53].

No nationally representative studies have been conducted among HIV-1 serodiscordant couples, but various partnership characteristics including partnership type, length of partnership, age disparity, race/ethnicity concordance, frequency of condom use, fertility desires, and frequency of outside partners have been found to effect HIV transmission risk among serodiscordant couples. [1,5,6,10] Clinical factors associated with the probability of HIV transmission through sexual intercourse specifically in serodiscordant couples include type of sexual intercourse [54], infection stage of the infected partner and susceptibility of the uninfected partner [34,55,56] and viral load in genital secretions of the infected person. [56,57] The use of condoms can reduce this risk of HIV transmission and estimates of the level of protection from consistent and correct condom use ranges from 60% to 96%, depending on multiple factors including known partner HIV status. [58]

Given these risk factors, to reduce transmission among HIV-1 serodiscordant couples, established effective prevention strategies include couples counseling and testing [59-61],

reduction of outside partners [1,9,11,29,35], male circumcision [23-26], consistent and correct condom use [29,58], delaying or avoiding pregnancy [43-46], ART for the HIV-1-uninfected partner [5,11] and PrEP for the HIV-1-uninfected partner. [61-63] It is important to note many of these established risk factors and prevention strategies to reduce HIV transmission in serodiscordant couples are observed within the context of randomized controlled trials, which may translate differently to 'real world' settings in terms of public health program implementation and findings. [82,83]

A better understanding of the drivers of HIV transmission within these high-risk couples over long periods of time remains important to prevent new infections. Given the many constraints of scaling-up and sustaining ART treatment to achieve viral load suppression, and the remaining risk of acquisition even when a positive partner is virally suppressed on ART [9,11,65], a gap remains in the integration of targeted, evidence-based non-ART based or ART complimentary prevention interventions.

### 1.2.2. Hormonal Contraception and Pregnancy in HIV-1 Serodiscordant Couples

In addition to the high prevalence of HIV serodiscordant couples, the population of east and southern Africa is growing at approximately 2.85% per year, with a fertility rate (TFR) of 4.7 children per woman. [64] HIV discordant couples must consider complex cultural expectations such as meeting family and social obligations concerning reproduction, individual fertility desires, and partnership dynamics which confound decisions about the risk of HIV transmission. [48] Although some studies have found pregnancy in the HIV-1-infected or uninfected female to be associated with two-fold increased risk of male to female and female to male HIV-1 transmission, [62] other studies that examined HIV acquisition and transmission separately, found more variable associations between pregnancy and post-partum periods and

HIV transmission. [42-45,61] The Partners in Prevention HSV/HIV Transmission Study found pregnancy increased the risk of female-to-male seroconversion but not male-to-female seroconversion. [36] With the widespread uptake of ART and prevalent extended PMTCT services, expectations of a longer life with reproductive capacity has led to increasing pregnancy rates among HIV positive women both independently, and within serodiscordant couples [36,38].

Pregnancy causes complex biological and behavioral changes in a woman and may change sexual behavior dynamics in the partnership. Simply trying to conceive increases the risk of HIV transmission or reinfection among serodiscordant couples [67-69] as well as may increase the burden of unplanned pregnancy in HIV serodiscordant couples [42-46]. Women who become pregnant may differ considerably from those who are not pregnant potentially causing confounding of observed associations in observational epidemiologic studies. Detangling these effects is difficult and involves the investigators' conceptualization of the causal pathway, determination of confounders and mediators, and analytic techniques. Because this can be somewhat subjective and can change with time, conception and pregnancy (term or otherwise) may confound and/or mediate the risk of HIV transmission [43-45].

Accessible safe contraception is vital for women's health as it can reduce maternal and infant mortality and morbidity and improve infant and maternal health. [40-41,69-70] In the context of HIV, effective contraception prevents pregnancy, negating the possibility of vertical HIV-1 transmission from mother to child. In 2013, approximately 33% of women in southern and East Africa reported current use of contraceptives. [70] Rwanda's reported contraceptive prevalence increased from 17% to 52% from 2005 to 2010, yet their prevalence of modern method only (includes COC, injectables, IUD, implants, condoms, and sterilization) was lower at 45%, while their total fertility rate of 4.5 remains relatively high. [13] Over the same time period,

unmet need for family planning services declined from 38% to 19%, [71] yet what proportion of this unmet need is among women living with HIV is unknown. According to a national study on unintended pregnancy and abortion in 2013, nearly half (47%) of all pregnancies in Rwanda were unintended. [71]

Hormonal contraception methods such as injectable depot medroxyprogesterone acetate (DMPA), norethisterone enanthate (NET-EN), and combined oral contraceptive pills (COCs) are increasingly used and critical to reducing unwanted pregnancy in high HIV prevalence areas in sub-Saharan Africa. [64,69,70] Currently there is inconclusive evidence on the association between HC use and HIV transmission and acquisition despite secondary analysis of RCTs, [37] recent rigorous longitudinal data analysis, [39] a large sample individual participant data meta-analysis [72] and a pooled meta-analysis of observational studies. [73] Some, but not all [74], high quality observational studies have demonstrated an increased risk for HIV-1 acquisition among women using DMPA specifically. [39-42]

Some factors found to effect women's contraceptive use include physical access, cost, lack of accurate information and limited knowledge of available services, as well as fertility preferences, religious traditions, partner communication, and fear of side effects. [76-80] Structural impediments such as clinical eligibility barriers, provider qualifications, bias, and inappropriate management of side effects also affect uptake and sustained use. [75,80,81] How these factors relate to non-use varies across time and settings. [80] In a multivariate decomposition analysis of Rwanda's 2010 DHS data, variables that significantly contributed to the increase in contraception prevalence were a woman's level of education, previous experience with child mortality, partner concordant fertility desires, and place of residence. [79] Yet this research was limited in that it analyzed government campaigns and programs and did not account

for supply side factors such as increased distribution points and stocks, and integration of family planning with HIV and other health services.

Given the complex nature of these reproductive issues, the need to better describe pregnancy and hormonal contraception use among women in HIV serodiscordant relationships over long periods of time during their reproductive age, the need to ensure current policies and programmatic development support women in serodiscordant relationships to safely plan a family with their partner.

### 1.2.3 Timing of ART Initiation by HIV-1 Serodiscordant Couples

ART significantly improves the health and survival of HIV-infected individuals, and reduces their infectiousness and likelihood of transmitting HIV to a sexual partner [5,11,29]. Unfortunately, many HIV-positive people in resource-limited countries who receive ART may not have a normal life expectancy, often because they start ART when they have lower than optimal CD4 counts. [84] HPTN 052, a multinational randomized clinical trial with HIV-1 serodiscordant couples, demonstrated that early initiation of ART (CD4 count between 350-550 cells/$mm^3$) reduced the risk of HIV transmission by 96% to the uninfected partner, compared to delayed ART initiation arm (CD4<=250 cells/$mm^3$) [11]. The viral load suppression observed in the index partner during the study was supported quarterly viral load monitoring and routine adherence counseling. As a result of this landmark study, couples voluntary testing and counseling guidelines (CVTC) were released in 2012 to include immediate ART initiation of HIV positive partners in stable, cohabitating HIV discordant relationships. [5] In order to meet this recommendation, it is necessary to understand where and when seropositive individuals are lost to pre-ART care or why they do not initiate ART when eligible.

Multiple studies in different settings have reported a reluctance to initiate ART by HIV-infected individuals [68,84,85,90]. Studies of retention in pre-ART care report substantial loss of patients at every point in the treatment cascade [89,91], starting with patients who do not return for their initial CD4 count results and ending with those who do not initiate ART despite eligibility. [90] Social constructs around HIV are important in understanding this attrition at each of these points of the care continuum, specifically the important decisions to refuse or initiate ART. Partners in stable HIV serodiscordant relationships may differ in the factors driving these important care decisions.

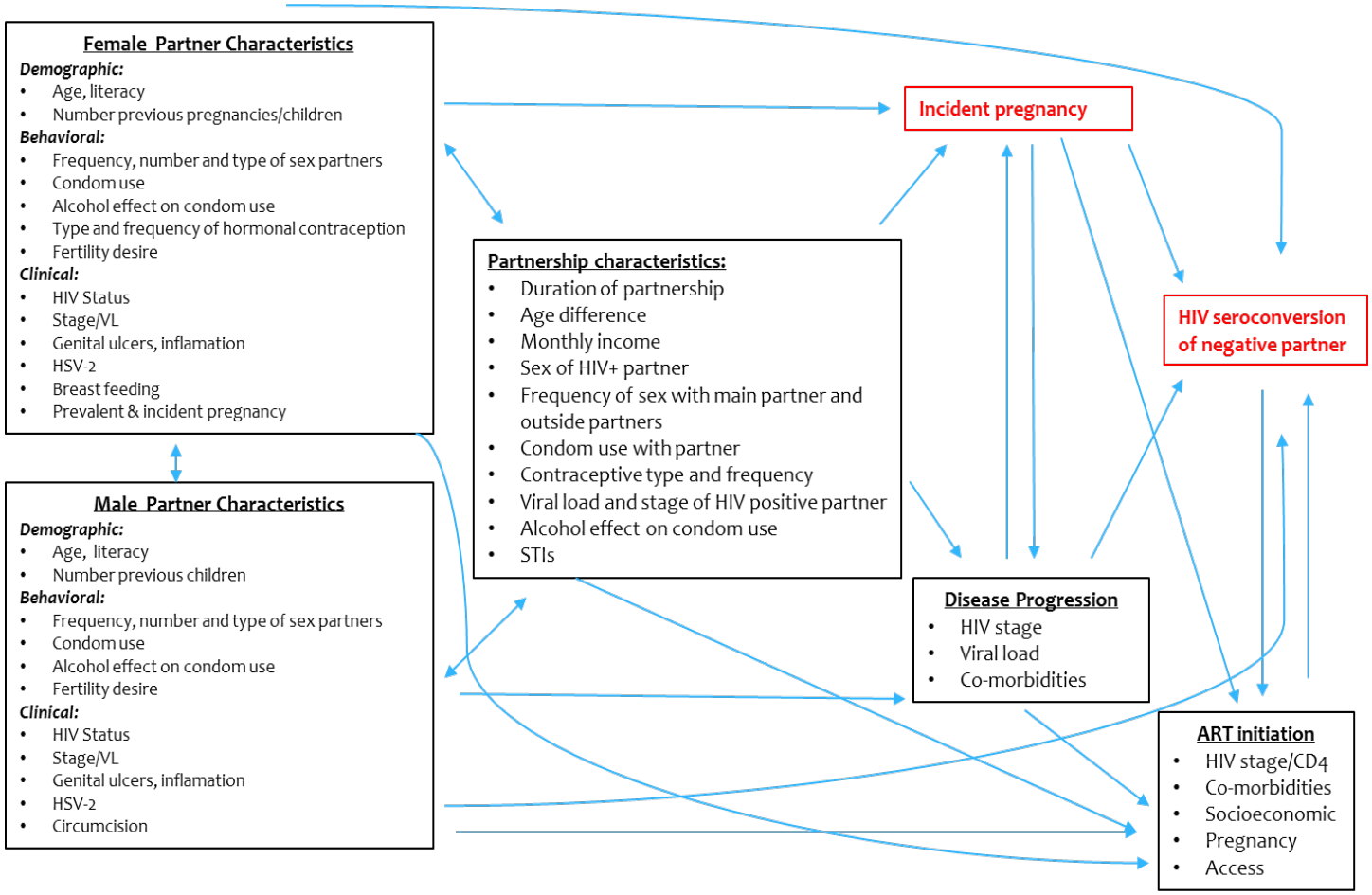
Looking specifically at HIV-1 serodiscordant couples, a Kenyan cohort study evaluated time to ART initiation in HIV-1 infected partners eligible for free ART to identify barriers to treatment. They observed a median time from meeting CD4 criteria until ART initiation of 8.9 months and found CD4 count and socioeconomic status were associated with delayed ART initiation. [84] A different qualitative study among heterosexual HIV serodiscordant couples in Kenya demonstrated a concerted interest in early initiation of ART to maintain health and prevent HIV transmission to uninfected partners. However, side effects, adherence to life long treatment, and the fear of stigma were prevalent themes as barriers to uptake. [85] Couples also noted a widespread belief that initiating therapy signified the final stage of AIDS, indicating potential challenges of getting positive partners that look and feel healthy in to treatment. [85] In the control arm of HPTN 052, nearly 20% of HIV-infected participants declined ART when offered after the trial demonstrated HIV protection. Many of these uninfected individuals stated that they were not ready to begin ART or believed their CD4 cell count was too high [106]. Other recent studies have suggested that higher CD4 cell counts are associated with delayed ART initiation or refusal [90-93].

A study in Kenya assessing the willingness of partners in serodiscordant couples to start PrEP gave their participants a hypothetical choice of starting either early ART or PrEP for HIV-1 prevention. They found 52.5% of HIV-1 infected participants preferred to initiate ART early and 56.9% of HIV-1 uninfected participants would preferred to use PrEP. [105] This finding is important as partners in serodiscordant couples may differ in their preferences for HIV care and prevention strategies. Despite access to regular health care, referrals to treatment centers, and free access to ART, all HIV positive individuals in serodiscordant couples are not initiating ART, indicating targeted approaches are needed to avoid delays in treatment initiation.

### 1.3 Conceptual Model

The conceptual model below (Figure 1) was adapted from a conceptual framework by Gorbach and Holmes [94] to describe how individual and partnership characteristics affect risk behaviors. While this conceptual framework was informed by both empirical and modeled evidence and draws on the Social Action Theory [95], it also integrates aspects from Crankshaw et al. and their research carried out in South Africa defining the constructs where sexual transmission of HIV must be considered for safe conception. [96] This adapted model integrates non-conception related and conception-related HIV risk behavior as well as clinical and treatment seeking factors by building on frameworks that integrate determinants across individual, heterosexual partnership and structural levels. [97,98] All of these components are factors in our outcomes of interest and this model guided our analyses.

**Figure 1: Conceptual framework of individual and partnership behavioral dynamics, disease progression, and HIV infection in heterosexual HIV-serodiscordant couples**



# Chapter II. Partner linked HIV-1 incident infection and predictors of transmission among serodiscordant couples in an open cohort study, Rwanda 2002-2011.

## 2.1 Abstract

**Objective:** Analyze individual and partnership predictors of linked HIV-1 transmission in heterosexual serodiscordant couples.

**Design:** Prospective open cohort of 1837 ART naive HIV-1 serodiscordant heterosexual couples in Kigali, Rwanda.

**Methods:** HIV-1 serodiscordant heterosexual couples were enrolled from couples counseling and testing sites in Kigali. Demographic, behavioral, and clinical exposures were measured in both partners at baseline and every three months. HIV-uninfected partners were re-tested every three-months at minimum. Genetic analysis classified incident HIV-1 infections as those acquired from the study partner as "linked infections." Partner linked incident HIV infections per 100 Couple Years (CYs) and HIV transmission rates by sex of HIV positive partner (M+F-/M-F+) were calculated. Baseline and time-varying predictors of HIV-1 transmission stratified by M+F-/M-F+ using mutivariable Cox models were estimated.

**Results:** Eighty-three partner linked incident HIV-1 infections occurred in the cohort (IR=2.33/100 CYs (95%CI: 1.86-2.90)) with 37 in women (IR=2.17/100 CY; 95%CI: 1.53-2.99) and 46 in men (2.49/100 CY; 95%CI: 1.83-3.33). In adjusted final model of linked HIV infection in females, baseline VL (aHR 2.33; 95%CI: 1.28-4.24), female genital inflammation (cHR 4.77; 95%CI: 1.72-13.21), and having unprotected sex

with study partner since last visit (cHR 3.29; 95%CI: 1.27-8.51) were significant predictors. Predictors of linked incident HIV-1 infection in males included baseline VL (aHR 2.14; 95%CI: 1.50-3.07), female genital inflammation (aHR 3.91; 95%CI: 1.71-8.94), any unprotected sex with study partner since previous visit (aHR 3.56; 95%CI: 1.48-8.56), and presence of sperm on a wet prep (aHR 3.35; 95%CI: 0.99-11.36).

**Conclusion:** Although relatively few linked incident infections were observed, predictors of linked HIV-1 transmission were similar in both M+F- and M-F+ couples, with only the addition of a few for male incident infection. Couple and individual level risk factor assessment is important. Safe sex and safer conception counseling need to be reinforced and made more accessible to this high-risk population. Genital symptoms such as inflammation and ulceration, particularly in women, should be routinely screened for and treated, or treated prophylactically for both partners in serodiscordant couples.

## 2.2 Introduction

While the annual number of new HIV infections among adults in sub-Saharan Africa has declined by 34% since 2001, sub-Saharan Africa continues to be disproportionally affected by HIV/AIDS with 70% of global incident infections occurring in this region in 2012. [1,2] Of these incident infections, a large proportion occurred in the context of stable heterosexual HIV-1 discordant relationships. [4,5] In modeled estimates using population-based data, annual estimated risk of HIV transmission from the infected to the uninfected partner in stable HIV serodiscordant couples was 30 per 100 person-years or more in Burundi, Rwanda, and Swaziland [17,18]. Another model, using data from Rwanda and Zambia, predicted 84.1% to 99.8% of infections among married or cohabiting adults occur within serodiscordant marital or cohabiting relationships, depending on sex of the index partner and residential area [2]. Population-based studies indicate the sex of the HIV-1 uninfected partner in these heterosexual relationships is equally likely to be female as male [1]. Given this high risk of HIV transmission within discordant partnerships [2-4] and the high frequency of these partnerships in sub-Saharan Africa, [1,9,11] targeting this population remains important to preventing new infections.

To reduce transmission among HIV serodiscordant couples, effective prevention strategies include couples counseling and testing [60-62], reduction of outside partners [1,12,29,35], male circumcision [23-26], consistent and correct condom use [59], delaying or avoiding pregnancy [43-46], early ART for the HIV infected partner [11], and PrEP for the uninfected partner. [63,64] Additional factors that have shown some effect on HIV transmission but need further investigation include fertility desires [36-39], hormonal

contraception use [40-42], pregnancy and/or postpartum periods [43-46], couples' age difference [47-49], and alcohol use among partners [50-53]. Importantly, many of these established risk factors that inform prevention strategies for HIV serodiscordant couples are observed within the context of RCTs, and may translate differently into 'real world' prevention programs and findings. [84]

An improved understanding of the drivers of HIV transmission within these high-risk couples over long periods of time remains important. Given the many constraints of scaling-up ART treatment to achieve sustained viral load suppression and the remaining risk of acquisition even when a positive partner is virally suppressed on ART [11], the need for targeted, evidence-based prevention interventions to compliment ART roll-out in HIV serodiscordant relationships are needed. We use 10 years of data from an ART naïve HIV-1 serodiscordant heterosexual cohort to measure risk and examine predictors of HIV transmission in Kigali, Rwanda.

## 2.3 Methods

**2.3.1** ***Study Participants****:* Heterosexual HIV serodiscordant couples (one partner is HIV-positive and the other HIV-negative) were invited to enroll in an open cohort study between 2002-2011, after being identified as eligible from couples (married or cohabiting) in Kigali, Rwanda who attended couples' voluntary HIV counseling and testing (CVCT) services. Couples either self-presented or enrolled after receiving an invitation from a community CVCT promoter. CVCT services included group counseling, rapid HIV testing, and post-test couples counseling with mutual disclosure of results. Couples were eligible to participate if they were confirmed HIV-1 serodiscordant, had been together at least 3

months and planned on staying in the Kigali region for at least a year. Couples were ineligible if either couple had a CD4 count <200 or either partner was currently on ART. Written informed consent was obtained from the couple jointly. Couples were censored if either partner died, the couple separated, either partner was lost to follow-up, or if the HIV positive partner started ART. ART initiation was based largely on clinical staging, Rwandan government policy and availability, and followed changing WHO guidelines for treatment initiation.

**2.3.2** ***Data Collection:*** Data was collected from a single clinical site in Kigali. Participants completed behavioral and medical history questionnaires and had a full physical examination including pelvic/genital exam (conducted by clinic physicians), and HIV and STI testing (gonorrhea, Chlamydia, Trichomonas) at baseline. Study visits every three months during follow-up included a physical exam, a blood sample for STI and HIV testing (negative partner), a vaginal swab wet mount (to determine bacteria, yeast cells, trichomoniasis, white blood cells to show an infection, or clue cells that show bacterial vaginosis (BV)), and assessment of prevalent and incident pregnancy by blood test and asking women how many months currently pregnant and pregnancy outcome since last visit. Clinical records were used to validate self-reported hormonal contraceptive use as it was given free on site during the participants' study enrollment. Questionnaires asked about ever and current use of methods at baseline and follow-up, which included categories for no method, condoms alone, COCPs (progesterone-only typically prescribed to breastfeeding women until child is 6 months old), DMPA injectables (150mg IM dosage), copper IUD, contraceptive implant (Norplant, Jadelle), or permanent methods

(hysterectomy/tubal ligation/vasectomy). Self-reported decision regarding current method, reason(s) for stopping/switching current method, and new contraception method were also captured at each three-month visit. Male circumcision was confirmed during baseline medical examination or physical examination after self-reported circumcision.

Study questionnaires included demographic and psychosocial data, sexual risk behaviors, medical history, and health services data. Data were collected by participant completion of written questionnaire in Kinyarwandan or English (preference of the participant) and face-to-face interviews were conducted if literacy was a problem.

Over time, some study procedures varied. From 2002-2006, HIV positive partners were seen quarterly after the initial follow-up at 1 month for routine physical and genital exams and laboratory screening for trichomonas and syphilis. All HIV-negative partners were tested for HIV at baseline, 1 month and each 3-month follow-up visit. From 2007-2011, physical exams and STI screenings were performed at baseline, annually, and when signs or symptoms were reported. Plasma banking for VL testing and p24 ELISA screening began in 2002. Beginning in January 2007, all HIV-negative partners were seen at visit months 0,1,2,3. Starting at visit month 3 and quarterly thereafter, a risk assessment was conducted to establish recent exposure within the partnership. Couples assessed as 'higher risk' were asked to come back at monthly intervals for repeat HIV testing until the next quarterly visit, at which time the risk assessment was repeated. 'Higher risk' was defined as having at least one of the following since last visit: self-reported unprotected sex, sperm or trichomonas on a wet prep, incident pregnancy, or incident syphilis. All follow-up visits included risk reduction counseling, access to contraception and free condoms. Free

outpatient health care was provided at the research clinic.

HIV-1 testing of HIV-negative partners was conducted using rapid serologic tests at each follow-up visit. [99] Blood plasma from the last antibody negative sample was tested by p24 ELISA and RNA polymerase chain reaction (PCR) to determine time of infection. Based on laboratory data available, date of HIV-1 infection was derived from one of the following: 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 VL positive/antibody negative test date. The molecular epidemiology of the incident transmission events that occurred during study follow-up was determined by the genetic characterization of HIV-1 strains. Linkage was assessed by comparing conserved PCR-amplified nucleotide sequences from each member of the couple to classify incident infections as "linked" to the study partner or acquired from outside the study couple "unlinked." [100-101]

**2.3.3** ***Variables of interest:*** Incident HIV-1 infection that was genetically linked to the study partner was the outcome of interest. Genetically unlinked infections were not included in this analysis. One incident infection with an indeterminate linkage result was classified as partner linked for this analysis. [101]

Baseline couple measures of interest included age disparity; years cohabiting; number of living children; HIV stage and Viral Load (log transformed) of the positive partner; circumcision status of male partner; and monthly income (USD). Individual exposures of interest at baseline included age; Kinyarwandan literacy; history of STI in the past year; number of sex partners in past year and lifetime; number of previous

pregnancies; current pregnancy; individual fertility desires, and being drunk in the past year.

Exposures of interest that were collected at each follow-up visit ("time-varying measures") included composite genital inflammation and genital ulceration indicators for each partner, self-reported number of protected and unprotected sex acts with the study partner since last visit, self-reported number of outside partners since last visit, both incident and prevalent pregnancy, current female contraception method, presence of sperm in the vaginal tract, and diagnosis or treatment of an STI for both partners.

Diagnosis of candida, BV, and trichomonas was done with vaginal wet preparations, which were also examined for presence of sperm as a biomedical measure of condomless sex. Rapid plasma reagin (RPR) was used for serologic diagnosis of syphilis. [102] Gonorrhea and chlamydia were diagnosed clinically by presence of endocervical or urethral discharge. Due to low sensitivity of gram staining, patients were empirically treated for both gonorrhea and chlamydia.

The dichotomous composite genital inflammation indicator was derived from individual time-varying measures (clinically diagnosed/treated or self-report) of genital inflammation (including cervical or vaginal inflammation in women); genital discharge (urethral discharge in men, vaginal or cervical discharge in women); inguinal adenopathy; or laboratory diagnosis or symptom-based treatment for trichomoniasis, gonorrhea, chlamydia, candida, or BV. Similarly, the composite genital ulceration variable was created from time-varying measures of chronic/recurrent or acute genital or perianal ulcers (clinically diagnosed/treated or self-report); ulceration observed in physical exam

(including erosion or friability of the cervix or vagina) treatment of chancroid or HSV-2; and/or incident positive RPR serology for syphilis.

**2.3.4** ***Data Analysis:*** Couple-years (CYs) of follow-up were computed for each couple from enrollment until either the couple was censored or the HIV-negative partner seroconverted. HIV incidence was calculated as the number of incident infections genetically linked to the study partner per CYs of follow-up. To evaluate possible cohort effects, HIV infection rates were calculated by months since enrollment and differences evaluated by log-rank tests. HIV transmission prior to study enrollment was examined by dichotomizing months since enrollment (0-3 vs. >3) and differences tested.

Kaplan Meier curves for all partner-linked seroconverters compared to non-seroconverters were compared to assess proportional hazards. These curves crossed, indicating that the rate of failure between the two groups crossed. Estimated hazards and plots of log(-log(Survival)) against log(time) confirmed the violation of the proportional hazards assumption after approximately 1200 days (40 months) of follow-up. Due to this violation and *a priori* hypothesis of differing risk factors for transmission based on the sex of seronegative partner, stratified analyses are presented by sex of incident HIV infection.

Exposures were stratified by sex of HIV-positive partner. Univariate descriptions of categorical variables (counts and percentages) and continuous variables (means and standard deviations) per couple (baseline variables, Table 1) or across all study intervals (time-varying, Table 2) are displayed. *A priori* covariates of interest (including age difference of partners, years cohabiting, education levels, monthly income, current and past STI history, baseline viral load of HIV positive partner and male circumcision) and those

significantly ($p<0.05$) associated with the incident HIV infection in bivariate analyses were considered for inclusion in multivariable models. Proportional hazards of time-independent variables were assessed graphically and with log-log plots of Schoenfeld residuals. Martingale residuals were used to evaluate functional forms of covariates. Multi-collinearity was assessed for all candidate variables through examination of the proportion of variance in a given predictor that was not explained by all of the other predictors (tolerance) and the review of the variance inflation factor. If multi-collinearity was present (condition indices > 25), variables with the greater attributable variance proportion (>0.5) and/or were not of interest a priori were dropped from the model. Multivariable Cox models were stratified by the sex of the HIV-positive partner to evaluate predictors of time to couple genetically linked incident HIV infection.

Missing data patterns were assessed for predictors of interest. If data was missing for a specific variable on a specific visit date but had a valid non-missing value in the visit before that matched the valid non-missing value for the visit date after, and other influential covariates remained constant, the missing value was then matched with those values. Goodness of model fit was assessed with log-likelihoods and using the Akaike information criterion (AIC) stepwise regression where entry criteria were set as 0.99 and staying in the model criteria 0.995. Adjusted hazard ratios (aHRs), 95% CIs, and p-values are reported (Table 3). All analyses were conducted with SAS v9.4 (Cary, NC).

**2.3.5** ***Ethics:*** The parent study was approved by the Office for Human Research Protections, the registered Institutional Review Boards at Emory University and the

Government of Rwanda. Secondary analyses were approved by the University of California, Los Angeles.

## 2.4 Results

Of the 1837 HIV-1 serodiscordant couples enrolled in this study, 1812 couples met eligibility criteria and were included in this analysis; 875 had a male partner that was HIV positive and female partner that HIV negative at baseline (M+F-) and 937 had a male partner that was HIV negative and a HIV positive female partner (M-F+). There were 83 study partner genetically linked ("linked") incident infections, 37 occurred among M+F- couples and 46 among M-F+ couples (Figure 1).

Average follow-up time for the 83 linked seroconverters was 453 days (median=315, SD=450) and differed from the 721 days of follow-up observed by the non-seroconverting couples (median=616, SD=560) ($p<.001$). The 37 M+F- seroconverted couples were followed for an average of 603 days (median=387, SD=509), compared to the 838 non-seroconverting M+F- couples that were followed for an average of 715 days (median=592, SD=558). The 46 M-F+ seroconverted couples were followed for an average of 332 days (median=228, SD=359), that differed significantly from the average of 727 days (median=638, SD=562) of follow-up for the 891 M-F+ couples that did not seroconvert. M+F- couples that experienced a female incident HIV infection had a significantly longer follow-up time ($p<.01$) than M-F+ couples with a male incident HIV infection.

The HIV-1 transmission rate for the cohort was 2.33/100 CYs (95%CI: 1.86-2.90). Of the 83 linked HIV incident infections, 37 occurred in women over 1704.6 CY

(IR=2.17/100 CY; 95%CI: 1.53-2.99) and 46 occurred in men over 1844.1 CY (2.49/100 CY; 95%CI: 1.83-3.33). HIV incidence rates in men were higher at 0-3 months (IR=5.89/100 CY; 95%CI: 3.22-9.89) compared to the subsequent period from 4 months through end of study (IR=2.04/100 CY; 95%CI: 1.42-2.89) (p<.001). Among woman, HIV incidence rates were slightly higher in the initial study period of 0-3 months (IR=2.74/100 CY; 95%CI: 1.01-5.96) compared to the following period from 4 months until end of study (IR=2.14/100 CY; 95%CI: 1.47-3.03), but this difference was not significant (Figure 2). Seroincidence rates did not differ by calendar time cohort (2002-2006 vs. 2007-2011).

In bivariate analysis, the following baseline characteristics were associated with female seroconversion (M+F- couples): younger age of women (cHR 0.91; 95%CI: 0.85-0.97), couple cohabiting for a shorter time period (cHR 0.89; 95%CI: 0.81-0.97), fewer numbers of living children (cHR 0.43; 95%CI: 0.25-0.75), higher log VL of the HIV positive male partner (cHR 2.89; 95%CI: 1.78-4.68), fewer previous pregnancies (cHR 0.74; 95%CI: 0.6-0.9), male partner having an STI in the year prior to enrollment (cHR 2.35; 95%CI: 1.18-4.71), higher log VL of the HIV positive male partner at baseline (cHR 2.89; 95%CI: 1.78-4.68), and the male partner reporting wanting a child (cHR 3.76 95%CI: 1.82-7.78) (Table 1).

Baseline characteristics associated with male seroconversion (M-F+ couples) in bivariate analysis include higher numbers of lifetime sex partners of the female partner (cHR 1.01; 95%CI: 1.003-1.01), female reporting being drunk in the past year (cHR 3.15; 95%CI: 1.47-6.77), higher WHO stage of the HIV positive female partner (Stage II: cHR 2.29; 95%CI: 1.12-4.65, stage III: cHR 2.62; 95%CI: 1.20-5.75), increasing log VL of the

HIV positive female partner (cHR 1.7 95%CI: 1.26-2.28), and male partner having an STI in the year prior to enrollment (cHR 2.75; 95%CI: 1.53-4.94). Being circumcised was associated with a decreased risk of HIV incident infection (cHR 0.21 95%CI: 0.07-0.69) (Table 1).

Bivariate time-varying exposures ($p<0.05$) predictive of increased risk of study partner linked HIV seroconversions in females (M+F- couples) include presence of female genital inflammation (cHR 4.78; 95%CI: 2.31-9.88), any unprotected sex with the study partner since last visit (cHR 3.9; 95%CI: 2.01-7.7), and more unprotected sex acts with the study partner (cHR 1.01; 95%CI: 1.00-1.03) (Table 2).

Bivariate time-varying exposures ($p<0.05$) predictive of increased risk of linked HIV seroconversions in males (M-F+ couples) include presence of female genital inflammation (cHR 3.71; 95%CI: 2.0-6.89), presence of male genital inflammation (cHR 4.41; 95%CI: 2.14-9.11), any unprotected sex with study partner since last visit (cHR 2.36; 95%CI: 1.30-4.27), more unprotected sex acts with the study partner (cHR 1.01; 95%CI: 1.00-1.02), and sperm present on wet prep (cHR 2.65; 95%CI: 1.03-6.85) (Table 2).

In final multivariable models, collinear baseline variables included woman's age, number of years cohabitating, number of living children, and number of previous pregnancies. Given our *a priori* assumptions and the variance inflation, only woman's age and number of previous pregnancies (when applicable) were kept in the models.

In the final model of linked incident HIV infection in females, baseline VL (aHR 2.33; 95%CI: 1.28-4.24), female genital inflammation (cHR 4.77; 95%CI: 1.72-13.21), and having unprotected sex with study partner since last visit (cHR 3.29; 95%CI: 1.27-8.51)

remained significant predictors after adjustment. The AIC to assess the goodness of model fit was 206.7, compared to the AIC of the reduced model of 247.2. (Table 3) In the final adjusted model of linked incident HIV infection in males, the baseline VL (aHR 2.14; 95%CI: 1.50-3.07), female genital inflammation (aHR 3.91; 95%CI: 1.71-8.94), any unprotected sex with study partner since previous visit (aHR 3.56; 95%CI: 1.48-8.56), and presence of sperm on a wet prep (aHR 3.35; 95%CI: 0.99-11.36) were significant predictors of transmission (Table 3). The AIC to assess the goodness of model fit was 236.4, compared to the AIC of the reduced model of 282.8.

## 2.5 Discussion

HIV transmission rates in this ART naïve cohort were similar to the 2.7 cases/100 person-years observed in the Partners in Prevention HSV/HIV Transmission Study and lower than the rate in two other observational cohorts of serodiscordant couples in Africa. [3,4,57,119] The lower rate in our study may be due to the quarterly couples counseling on reducing risk behaviors and free condoms. Higher incidence rates in the first 3-month study period of (time on study) were observed in men compared to women. Incident HIV infections detected during the first 3 months on study may imply prevalent infection (pre-study transmission) since partner testing was criteria for study entry, while the reduction after 3 months could reflect an effect of the couples testing and counseling intervention at baseline. Although transmission rates decreased significantly in men by calendar time, this was not observed in women. An increase in the HIV incidence rates in females at the end of follow-up was unexpected, yet our confidence intervals overlapped with those from the previous time period indicating no true difference.

It has been suggested women seroconvert more quickly due to increased biological susceptibility [42] and more social vulnerability (4). However, these explanations are based on findings from large, low risk and homogeneous study populations with little variability in their behavior. The difference in transmission rates showed female-to-male transmission was slightly faster compared to the male-to-female rate in our cohort, although not significant (HR: 1.17; 95%CI: 0.76-1.8), so at the very least demonstrated no difference between the groups was observed. This may be due to the diversity of characteristics and behaviors in our cohort from the high proportion of women with prevalent pregnancy at baseline (typically women are excluded if pregnant from studies) to vastly different number of lifetime sexual partners for HIV+ women. These characteristics may also infer different dynamics of transmission within couples in our study. Assuming all serodiscordant partnerships have the same risk profile due to being 'stable,' based on a defined amount of time, has potential to introduce residual confounding as couples together for 4 months may be qualitatively different than couples together for 6 years and have important implications for specific prevention interventions given the type of 'stable serodiscordant partnership.'

Interestingly, incident infections in males tended to not be associated with many *a priori* individual demographic variables of interest such as age, income or education levels but were found to be associated with partner characteristics such as number of female lifetime sex partners, female being drunk in the last year, and female viral load and stage at baseline. We saw a large range of numbers of reported lifetime sex partners in female partners of men that seroconverted (mean=18.1) compared to those that did not (mean=4.4). The non-normal distribution of number of female lifetime sex partners (Figure

3) may suggest heterogeneity among the women who were HIV positive at baseline that is not adequately captured in our risk factor variables. Coupled with a tradition of wearing condoms with outside partners but not necessarily with the married/cohabitating partner among some populations [19], this could lead to an increase risk for these male uninfected partners. Only the male's circumcision status (protective) and having an STI in the past year were independently associated with incident infection in males.

Consistent with other findings [3,7,9,12] having more living children and the number of previous pregnancies were protective of incident infection in women. Among M+F- couples, male and female desires for another child were significant in bivariate analyses but did not persist in the multivariable model most likely due to missing data resulting in small numbers. Unprotected sex with study partner since last visit was a predictor of seroconversion in both M+F- and M-F+ couples which may also, in light of the low reported use of any hormonal contraception method in the time interval of estimated seroconversion, indicate a more 'recent' desire to get pregnant than a baseline fertility measure. It is not uncommon for many HIV-1 serodiscordant couples to have multiple children, with both the infected and uninfected partners often reporting desires for additional children post status disclosure [29-32]. In one study, over half of serodiscordant couples recruited from HIV-1 care centers in Uganda wished to have children in the future [26]. Within a cultural context, a partners' desire for more children may outweigh a known risk (unprotected sex) to achieve this goal. Indeed, the incident pregnancy rate in our cohort was approximately 13/100CYs, not including 334 prevalent pregnancies at baseline. Although slightly lower than the annual pregnancy rates in the Partners in Prevention

HSV/HIV Transmission Study (16%) but higher than that observed in an observational cohort in Kenya (9.7%) [12], these rates seem high in light of this cohort being ART naïve, having free access to both condoms and hormonal contraceptives, and receiving quarterly risk reduction counseling.

Genital inflammation in females is a significant predictor of HIV transmission in both men and women in unadjusted analyses. In adjusted analysis, this large effect remains in predicting HIV transmission in both males and females. The Partners in Prevention Study looked to address this issue and observed a protective effect of daily acyclovir treatment in reducing genital ulcer disease in the intervention group (aRR 0.39; 95% CI, 0.32 -0.48; P<0.001) [65], although an overall reduction in HIV-1 transmission among serodiscordant couples was not observed. Novel strategies in addition to incorporating prophylactic treatment for genital inflammation and ulceration to reduce the risk of HIV transmission are needed.

***2.5.1 Limitations:*** Limitations included the recruitment and/or self-selection into the cohort study from a couples counseling and testing center, potentially introducing a selection bias. These couples were already seeking counseling and testing services so they may differ qualitatively from other serodiscordant couples that did not seek counseling and testing services, making their results more likely to generalize to more "health motivated" couples. Data was also collected by questionnaires or interviews administered by clinical nursing staff, likely leading to some social desirability bias on sensitive issues around outside partners, frequency of sex, and condom use. Funding changes and study priorities lead to some changes in data collected over time; however, we do not expect those differences to

be differential by linked HIV-1 transmission. The potential for confounding by unmeasured factors such as condom use with outside partners, changing social norms around drinking alcohol, and time-varying fertility desires exists. The inclusion of women with high numbers of lifetime sex partners may have had different risk profiles than women with fewer, more "normative" lifetime sex partners, potentially introducing additional important unmeasured confounding.

This was an ART naïve cohort with initiation of ART of the positive partner as criteria for censoring. ART was scaled-up nationally during study follow-up time making it possible ART availability had differential censoring effects on couples with linked incident infection compared with non-seroconverting couples. This, coupled with PMTCT scale-up, may have led to more rapid start of ART and thus censoring of couples with an HIV-positive female partner. Yet in sensitivity analyses, we did not see a significant difference of follow-up time by HIV-positive women who were pregnant during study versus those that were not.

Study attrition was relatively low and may be due to the study being conducted in Kigali, a highly concentrated urban population with low migration. The low attrition may also have been associated with benefits couples received from being in the study, such as access to comprehensive medical care and reproductive health services. The long average follow-up time may have had a "healthy couple" effect, whereby the healthiest couples remained on study.

Stratified analyses gave us less power to detect the true differences. Missing data in some key covariates affected our precision [107]. In cursory missing data analyses, we see

missing data is not likely to be independent of other covariates. Because of this, neither multiple imputation nor maximum likelihood methods that assume the data is missing at random (MAR) or missing completely at random (MCAR) hold. The observed missing not at random (MNAR) pattern likely necessitates more advanced G-estimation methods to model the MNAR in further analyses.

## 2.6 Conclusion

Given the high risk of HIV transmission within serodiscordant couples, the relatively low linked HIV-1 incidence rate after 3 months may indicate the effectiveness of couples HIV testing and counseling in identifying serodiscordant status and helping identify both individual and partner risk behaviors to modify to reduce risk of transmission. With the widespread rollout of ART, not only targeting HIV prevention strategies specific to HIV-1 serodiscordant couples, but also integrating these into their routine ART care is crucial to preventing new infections in this population. Both the desire to have children and the number of incident pregnancies demonstrates this persists during identification of HIV discordant status making routine fertility counseling over time essential. Couples not only need counseling about options, but the safe conception methods made easily accessible to them. Clinically, genital inflammation should be regularly screened for among discordant couples to reduce risk of inflammation, ulceration, and viral shedding to ultimately reduce the risk of HIV acquisition or transmission. The study also demonstrated the value of collecting bio-behavioral data on both partners is essential to understanding transmission dynamics by identifying both individual and partnership level risk behaviors.

## 2.7 Figures and Tables

**Figure 1. Study population and seroincidence rates per 100 couple years by sex of HIV positive partner among HIV-1 serodiscordant couples in Rwanda.**

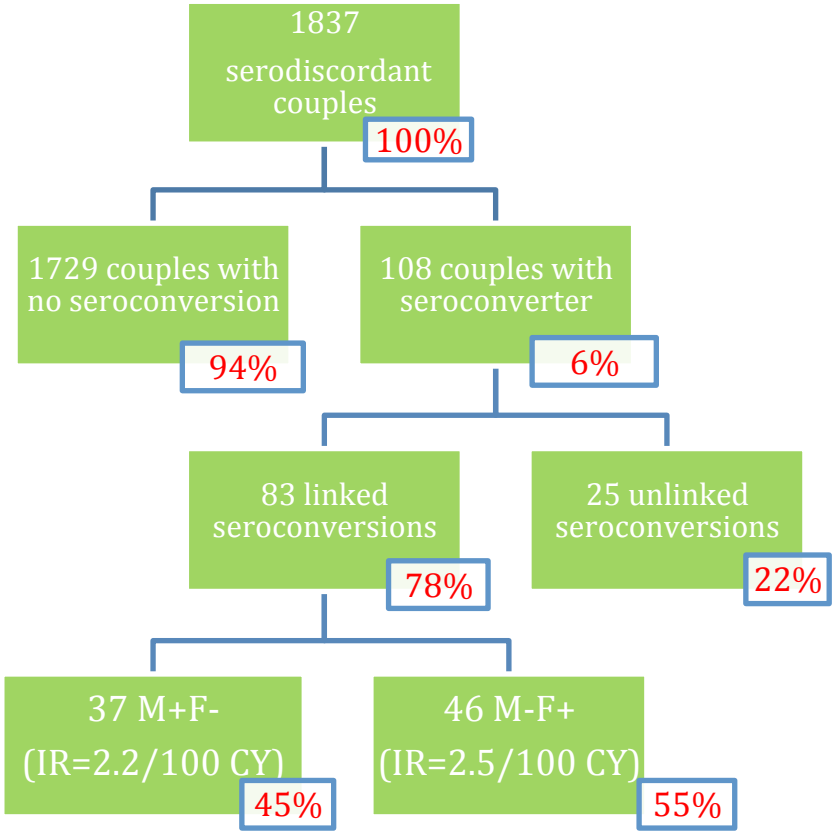


**Figure 2. Study partner linked HIV seroincidence rates and 95% confidence intervals by sex and months since study enrollment, 2002-2011.**

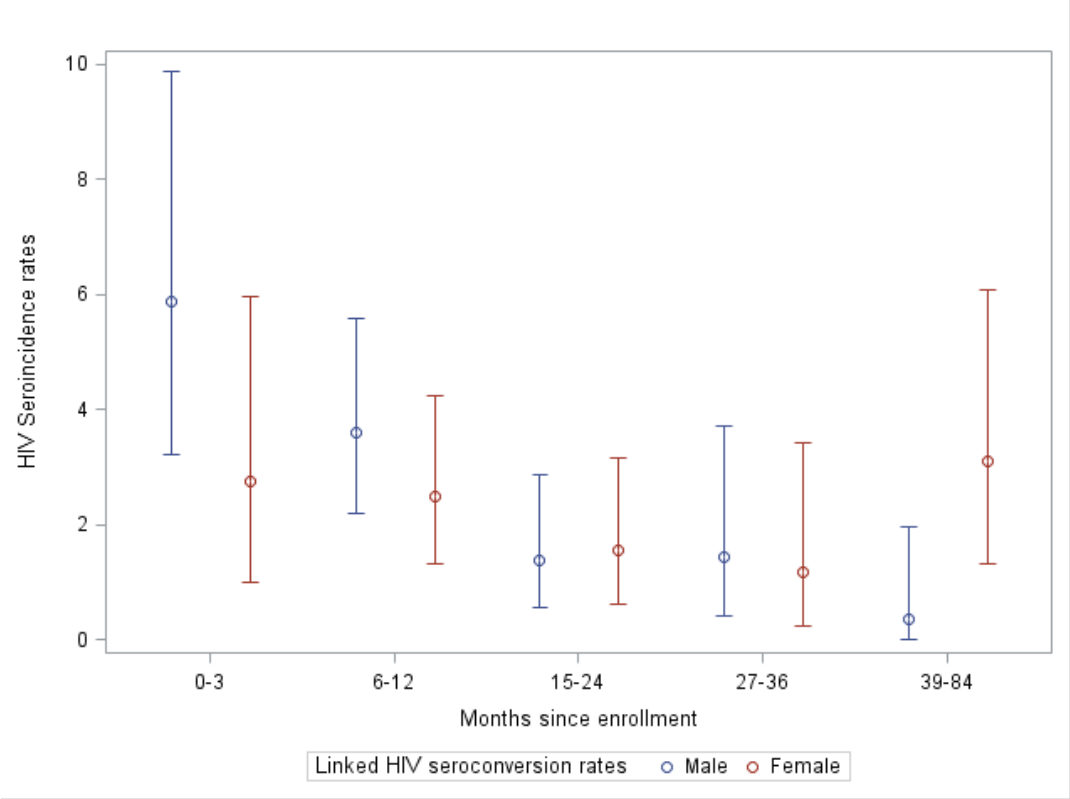
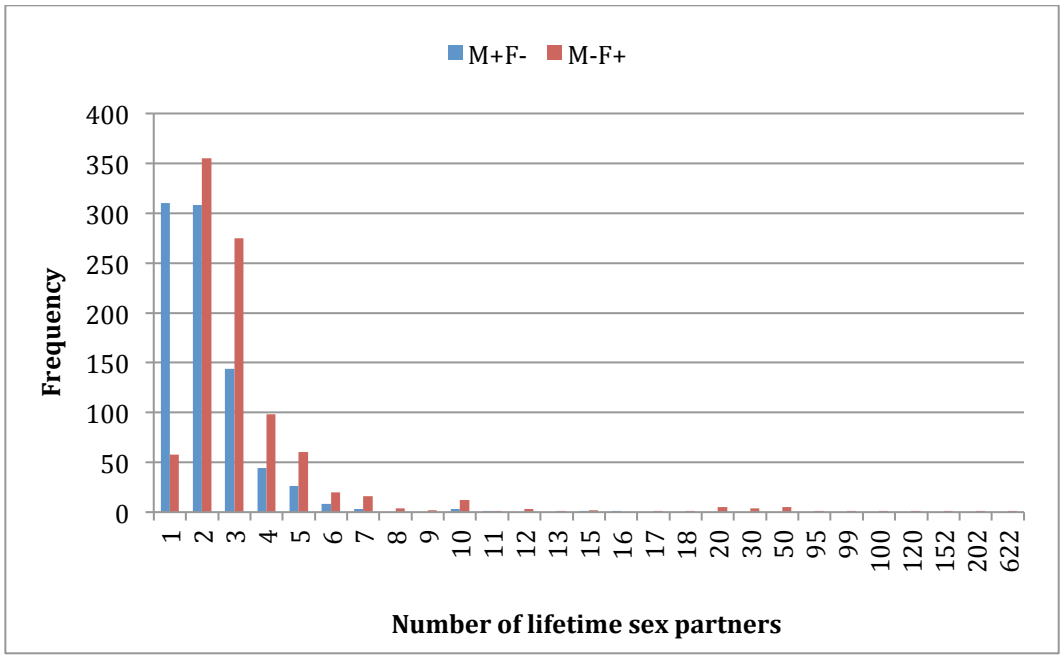


**Figure 3. Number of lifetime sex partners of females in stable serodiscordant relationships, stratified by gender of HIV positive partner.**



## Table 1. Descriptive analyses of baseline covariates by seroconversion outcomes among Rwandan HIV-1 discordant couples

| | M+F- Couples | | | | | | | M-F+ Couples | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Non-seroconverters (N = 838) | | Linked seroconverters (N =37) | | cHR | 95%CI | | Non-seroconverters (N =891) | | Linked seroconverters (N =46) | | cHR | 95%CI | |
| | N | % | N | % | | | | N | % | N | % | | | |
| **Demographics** | | | | | | | | | | | | | | |
| **Age of man*** (per year increase) | 36.6 | 7.6 | 34.7 | 7.7 | 0.97 | 0.93 | 1.01 | 34.4 | 8.6 | 35.1 | 10.2 | 1.01 | 0.97 | 1.04 |
| **Age of woman*** (per year increase) | 29.5 | 6.3 | 26.3 | 5.7 | 0.91 | 0.85 | 0.97 | 29.2 | 5.9 | 28.7 | 6.7 | 0.99 | 0.94 | 1.04 |
| **Years age difference*** (per year increase) | 7.7 | 6.1 | 8.8 | 6.9 | 1.03 | 0.98 | 1.09 | 6.3 | 6.6 | 8.4 | 6.9 | 1.03 | 0.99 | 1.07 |
| **Years cohabiting*** (per year increase) | 6.8 | 5.6 | 4.4 | 4.6 | 0.89 | 0.81 | 0.97 | 5.3 | 4.6 | 5.3 | 5.0 | 0.99 | 0.93 | 1.06 |
| **No. living children*** (per child increase) | 2.2 | 1.8 | 0.8 | 0.8 | 0.43 | 0.25 | 0.75 | 1.5 | 1.4 | 1.3 | 1.5 | 0.87 | 0.61 | 1.23 |
| **Monthly household income-USD*** (per dollar increase) | 64.5 | 75.2 | 74.7 | 77.6 | 1.00 | 1.00 | 1.01 | 61.7 | 62.0 | 62.3 | 58.0 | 1.00 | 1.00 | 1.01 |
| **Female reads Kinyarwanda** | | | | | | | | | | | | | | |
| Yes, easily | 533 | 64 | 27 | 73 | ref | | | 570 | 64 | 29 | 63 | ref | | |
| With difficulty/not at all | 301 | 36 | 10 | 27 | 0.61 | 0.30 | 1.26 | 320 | 36 | 17 | 37 | 1.01 | 0.56 | 1.85 |
| **Male reads Kinyarwanda** | | | | | | | | | | | | | | |
| Yes, easily | 623 | 75 | 26 | 70 | ref | | | 630 | 71 | 32 | 70 | ref | | |
| With difficulty/not at all | 211 | 25 | 11 | 30 | 1.40 | 0.69 | 2.84 | 260 | 29 | 14 | 30 | 1.11 | 0.59 | 2.07 |
| **Sexual History** | | | | | | | | | | | | | | |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Female number lifetime sex partners*** (per partner increase) | 5.8 | 104.2 | 2.2 | 1.2 | 1 | 0.98 | 1.01 | 4.40 | 11.60 | 18.10 | 93.40 | 1.01 | 1.00 | 1.01 |
| **Male number lifetime sex partners*** (per partner increase) | 11.3 | 28.3 | 6.1 | 3.8 | 0.96 | 0.9 | 1.03 | 10.20 | 26.70 | 5.60 | 5.10 | 0.97 | 0.93 | 1.01 |
| **Female number sex partners last year*** (per partner increase) | 1.1 | 0.4 | 1.1 | 0.2 | 1.28 | 0.69 | 2.36 | 1.10 | 0.90 | 1.10 | 0.30 | 0.97 | 0.64 | 1.47 |
| **Male number sex partners last year*** (per partner increase) | 1.5 | 2 | 1.3 | 0.7 | 0.9 | 0.55 | 1.46 | 1.40 | 0.90 | 1.40 | 0.80 | 1.03 | 0.74 | 1.42 |
| **Male drunk in last year** | | | | | | | | | | | | | | |
| Yes | 262 | 29 | 18 | 39 | 1.8 | 0.94 | 3.41 | 268 | 30 | 18 | 38 | 1.41 | 0.78 | 2.55 |
| No | 628 | 71 | 28 | 61 | ref | | | 639 | 70 | 29 | 62 | ref | | |
| **Female drunk in last year** | | | | | | | | | | | | | | |
| Yes | 43 | 5 | 1 | 2 | 0.56 | 0.08 | 4.07 | 49 | 6 | 8 | 17 | 3.15 | 1.47 | 6.77 |
| No | 789 | 95 | 36 | 98 | ref | | | 841 | 94 | 38 | 83 | ref | | |
| **Family planning** | | | | | | | | | | | | | | |
| **No. previous pregnancies*** | 3.3 | 2.2 | 2.4 | 2.1 | 0.74 | 0.6 | 0.9 | 3 | 2 | 3.2 | 2.2 | 1.01 | 0.87 | 1.16 |
| **Pregnant at baseline** | | | | | | | | | | | | | | |
| Yes | 265 | 32 | 14 | 41 | 1.67 | 0.84 | 3.31 | 210 | 24 | 11 | 24 | 1.05 | 0.53 | 2.08 |
| No | 553 | 68 | 20 | 59 | ref | | | 663 | 76 | 34 | 26 | ref | | |
| **Female want child** | | | | | | | | | | | | | | |
| Yes | 44 | 8 | 8 | 22 | 6.68 | 1.67 | 8.03 | 56 | 9 | 6 | 18 | 2.35 | 0.97 | 5.69 |
| Don't know/No | 513 | 92 | 29 | 78 | ref | | | 549 | 91 | 28 | 82 | ref | | |
| **Male want child** | | | | | | | | | | | | | | |
| Yes | 47 | 9 | 10 | 27 | 3.76 | 1.82 | 7.77 | 63 | 11 | 6 | 18 | 1.66 | 0.68 | 4.01 |
| Don't know/No | 479 | 91 | 27 | 73 | ref | | | 501 | 89 | 27 | 82 | ref | | |
| **Clinical** | | | | | | | | | | | | | | |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **HIV stage of positive partner** | | | | | | | | | | | | | | |
| Stage I | 279 | 33 | 15 | 41 | ref | | | 395 | 44 | 13 | 28 | ref | | |
| Stage II | 354 | 42 | 13 | 35 | 0.75 | 0.36 | 1.58 | 318 | 36 | 21 | 46 | 2.29 | 1.12 | 4.65 |
| Stage III & IV | 205 | 25 | 9 | 24 | 0.96 | 0.42 | 2.2 | 178 | 20 | 12 | 26 | 2.62 | 1.20 | 5.75 |
| **Viral Load of positive partner** (log10 copies/ml)* | 4.1 | 1 | 4.8 | 0.7 | 2.89 | 1.78 | 4.68 | 3.7 | 1 | 4.3 | 1.2 | 1.7 | 1.26 | 2.28 |
| **Circumcised male partner** | | | | | | | | | | | | | | |
| Yes | 143 | 17 | 4 | 11 | 0.62 | 0.22 | 1.75 | 221 | 25 | 3 | 7 | 0.21 | 0.07 | 0.69 |
| No | 695 | 83 | 33 | 89 | ref | | | 667 | 75 | 43 | 93 | ref | | |
| **Female had STI in last year** | | | | | | | | | | | | | | |
| Yes | 286 | 35 | 15 | 44 | 1.27 | 0.65 | 2.51 | 479 | 55 | 31 | 69 | 1.81 | 0.96 | 3.4 |
| No | 532 | 65 | 19 | 56 | ref | | | 394 | 45 | 14 | 31 | ref | | |
| **Male had STI in last year** | | | | | | | | | | | | | | |
| Yes | 311 | 38 | 21 | 62 | 2.35 | 1.18 | 4.71 | 239 | 27 | 24 | 53 | 2.75 | 1.53 | 4.94 |
| No | 507 | 62 | 13 | 38 | ref | | | 634 | 73 | 21 | 47 | ref | | |

USD: United States Dollar; STI: sexually transmitted infection; cHR: crude hazard ratio; CI: confidence interval; VL: viral load

* continuous variable, mean and standard deviation reported

**Table 2. Descriptive analyses of time-varying covariates by HIV seroconversion outcomes in Rwandan HIV-1 discordant couples**

| | PARTNER LINKED HIV INFECTION IN FEMALES | | | | | | | PARTNER LINKED HIV INFECTION IN MALES | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Non-transmitting intervals | | Linked transmission interval | | cHR | 95%CI | | Non-transmitting intervals | | Linked transmission interval | | cHR | 95%CI | |
| | n= 9415 | % | n= 37 | % | | | | n= 7729 | % | n= 46 | % | | | |
| **Clinical Characteristics** | | | | | | | | | | | | | | |
| Genital inflammation-Female | | | | | | | | | | | | | | |
| Yes | 843 | 9% | 11 | 30% | 4.78 | 2.31 | 9.88 | 1164 | 15% | 18 | 39% | 3.71 | 2.00 | 6.89 |
| No | 8551 | 91% | 26 | 70% | ref | | | 6521 | 85% | 28 | 61% | ref | | |
| Genital ulcer-Female | | | | | | | | | | | | | | |
| Yes | 224 | 2% | 2 | 5% | 2.20 | 0.53 | 9.21 | 422 | 6% | 5 | 11% | 2.06 | 0.80 | 5.32 |
| No | 8961 | 98% | 35 | 95% | ref | | | 6782 | 94% | 41 | 89% | ref | | |
| Genital inflammation-Male | | | | | | | | | | | | | | |
| Yes | 587 | 11% | 5 | 15% | 1.65 | 0.63 | 4.35 | 400 | 7% | 10 | 22% | 4.41 | 2.14 | 9.11 |
| No | 4902 | 89% | 28 | 85% | ref | | | 5702 | 93% | 35 | 78% | ref | | |
| Genital ulcer-Male | | | | | | | | | | | | | | |
| Yes | 340 | 6% | 3 | 9% | 1.74 | 0.53 | 5.76 | 224 | 4% | 3 | 8% | 2.05 | 0.62 | 6.75 |
| No | 5146 | 94% | 31 | 91% | ref | | | 5870 | 96% | 37 | 92% | ref | | |
| **Sexual Behavior** | | | | | | | | | | | | | | |
| Any unprotected sex with study partner since last visit | | | | | | | | | | | | | | |
| Yes | 2354 | 27% | 22 | 60% | 3.90 | 2.01 | 7.70 | 2098 | 31% | 24 | 53% | 2.36 | 1.30 | 4.27 |
| No | 6359 | 73% | 15 | 40% | ref | | | 4603 | 69% | 21 | 47% | ref | | |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number unprotected sex acts with study partner since last visit* | 3.7 | 13.7 | 8.4 | 17.7 | 1.01 | 1.00 | 1.03 | 5.4 | 18.7 | 13 | 28.9 | 1.01 | 1.00 | 1.02 |
| Number of sexual partners since last visit* | 1.0 | 0.5 | 1.0 | 0.0 | 2.26 | 0.49 | 10.36 | 1.0 | 0.6 | 1.0 | 0.22 | 0.59 | 0.16 | 2.25 |
| Sperm present on wet prep | | | | | | | | | | | | | | |
| Yes | 319 | 4% | 1 | 3% | 1.14 | 0.15 | 8.54 | 331 | 5% | 5 | 12% | 2.65 | 1.03 | 6.85 |
| No | 8381 | 96% | 28 | 97% | ref | | | 6635 | 95% | 38 | 88% | ref | | |
| **Family Planning** | | | | | | | | | | | | | | |
| Pregnant during interval | | | | | | | | | | | | | | |
| Yes | 805 | 13% | 7 | 22% | 1.94 | 0.823 | 4.57 | 718 | 13% | 6 | 20% | 1.57 | 0.63 | 3.89 |
| No | 5546 | 87% | 25 | 78% | ref | | | 4826 | 87% | 24 | 80% | ref | | |
| Breastfeeding during interval | | | | | | | | | | | | | | |
| Yes | 3682 | 50% | 16 | 47% | 0.94 | 0.48 | 1.86 | 1046 | 15% | 7 | 16% | 0.96 | 0.43 | 2.18 |
| No | 3732 | 50% | 18 | 53% | ref | | | 5705 | 85% | 37 | 84% | ref | | |
| Contraceptive method used at last visit | | | | | | | | | | | | | | |
| Non-hormonal** | 8197 | 87% | 32 | 84% | ref | | | 6343 | 83% | 41 | 89% | ref | | |
| Implant | 502 | 6% | 0 | 0% | - | - | - | 347 | 5% | 0 | 0% | - | - | - |
| Injectables | 595 | 6% | 5 | 13% | 2.09 | 0.8 | 5.47 | 818 | 11% | 4 | 9% | 0.84 | 0.30 | 2.36 |
| OCPs | 92 | 1% | 0 | 0% | - | - | - | 127 | 2% | 1 | 2% | 1.12 | 0.16 | 9.00 |

OCP: oral contraceptive pill; cHR: crude Hazard Ratio; CI: confidence interval

* Continuous variable, mean and standard deviation reported

**IUD, condoms alone, permanent method, or none

^p-values are 2-tailed

**Table 3. Multivariable models of predictors of time to linked HIV transmission among Rwandan men and women in HIV-1 discordant relationships**

| FEMALE HIV INCIDENT INFECTION | | | | |
|---|---|---|---|---|
| | **aHR** | **95%CI** | | **p-value** |
| ***Baseline*** | | | | |
| Female age | 1.01 | 0.93 | 1.10 | 0.81 |
| Number of previous pregnancies | 0.88 | 0.65 | 1.17 | 0.37 |
| Male partner had STI in past year | 1.09 | 0.46 | 2.60 | 0.85 |
| Man wants child | 2.08 | 0.62 | 6.96 | 0.24 |
| Woman wants child | 1.27 | 0.32 | 4.95 | 0.74 |
| Baseline Viral Load (log transformed) | 2.33 | 1.28 | 4.24 | <0.01 |
| ***Time-varying*** | | | | |
| Genital inflammation in female | 4.77 | 1.72 | 13.21 | <0.01 |
| Any unprotected sex with study partner since last visit | 3.29 | 1.27 | 8.51 | 0.01 |
| Number of unprotected sex acts since last visit | 1.02 | 0.98 | 1.06 | 0.28 |
| **MALE HIV INCIDENT INFECTION** | | | | |
| | **aHR** | **95%CI** | | **p-value** |
| ***Baseline*** | | | | |
| Female age | 1.03 | 0.96 | 1.09 | 0.45 |
| Number of lifetime sex partners of female | 0.82 | 0.24 | 2.80 | 0.75 |
| Male had STI in past year | 2.07 | 0.94 | 4.58 | 0.07 |
| Circumcised male partner | 0.24 | 0.06 | 1.05 | 0.06 |
| Female ever drunk in past year | 0.61 | 0.20 | 1.90 | 0.39 |
| HIV Stage II v. I | 1.24 | 0.48 | 3.17 | 0.66 |
| HIV Stage III/IV v. I | 0.62 | 0.20 | 1.98 | 0.42 |
| Viral Load (log transformed) | 2.14 | 1.50 | 3.07 | <0.01 |
| ***Time-varying*** | | | | |
| Genital inflammation in female partner | 3.91 | 1.71 | 8.94 | <0.01 |
| Genital inflammation in male partner | 2.54 | 0.82 | 7.90 | 0.11 |
| Any unprotected sex with study partner since last visit | 3.56 | 1.48 | 8.56 | <0.01 |
| Number of unprotected sex acts since last visit | 1.01 | 0.99 | 1.02 | 0.45 |
| Sperm present on wet prep at visit | 3.35 | 0.99 | 11.36 | 0.05 |

aHR: adjusted Hazard Ratio; CI: confidence interval; ^p-values are 2-tailed

# Chapter III. A Closer Look: Describing Pregnancy and Hormonal Contraception in a 10-year cohort of HIV-1 Serodiscordant Couples in Rwanda

## 3.1 Abstract

**Objectives:** To describe incident pregnancy, hormonal contraception use, and fertility desires among ART naïve heterosexual HIV-1 serodiscordant couples.

**Methods:** HIV-1 serodiscordant heterosexual couples were enrolled from couples counseling and testing sites in Kigali. Demographic, behavioral, and clinical exposures including type of contraception use were measured in both partners at baseline and every three months. Prevalent and incident pregnancy were reported and confirmed with urine test at each three-month visit. Incident pregnancy rates were calculated for the cohort and by sex of the positive partner (M+F-/M-F+). Number of incident pregnancies in women by HIV status and contraception use during interval of conception was described. Hormonal contraception ever use by woman's HIV status was described by baseline covariates and multivariable regression models were estimated.

**Results:** The overall pregnancy incidence rate for the cohort 12.7/100 PY (95%CI: 11.3-14.1). In M-F+ couples, the pregnancy incidence rate was 13.2/100 PY (95%CI: 11.3-15.3) and 12.1/100 PY (95%CI: 10.2-14.1) in M+F- couples. 34% of HIV-1 positive women had ever used HC, 26% of HIV- women who did not seroconvert had ever used HC and 25% of HIV- women who seroconverted during study were HC ever users. In adjusted regression models, among HIV+ women HC ever use was associated with being younger (aRR 0.97; 95% CI: 0.95-0.99), ability to read Kinyarwandan easily (aRR 1.28; 95% CI: 1.06-1.55),

and not having an STI in the past year (aRR 0.80; 95%CI: 0.67-0.95). Among HIV negative women who did not seroconvert, HC ever use was associated with younger age (aRR 0.98; 95% CI: 0.96-1.0) and not being pregnant at baseline (aRR 0.72; 95% CI: 0.55-0.94). Across HIV groups, injectables were the most frequently used type of hormonal contraception at last visit and during most of study follow-up. Among HIV+ women who became pregnant, 1% used injections, 1% used OCP and 1% had an IUD, while the vast majority (97%) reported condoms or no method of contraception at time of conception. Two percent of HIV- women who never seroconverted reported using an injection and 1% OCP during the interval of conception, while all HIV- women who eventually seroconverted reported no method or condoms only.

**Conclusions:** Hormonal contraception use among women in serodiscordant couples was low given the counseling and availability. High pregnancy rates, particularly among the HIV+ women, suggest the need for more effective and widely available safe conception methods.

## 3.2 Background

The population of east and southern Africa is growing at approximately 2.85% per year, with a total fertility rate (TFR) of 4.7 children per woman. [65] Pregnancy causes complex biological and behavioral changes in a woman and often sexual behavior changes in the partnership. Simply trying to conceive may increase the risk of HIV transmission in HIV serodiscordant couples. [68,69] HIV serodiscordant couples must consider complex cultural expectations such as meeting family and social obligations concerning reproduction, individual fertility desires, and partnership dynamics which confound decisions about the risk of HIV transmission. [65] Although some studies have found pregnancy in the HIV-1-infected or uninfected female to be associated with two-fold increased risk of male-to-female and female-to-male HIV-1 transmission, [63] other studies have found more variable associations between pregnancy and post-partum periods and HIV transmission when looking at acquisition and transmission separately. [43-47] The Partners in Prevention HSV/HIV Transmission study found pregnancy increased the risk of female-to-male seroconversion but not male-to-female seroconversion. [36]

With the widespread uptake of ART and prevalent PMTCT, expectations of a longer life with an improved quality and reproductive capability have led to increasing pregnancy rates among women living with HIV, including among serodiscordant couples. [36] Unplanned pregnancy remains an issue for both HIV positive and HIV negative women in serodiscordant couples in Africa [64,67,69,70], and in Rwanda specifically [71].

Accessible and effective contraception is essential for women's health as it can reduce maternal and infant mortality and morbidity and improve infant and maternal

health. [40-41,70] In the context of HIV, safe and effective contraception prevents pregnancy, negating the possibility of vertical HIV-1 transmission from mother to child. Specifically, HC methods such as injectable depot medroxyprogesterone acetate (DMPA), norethisterone enanthate (NET-EN), and combined oral contraceptive pills (COCs) are increasingly used and critical to reducing unwanted pregnancy in high HIV prevalence areas in sub-Saharan Africa. [40-41] The WHO consultation and guidelines produced in 2012 recommended no restrictions on the use of hormonal contraceptive methods by women with or at high risk of HIV infection and remained unchanged in 2014 after a review of the new evidence. [64] The only addition was a recommendation that given uncertainty in the current literature, women at high risk of HIV on progestogen-only injectable contraceptives should be informed it may or may not increase their risk of HIV acquisition and be counseled and have access to dual HIV preventive measures. [64] Currently, there is inconclusive evidence on the association between HC use and HIV infection despite multiple observational studies and secondary analysis of RCTs, [40] recent rigorous longitudinal data analysis, [39] a large sample individual participant data meta-analysis [73] and a pooled meta-analysis of observational studies. [74] Some, but not all [75], high quality observational studies have demonstrated an increased risk for HIV-1 acquisition among women using DMPA specifically. [74]

Given the complex nature of these reproductive issues, the need to better describe pregnancy and hormonal contraception use among women, specifically in stable HIV serodiscordant relationships, over long periods of time during their reproductive age remains. Detangling the risk factors involved in conception or actively trying not to

conceive when the woman or the man is HIV positive is difficult and involves the investigators' conceptualization of each causal pathway separately to determine confounders and mediators and appropriate analytic techniques. As this can be somewhat subjective and can change over time, conception and pregnancy may confound and/or mediate the risk of HIV transmission [45,46]. We describe the HC and pregnancy experience of women in ART naïve HIV-1 serodiscordant relationships in Rwanda to better inform both potential conceptual frameworks to guide analyses and targeted public health programs.

## 3.3 Methods

***3.3.1 Study Participants:*** Heterosexual HIV-1 serodiscordant couples (one partner is HIV-positive and the other HIV-negative) were invited to enroll in an open cohort study between 2002-2011, after being identified as eligible from couples (married or cohabiting) in Kigali, Rwanda who attended couples' voluntary HIV counseling and testing (CVCT) services. Couples either self-presented or presented after receiving an invitation from a community CVCT promoter. CVCT services included group counseling, rapid HIV testing, and post-test couples counseling with mutual disclosure of results. Couples were eligible to participate if they were confirmed HIV-1 serodiscordant, had been together at least 3 months and planned on staying in the Kigali region for at least a year. Couples were ineligible if either couple had a CD4 count <200 or either partner was on ART. Written informed consent was obtained from the couple jointly.

***3.3.2 Variables of interest:*** Incident pregnancy was identified by clinically confirmed pregnancy or a positive pregnancy test at a follow-up visit (every 3 months or if client-

initiated an interim visit). Pregnancy conception interval was calculated as the study interval prior to the clinically confirmed first report of incident pregnancy.

Hormonal contraceptive use during study follow-up time was dichotomized to create an ever vs. never use of hormonal contraceptives variable for comparison across three groups of women. Women with at least one reported time interval of using of injectables including depo-medroxyprogesterone acetate (DMPA) and norethisterone enanthate (NET-EN), oral contraceptive pills (OCPs), or contraceptive implant, during follow-up were classified as "HC ever users." Women that reported using only condoms, an intrauterine device (IUD), a permanent method (hysterectomy or tubal ligation), and/or no contraceptive method across all study periods were classified as "HC never users." Women were stratified by their HIV status at baseline and the subgroup of HIV negative women who seroconverted (including both linked and unlinked transmissions) during the study made up a third comparison group.

Baseline couple measures of interest included age disparity; years cohabiting; number of previous pregnancies; and monthly income (USD). Individual exposures of interest at baseline included age of each partner; female Kinyarwandan literacy; history of STI in the past year of each partner; female age at sexual debut; female number of sex partners in past year and lifetime; number of previous pregnancies; current pregnancy; fertility desires of each partner, if either partner was drunk in the past year, and serologic confirmation of herpes simplex virus 2 (HSV-2) in the female partner.

***3.3.3 Data Collection:*** Data was collected from a single clinical site in Kigali. From 2002 through 2006, both partners were seen quarterly after the initial follow-up at 1 month for

routine physical and genital exams, a blood sample for STI and HIV testing (negative partner), a vaginal swab wet mount (to determine bacteria, yeast cells, trichomoniasis, white blood cells to show an infection, or clue cells that show BV), and assessment of prevalent and incident pregnancy by blood test and asking women how many months currently pregnant and pregnancy outcome since last visit. Self-reported hormonal contraceptive use was validated by clinical records maintained at the site as it was given free on site during the participant's study enrollment.

Questionnaires asked about ever and current use of methods at baseline and follow-up, which included categories for no method, condoms alone, combined COPs, DMPA injectables (150mg IM dosage), copper IUD, contraceptive implant (Norplant, Jadelle), or permanent methods (hysterectomy/ tubal ligation/vasectomy). Self-reported decision regarding current method, reason(s) for stopping/switching current method, and new contraception method were also captured at each three-month visit.

Beginning in January 2007 through 2011, all HIV-negative partners were seen at visit months 0, 1, 2, and 3. Starting at visit month 3 and quarterly thereafter, a risk assessment was conducted to establish recent exposure within the partnership. Couples assessed as 'higher risk' were asked to come back monthly for repeat HIV testing until the next quarterly visit, at which time the risk assessment was repeated. 'Higher risk' was defined as having at least one of the following since last visit: self-reported unprotected sex, sperm or trichomonas on a wet prep, incident pregnancy, or incident syphilis.

Study questionnaires included demographic and psychosocial data, sexual risk behaviors, medical history, and health services data. Data were collected by participant

completion of written questionnaire in Kinyarwandan or English (depending on preference of the participant) and face-to-face (FTF) interviews were conducted if literacy was a problem or preferred.

Couples were censored if either partner died, the couple separated, either partner was lost to follow-up, or if the HIV-positive partner started ART. All follow-up visits included risk reduction counseling, access to hormonal and permanent contraception methods and free condoms. Free outpatient health care was provided at the research clinic.

***3.3.4 Data Analysis:*** Person-years (PYs) at risk of pregnancy were computed for each woman from enrollment until either the couple was censored or the HIV-negative partner seroconverted. Time excluded from the time at risk were all days a woman was pregnant and, if had a live birth and indicated breastfeeding, an additional six months postpartum to account for the reduction of fertility while breastfeeding. Incident pregnancy rate was calculated as the number of incident pregnancies per CYs of follow-up time at risk.

Descriptions of baseline categorical variables (counts and percentages) and continuous variables (means and standard deviations) were stratified across HIV groups (HIV+, HIV-, and HIV seroconverters) (Table 1). To compare differences between HC ever/never users within HIV stratified groups, two sample t-tests were performed on continuous variables and Pearson's Chi-square test for independence conducted on categorical variables, and p-values presented. Baseline factors associated with HC ever use ($P<.05$) in bivariate analyses were used in multivariable regression models to estimate their association to HC ever use stratified by HIV status group (Table 2).

Contraceptive method reported at time of conception was reported by HIV serostatus of woman (Figure 1).

***3.3.5: Ethics:*** 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 Government of Rwanda.

## 3.4 Results

There were 334 incident pregnancies over 2640 person-years (PY) making the overall pregnancy incidence rate for the cohort 12.7/100 PY (95%CI: 11.3-14.1). One hundred eighty two pregnancies occurred in HIV+ women (M-F+) over 1379 PY (IR=13.2/100 PY; 95%CI: 11.3-15.3) and 152 occurred in HIV- women (M+F-) over 1261 PY (12.1/100 PY; 95%CI: 10.2-14.1).

Of the 1837 women in serodiscordant relationships, 52% were HIV-1 positive (n=955), 45% of the women were HIV-1 negative and never seroconverted (n= 838), and 3% of the women were HIV-1 negative at baseline who seroconverted during the study (n=44). Among HIV-1 positive women, 34% (n=328) were HC ever users and 66% (n=627) were HC never users. Among HIV-1 negative women who did not seroconvert, 26% (n=215) were HC ever users and among women who seroconverted during study, 25% (n=11) were HC ever users and (Table 1).

Among HIV positive women, women who were HC ever users differed significantly from HC never users at baseline by age (mean age 28 vs. 30), years cohabiting with their partner (mean years 4.7 vs. 5.6), ease with which read Kinyarwandan (69% vs. 61%). HIV+ HC ever users also had a lower mean number of lifetime sex partners (3.5 vs.

5.9), and were less likely to have an STI in the past year (50% vs. 59%) than HC never users (Table 1).

Among HIV negative women who never seroconverted, fewer HC ever users were pregnant at baseline (26% vs. 35%). HIV negative women who eventually seroconverted and were HC ever users only differed from their counterparts if their male partners had an STI in the previous year (82% vs. 47%) (Table1).

In bivariate regression models, HC ever use by HIV+ women was associated with a younger woman (cRR 0.97; 95%CI: 0.96-0.98), fewer years cohabitating with partner (cRR 0.98; 95%CI: 0.96-1.0), woman's ability to read Kinyarwandan easily (cRR 1.26; 95%CI: 1.04-1.53), and the woman partner having an STI in the past year (cRR 0.79; 95%CI: 0.66-0.94). HC ever use in HIV- women who did not seroconvert, was not significantly associated with any covariates of interest.

In adjusted regression models, among HIV+ women HC ever use was associated with being younger (aRR 0.97; 95% CI: 0.95-0.99), ability to read Kinyarwandan easily (aRR 1.28; 95% CI: 1.06-1.55), and not having an STI in the past year (aRR 0.80; 95%CI: 0.67-0.95). Among HIV negative women who did not seroconvert, HC ever use was associated with younger age (aRR 0.98; 95% CI: 0.96-1.0) and not being pregnant at baseline (aRR 0.72; 95% CI: 0.55-0.94). In HIV- women who seroconverted, HC ever use was not associated with any of our covariates of interest due to small sample size (Table 2).

Pregnancy during follow-up was not uncommon, with 15% of HIV+ (n=146) positive women becoming pregnant with 182 incident pregnancies during follow-up as well as 15% of HIV- women (n=128) experiencing 143 incident pregnancies, and 18% (n=9) of

HIV negative women that seroconverted having 9 incident pregnancies (Figure 1). The most multigravida women were found in the HIV+ group, with 19 women having two pregnancies during follow-up and 3 HIV+ women being pregnant 3 separate times. Comparatively, eighteen HIV- women and one woman who seroconverted had a second pregnancy (data not shown).

Among HIV+ women who were pregnant, 1% (n=2) reported using an injection method, 1% (n=2) reported use of OCP, 1% (n=2) had an IUD, and 97% (=140) reported using condoms and/or no contraceptive method during the interval of conception. Two percent (n=2) of HIV- women reported using an injection and 1% OCP during the interval of conception. Among HIV- women who seroconverted, only condoms or no method was reported at time of conception for fall pregnancies (Figure 1).

## 3.5 Discussion

We described pregnancy rates, incident pregnancies and women who ever used hormonal contraception in urban Rwandan women in ART naïve HIV-1 serodiscordant relationships. From a practical standpoint, understanding pregnancy rates and contraceptive use over long periods of time helps us identify factors that can inform safer conception and fertility programs for HIV serodiscordant couples. While other studies found factors affecting women's contraceptive use included physical access, cost, lack of accurate information and limited knowledge of available services [75], this study was based in couples counseling and testing services and each follow-up visit included counseling and free provision of most all modern methods of contraception (various hormonal, permanent, condoms) as well as any additional clinical care necessary for HC use. This provided an

environment in which such previously stated structural barriers were minimized leaving us to assess more behavioral and clinical factors associated with use.

In one study of serodiscordant couples in Rwanda and Zambia, less than two-thirds of women in HIV-1 serodiscordant couples had ever used any contraceptive method. [78] Many of these women cited fear of side effects as the barrier to use, regardless of a high awareness of HC. [78] We observed a relatively low prevalence of hormonal contraceptive methods overall, at last visit, and high prevalence of condoms/no contraceptive method used. We believe the low HC use could reflect a desire for pregnancy as the relatively high number of incident pregnancies across all HIV groups, even in light of the HC use, necessitates a more in-depth look at HC failure rates and potential user errors such as elongated time between visits or frequency and type of method switching. The desire for pregnancy regardless of risk associated with possible HIV infection may be more important or could be influenced by larger unmeasured cultural factors such as stigma with couples without children, or even a potential desire to grow families following the 1994 Genocide in the earlier study years. One intervention with heterosexual HIV serodiscordant couples in Kenya providing family planning counseling and free contraception led to significant declines in pregnancy and sustained high reports of condom use [112]. Thus, it is important to ascertain fertility desires, discuss risks while trying to conceive and during pregnancy, and provide contraceptive options, including long-acting methods such as IUDs and implants for couples that do not want to have children.

Fertility preferences, religious traditions, partner communication, and fear of side effects [76-79] have been found to be associated with Rwandan woman's adoption of contraception methods in previous studies. We found woman's fertility intentions at baseline did not differ by HC ever versus never user in any of the three groups of women. Among couples that were pregnant during follow-up, the majority of both the female and male partners across HIV groups had expressed the intention to not have children at baseline. To explore this further we examined male and female and concordant fertility desires at baseline and found they did not predict did not reflect any HC use during follow-up nor incident pregnancy (data not shown). This could be a reflection of couples, newly aware of their serodiscordant status, answering questions about future fertility desires that are very complex. Unfortunately, fertility desires were not formally recorded during follow-up to better understand if the pregnancies observed were intended or unintended.

According to a national study on unintended pregnancy and abortion in Rwanda in 2013, nearly half (47%) of all pregnancies in the country were unintended. [71] Among women with and at-risk for HIV-1 infection, dual contraception of using both condoms and an effective hormonal contraceptive is widely accepted as an important standard for preventing unintended pregnancies and HIV transmission. [64] However, couples may stop using condoms when they commit to a partner and if they are trying to conceive [35]. We were unable to look at dual protection over time. Very limited resources for HIV serodiscordant couples that wished to get pregnant and deliver safely existed in Rwanda during the study, particularly the first few years. Safe conception practices have become

more prevalent over time, but remain a gap in HIV prevention and/or ART care services for this important high-risk population. [15,16,19]

Although some studies have found pregnancy in the HIV-1-infected or uninfected female to be associated with two-fold increased risk of male to female and female to male HIV-1 transmission [63], other studies have found more variable associations when they looked at HIV-1 acquisition and transmission separately [43-47]. Among the 182 HIV+ women in our study with an incident first pregnancy, there were 10 linked seroconversions in their male partners, but only 5 of these were genetically linked, indicating risk of HIV as well as risk of pregnancy from outside partners must be included in couples counseling services. Culturally specific beliefs and practices such as the need to feed the fetus sperm from the father for it to grow during gestation [108] or the Rwandan practice to have sex one week after giving birth [109] are important to address in counseling interventions but also warrant further investigation as they may be key risk behaviors that drive HIV transmission in either the pre-natal period or in the subsequent postpartum sexual practices.

Limitations to our study included the crude outcome of HC never vs. HC ever users, limiting our ability to look at time-varying factors due to temporality resulting in correlations. Also the HC ever/never outcome only measures at least one 3-month time interval of exposure to HC and it is possible in-depth analyses of long-term users of HC and those that "tried" HC or switched frequently may be more informative to targeting safe conception practices in important sub-populations for clinical scoring tools and prediction models [38]. Yet due to the overall low overall prevalence of HC ever use, we do not expect the ability to infer much more given small numbers.

The amount of study time differed by HC use in both HIV+ and HIV- groups. Because this study offered free hormonal contraction (implants, injections, OCPs) it may have been that HC users were incentivized to stay in the study to receive free hormonal contraception and access to other reproductive and medical services, creating a differential follow-up by HC user group. We don't believe this differential follow-up to be great as average study group by both groups was quite long and the prevalence of HC use was quite low. The recruitment/self-selection into the cohort study from a couples counseling and testing center couple potentially introduce selection bias. Data was collected by questionnaires administered by clinical nursing staff, which may have resulted in social desirability bias on sensitive issues around outside partners, frequency of sex, and condom use. It is also possible that ART scale-up in Rwanda in 2007 may have changed HC behaviors, but because this was an ART naïve cohort and couples were censored at ART initiation, we don't believe there would be differential use of HC in this ART naïve cohort, but it may have influenced study follow-up time.

## 3.6 Conclusions

Our study demonstrated that any exposure to hormonal contraception use remained relatively low in a 10-year cohort, potentially reflecting more "real-life" choices of HIV-serodiscordant couples in a pre-ART period. We observed important differences between HC ever users in each stratified group of women, indicating prediction models and scoring tools need to assess pregnancy risk differently depending on the sex of the HIV+ partner in the serodiscordant relationship and have a time component. With the prevalent scale-up of ART in Rwanda, safe contraception methods to limit unintended pregnancies and

interventions to limit failed hormonal contraceptive method use are needed as much as simple but safe methods to get pregnant when desired. Aligning the progression of stable couples' fertility desires over time with their progression of ART experiences (viral load suppression, toxicity and/or co-morbidities, non-adherence, aging out of reproductive period, etc.) could facilitate an integrated safer conception tool for ART programs. Just as contraception failures may result in pregnancy, treatment failures may result in new infections of partners when dual protection contraceptive methods are not used, so understanding the dynamic influences of family planning in the context of HIV, and now prevalent ART, remains a pertinent area of investigation for heterosexual serodiscordant couples.

## 3.7 Figures and Tables

**Figure 1. Pregnancy and contraception method use during time of conception of Rwandan women in HIV serodiscordant relationships, by HIV status, 2002-2011.**

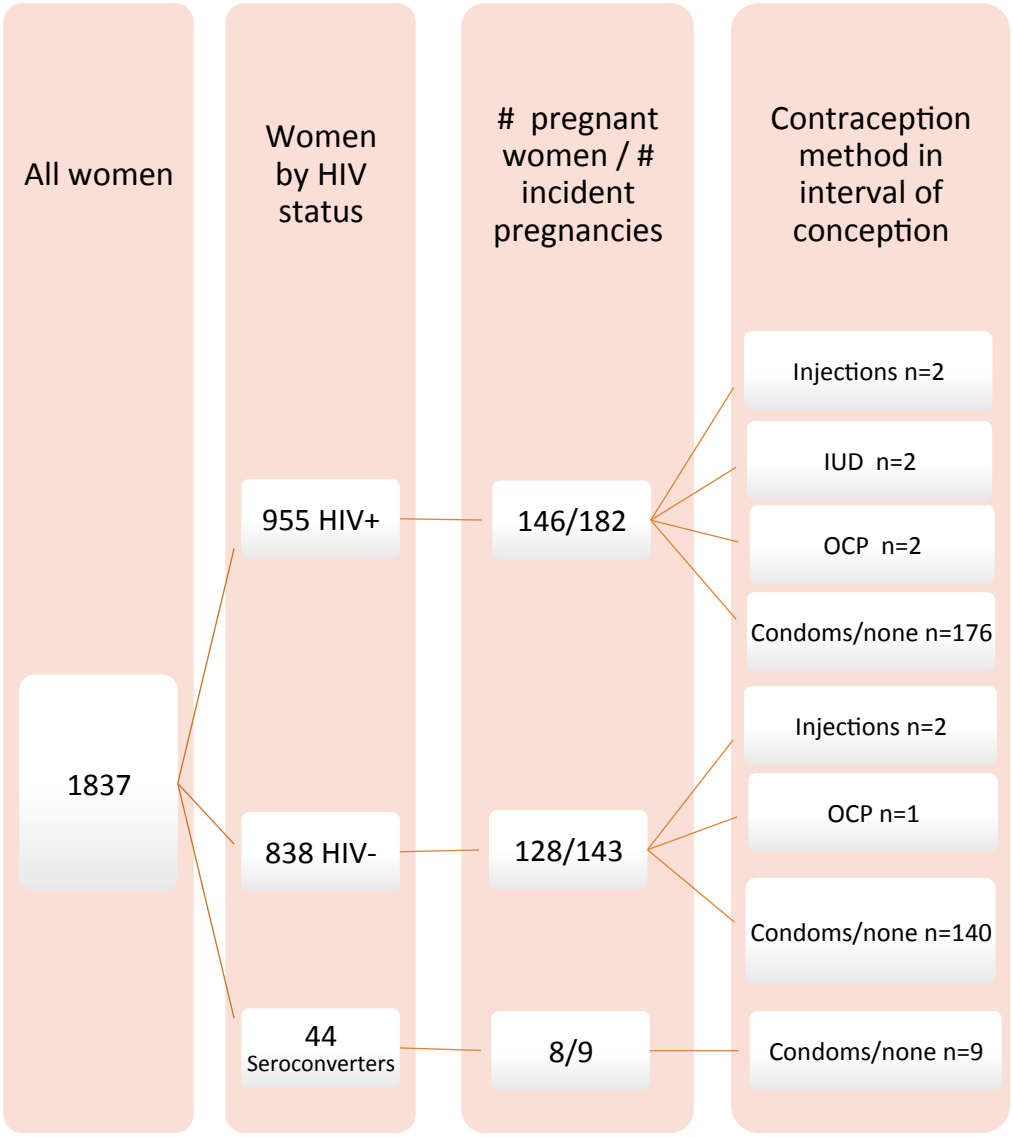


**Table 1. Descriptive statistics of baseline variables by hormonal contraception use by HIV status of women in HIV serodiscordant relationships in Rwanda, 2002-2011**

| | HIV + (n=955) | | | | HIV- nonseroconverters (n=838) | | | | HIV Seroconverters (n=44) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | HC Never User (N=627) | | HC Ever User (N =328) | | HC Never User (N=623) | | HC Ever User (N =215) | | HC Never User (N=33 ) | | HC Ever User (N =11) | |
| | N | % | N | % | N | % | N | % | N | % | N | % |
| *Demographics* | | | | | | | | | | | | |
| Woman's age** (mean, SD) | 29.7 | 6.3 | 27.9 | 5.1 | 29.7 | 6.4 | 28.8 | 5.8 | 26.0 | 6.2 | 27.9 | 4.6 |
| Age difference with partner in years (mean, SD) | 6.7 | 6.2 | 7.0 | 6.6 | 7.8 | 6.2 | 7.6 | 5.9 | 8.9 | 7.3 | 12.0 | 9.2 |
| Years cohabiting with partner* (mean, SD) | 5.6 | 4.9 | 4.7 | 3.8 | 6.9 | 5.8 | 6.4 | 5.2 | 4.2 | 4.1 | 5.2 | 5.0 |
| Monthly household income (mean, USD) | 61.3 | 57.8 | 62.1 | 67.9 | 65.6 | 76.3 | 61.3 | 72.1 | 74.3 | 73.0 | 59.8 | 73.7 |
| Woman reads Kinyarwanda* | | | | | | | | | | | | |
| Yes, easily | 380 | 61% | 226 | 69% | 395 | 64% | 138 | 64.0 | 22 | 67% | 9 | 82.0 |
| With difficulty/not at all | 246 | 39% | 102 | 31% | 224 | 36% | 77 | 36.0 | 11 | 33% | 2 | 18.0 |
| Drunk in past year | | | | | | | | | | | | |
| Yes | 32 | 5% | 11 | 5% | 32 | 5% | 11 | 5% | 2 | 6% | 0 | 0% |
| No | 586 | 95% | 203 | 95% | 586 | 95% | 203 | 95% | 31 | 94% | 11 | 100% |
| Male partner drunk in past year | | | | | | | | | | | | |
| Yes | 187 | 30% | 99 | 30% | 210 | 34% | 74 | 34% | 19 | 58% | 4 | 36% |
| No | 439 | 70% | 229 | 70% | 409 | 66% | 141 | 66% | 14 | 42% | 7 | 64% |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Family planning characteristics* | | | | | | | | | | | | |
| Number previous pregnancies | 3 | 2.1 | 3.1 | 1.8 | 3.4 | 2.2 | 3.3 | 2.0 | 2.4 | 2.0 | 3 | 2.1 |
| Currently pregnant* | | | | | | | | | | | | |
| Yes | 147 | 24% | 79 | 24% | 209 | 35% | 56 | 26% | 12 | 40% | 4 | 36% |
| No | 466 | 76% | 244 | 76% | 396 | 65% | 157 | 74% | 18 | 60% | 7 | 64% |
| Female want child* | | | | | | | | | | | | |
| Yes | 50 | 11% | 13 | 7% | 32 | 8% | 12 | 9% | 7 | 21% | 1 | 9% |
| Don't know/No | 413 | 89% | 181 | 93% | 388 | 92% | 125 | 91% | 26 | 79% | 10 | 91% |
| Male want child* | | | | | | | | | | | | |
| Yes | 51 | 12% | 20 | 12% | 38 | 9% | 9 | 7% | 8 | 24% | 3 | 27% |
| Don't know/No | 391 | 88% | 152 | 88% | 364 | 91% | 115 | 93% | 25 | 76% | 8 | 73% |
| *Sexual History & Clinical* | | | | | | | | | | | | |
| Age of Sexual Debut | 18.2 | 2.9 | 18 | 3 | 19.2 | 3.3 | 18.9 | 3.2 | 18.2 | 2.8 | 19.4 | 2.8 |
| Number lifetime sex partners* (mean, SD) | 5.9 | 28.5 | 3.5 | 4.6 | 7.1 | 121.2 | 2.1 | 1.3 | 2.3 | 1.2 | 1.7 | 0.8 |
| Number sex partners last year (mean, SD) | 1.2 | 1.1 | 1.1 | 0.4 | 1.1 | 0.5 | 1 | 0.2 | 1.1 | 0.3 | 1 | 0 |
| Had STI in past year* | | | | | | | | | | | | |
| Yes | 360 | 59% | 161 | 50% | 220 | 36% | 66 | 31% | 12 | 40% | 4 | 36% |
| No | 253 | 41% | 162 | 50% | 385 | 64% | 147 | 69% | 18 | 60% | 7 | 64% |
| Male partner had STI in past year* | | | | | | | | | | | | |
| Yes | 181 | 30% | 90 | 28% | 237 | 39% | 74 | 35% | 14 | 47% | 9 | 82% |
| No | 432 | 70% | 236 | 72% | 368 | 61% | 139 | 65% | 16 | 53% | 2 | 18% |
| HSV-2 | | | | | | | | | | | | |
| Positive | 434 | 76% | 255 | 78% | 363 | 66% | 150 | 73% | 24 | 77% | 10 | 91% |
| Negative/Doubtful | 139 | 24% | 73 | 22% | 185 | 34% | 55 | 27% | 7 | 23% | 1 | 9% |

USD: United States Dollar; STI: sexually transmitted infection; HSV-2: herpes simplex virus 2

* $p<0.05$ in two sample t-test assuming unequal variance (Satterthwaite) for continuous variables and chi-squares for categorical variables

** $p<0.0001$ in two sample t-test assuming unequal variance (Satterthwaite) for continuous variables and chi-squares for categorical variables

**Table 2. Crude and adjusted models of predictors of ever using hormonal contraception by HIV status of women in HIV-1 serodiscordant relationships in Rwanda, 2002-2011**

| | HIV+ HC Ever Users | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Crude | | | | Adjusted | | | |
| | cRR | 95%CI | | p-value | aRR | 95%CI | | p-value |
| Age of woman | 0.97 | 0.95 | 0.98 | <.0001 | 0.97 | 0.95 | 0.98 | <.0001 |
| Years cohabiting with partner | 0.98 | 0.96 | 1.00 | 0.02 | 1.01 | 0.98 | 1.03 | 0.56 |
| Reads Kinyarwanda easily | 1.26 | 1.04 | 1.53 | 0.02 | 1.28 | 1.05 | 1.55 | 0.01 |
| Had an STI in the past year | 0.79 | 0.66 | 0.94 | 0.01 | 0.80 | 0.67 | 0.95 | 0.01 |
| | HIV- non-seroconverters HC Ever Users | | | | | | | |
| | Crude | | | | Adjusted | | | |
| | cRR | 95%CI | | p-value | aRR | 95%CI | | p-value |
| Age of woman | 0.98 | 0.97 | 1.00 | 0.08 | 0.98 | 0.96 | 1.00 | 0.06 |
| Currently pregnant | 0.75 | 0.57 | 0.97 | 0.03 | 0.72 | 0.55 | 0.94 | 0.02 |
| | HIV- Seroconverter HC Ever Users | | | | | | | |
| | Crude | | | | Adjusted | | | |
| | cRR | 95%CI | | p-value | aRR | 95%CI | | p-value |
| Age of woman | 1.04 | 0.96 | 1.12 | 0.34 | 1.05 | 0.97 | 1.14 | 0.26 |
| Male partner had STI in past year | 3.52 | 0.87 | 14.32 | 0.08 | 3.87 | 0.96 | 15.59 | 0.06 |

STI: sexually transmitted infection; cRR: crude risk ratio; CI: confidence interval

# Chapter IV. Predictors of ART initiation among Heterosexual HIV-1 serodiscordant couples in an ART naïve cohort in Rwanda.

## 4.1 Abstract

**Objective:** Evaluate the predictors of time to ART initiation among ART naïve HIV-1 serodiscordant heterosexual couples in an open cohort in Kigali, Rwanda.

**Methods:** HIV-1 serodiscordant heterosexual couples were enrolled from couples counseling and testing sites in Kigali from 2002-2011. Demographic, behavioral, and clinical exposures were measured in both partners at baseline and every three months. HIV-uninfected partners were re-tested every three-months at minimum. Couples were censored if they separated, one partner died, the uninfected partner seroconverted, or the positive partner initiated ART. Baseline and time-varying predictors of time to ART initiation stratified by sex of the positive partner using multivariable Cox models were estimated.

**Results:** Of the 1837 heterosexual HIV-1 serodiscordant couples included in the cohort (882 M+F- and 955 M-F+), 30% (n=544) had an HIV-1 positive partner initiate ART. Of the ART initiating couples, 39% had a seropositive male partner (M+F-) and 61% had a seropositive female partner (M-F+). Shorter time to ART initiation in M+F- couples was predicted by log10 viral load of positive partner at baseline (aHR1.54; 95%CI:1.01-2.34), while both log10 viral load of positive partner at baseline (aHR1.43; 95%CI:1.02-2.02) and WHO stage IV (aHR 4.85; 95%CI:1.45-16.26) predicted time to ART initation in M-F+ couples.

**Conclusion:** As expected, clinical values were the main predictors of time to ART initiation. Due to limited data we were unable to look at those that were clinically eligible compared to those who initiated ART over time.

## 4.2 Background

The importance of initiating Antiretroviral therapy (ART) in HIV positive individuals to improve survival, reduce morbidities, and reduce transmission to sexual partners is well established. [11,29,83] While structural and system-level barriers to ART initiation have been traditional impediments, vast improvements in the availability of affordable treatment have rapidly changed these barriers. [5,68] Unfortunately, HIV-positive people in resource-limited countries who receive ART are still more likely to not have a normal life expectancy, often because they start ART with a sub-optimal CD4 count.

HPTN 052, a multinational randomized clinical trial with HIV-1 serodiscordant couples, demonstrated that early initiation of ART (CD4 count between 350-550 cells/mm$^3$) reduced the risk of HIV transmission by 96% to the uninfected partner, compared to delayed ART initiation arm (CD4<=250 cells/mm$^3$) [11]. Importantly, the viral suppression observed in the index partner was supported by quarterly viral load monitoring and routine adherence counseling. Additional observational studies of ART provision to infected partners in HIV serodiscordant couples in 'real world settings' found between 80-92% reductions in HIV-1 transmission [8,30]. While risk reduction in discordant couples must be a multifaceted approach, viral load suppression plays a large role in reducing transmission risk if it can be sustained over the long-term. [8,15] As a

result of these studies, couples voluntary testing and counseling guidelines (CVTC) were released in 2012 to include immediate ART initiation of HIV positive partners in stable, cohabitating HIV discordant relationships. [4] Complex and not well-defined factors may lead to both non-timely initiation of ART when eligible as well as sustained adherence to achieve viral load suppression among positive partners in discordant relationships. In order to meet this recommendation, it is necessary to understand where and when patients are lost to pre-ART care or why they do not initiate ART.

Multiple studies in different settings have reported a reluctance to initiate ART by HIV-infected individuals [6,7,87,88]. Studies of retention in pre-ART care report substantial loss of patients at every point in the treatment cascade [89], starting with patients who do not return for their initial CD4 count results and ending with those who do not initiate ART despite eligibility. [90] Willingness of HIV-1-infected partners to initiate ART when they are asymptomatic and/or non-symptomatic but know their positive status is an important aspect of reaching all high-risk couples. One study in South Africa observed only 39% of eligible HIV positive individuals had initiated ART 1 year after being diagnosed and having an eligible CD4 count. [89,90] More recently, in a Kenyan cohort study, 37% of HIV-1 infected partners eligible for ART did not initiate within 1 year of referral for free treatment [84]. Higher CD4 count (>100 cells/mm3) and lower socioeconomic status, measured in home ownership and rent cost, were strong predictors of non-initiation of ART [84]. Other studies have found fear of ART side effects, stigma and status disclosure, concerns over sustainability of care, food insecurity, and preference for alternative medicines pose barriers to ART initiation and ongoing adherence [22,88].

Initiatives to promote provider-initiated testing have increased the number of people who know their HIV status, but patients tested in this way tend to have a lower rate of linkage to ongoing HIV care and ART initiation compared to those who initiate testing on their own [93] or as a couple if they are in a stable relationship. [113]

These findings highlight the evolving need to better understand factors leading individuals and couples to initiate ART and using this information to guide targeted programs to both initiate and retain people on treatment to achieve and sustain viral suppression over time. We focused on factors that may predict time to ART initiation in an attempt to better understand important factors in getting positive partners in serodiscordant couples into dare. Because these couples were aware of eachothers' status and remained together over longer periods of time, their time to ART initiation are particularly informative. In the current environment of diminishing global resources and continued implementation challenges, it is important to create additive prevention interventions and targeted ART adherence interventions for serodiscordant couples. Understanding the complicated dynamics of clinical and socio-cultural factors associated with ART initiation will help inform future trials and operational aspects of delivering targeted prevention and treatment programs specific to this high-risk population.

## 4.3 Methods

***4.3.1. Study Participants:*** Heterosexual HIV-1 serodiscordant couples were invited to enroll in an open cohort study between 2002-2011, after being identified as eligible from couples (married or cohabiting) in Kigali, Rwanda who attended couples' voluntary HIV counseling and testing (CVCT) services. Couples either self-presented or presented after

receiving an invitation from a community CVCT promoter. Couples were eligible to participate if they were confirmed HIV-1 serodiscordant, had been together at least 3 months and planned on staying in the Kigali region for at least a year. Couples were ineligible if either couple had a CD4 count <200 or either partner was on ART. Written informed consent was obtained from the couple jointly. Couples were censored if either partner died, the couple separated, the positive partner started ART, or if either partner was lost to follow-up.

***4.3.2. Data Collection:*** Data was collected from a single clinical site in Kigali. Participants completed behavioral and medical history questionnaires and had a full physical examination including pelvic/genital exams, and HIV and STI testing (gonorrhea, Chlamydia, Trichomonas) at baseline. Study visits every three months during follow-up included a physical exam, a blood sample for STI and HIV testing (negative partner), a vaginal swab wet mount (to determine bacteria, yeast cells, trichomoniasis, white blood cells to show an infection, or clue cells that show BV), and assessment of prevalent and incident pregnancy by blood test and asking women how many months currently pregnant and pregnancy outcome since last visit. Self-reported hormonal contraceptive use was validated by clinical records maintained at the site as all hormonal contraception was given free during the participant's study enrollment. Male circumcision was confirmed during baseline medical examination or physical examination after self-reported circumcision.

From 2007-2011, physical exams and STI screenings were performed at baseline, annually, and when signs or symptoms were reported. All HIV-negative partners were seen at visit months 0,1,2,3. Starting at visit month 3 and quarterly thereafter, a risk assessment

was conducted to establish recent exposure within the partnership. Couples assessed as 'higher risk' were asked to come back at monthly intervals for repeat HIV testing until the next quarterly visit, at which time the risk assessment was repeated. 'Higher risk' was defined as having at least one of the following since last visit: self-reported unprotected sex, sperm or trichomonas on a wet prep, incident pregnancy, or incident syphilis. All follow-up visits included risk reduction counseling, access to contraception and free condoms.

At each study visit, individuals were asked if the HIV positive partner had initiated ART. Reports of ART initiation were confirmed by clinic staff and the couple was censored from the study. Referrals for ART treatment and care were given at the study site based on clinical assessment. Study questionnaires included demographic and psychosocial data, sexual risk behaviors, medical history, and health services data. Data were collected by participant completion of written questionnaire in Kinyarwandan or English (depending on preference of the participant) and face-to-face (FTF) interviews were conducted if literacy was a problem or preferred. Free outpatient health care was provided at the research clinic.

***4.3.3. Variables of interest:*** ART initiation by the seropositive partner in the serodiscordant relationship is our outcome of interest. The definition of ART initiation does not include short-course use of antiretroviral drugs for PMTCT of HIV during pregnancy or breastfeeding. We assessed ART initiation whether the participant had an eligible CD4 count or not.

During the study timeframe, 2003-2011, the Rwandan government criteria for ART initiation changed. National scale-up of free ART by the Government of Rwanda began in January 2004. [117] From 2004 and 2007, adults and children were eligible for ART if they were HIV positive and had a diagnosis (1) of World Health Organization (WHO) clinical stage IV, irrespective of CD4+ cell count, (2) WHO clinical stage III and a CD4+ cell count <350 cells per microliter, or (3) WHO clinical stage I or II and a CD4+ T-lymphocyte count <200 cells per microliter. From 2007, criteria included WHO clinical stage IV without consideration of CD4 count or WHO Stage I, II, III with CD4 < 350/mm3. At the end of 2011, Rwandan ART guidelines changed to incorporate new criteria to start ART; WHO Stage 3 or 4 regardless CD4 cell count, or WHO Stage 1 or 2 with CD4 < 350/mm3, any patient with HIV-TB coinfection regardless CD4 cell count, any patient with HIV-Hepatitis B co-infection, and any HIV-Positive partner of a serodiscordant couple regardless CD4 and WHO stage. These changes were made at the time our study was completed so official implementation had no occurred. Reported ART initiation dates were confirmed with the treatment clinic and the couple was censored from the study.

Baseline couple measures of interest included age disparity; years cohabiting; number of living children; HIV stage and VL (log transformed) of the positive partner; and monthly income (USD). Individual exposures of interest at baseline included age; Kinyarwandan literacy; number of years living in Kigali; number of sex partners in past year and lifetime; number of previous pregnancies; current pregnancy; individual fertility

desires; being drunk in the past year; presence of any lab confirmed STI in either partner; and serologic confirmation of herpes simplex virus 2 (HSV-2).

Time-varying exposures of interest included individual composite genital inflammation and genital ulceration indicators, self-reported number of unprotected sex acts with the study partner since last visit, self-reported number of outside partners since last visit, pregnancy (both incident and prevalent), current female contraception method, current clinically diagnosed pulmonary Tuberculosis (TB), and current diagnosis or treatment of an STI.

Diagnosis of candida, BV and trichomonas was done with vaginal wet preparations, which were also examined for presence of sperm as a biomedical measure of condomless sex. RPR was used for serologic diagnosis of syphilis. HSV-2 was diagnosed serologically with Focus Herpes Select 21IgG and Gonorrhea and chlamydia were diagnosed clinically by presence of endocervical or urethral discharge. Due to low sensitivity of gram staining, patients were empirically treated for both gonorrhea and chlamydia.

The dichotomous composite genital inflammation indicator was derived from individual time-varying measures (clinically diagnosed/treated or self-report) of genital inflammation (including cervical or vaginal inflammation in women); genital discharge (male urethral discharge, female vaginal or cervical discharge); inguinal adenopathy; or laboratory diagnosis/symptom-based treatment for trichomoniasis, gonorrhea, chlamydia, candida, or BV. Similarly, the composite genital ulceration variable was created from time-varying measures of chronic/recurrent or acute genital or perianal ulcers (clinically

diagnosed/treated or self-report); ulceration observed in physical exam; treatment of chancroid or HSV-2; and/or incident positive RPR serology for syphilis.

***4.3.4. Data Analysis:*** Couple time of follow-up was computed for each couple from enrollment until either the couple was censored or ART was initiated. Kaplan Meier curves for all couples initiating ART compared to those censored for other reasons, by sex of seropositive partner, were compared to assess proportional hazards. To evaluate possible cohort effects, years of ART initiation were grouped, stratified by sex of positive partner, and differences evaluated by log-rank tests.

Exposures were stratified by sex of HIV-positive partner. Univariate descriptions of categorical variables (counts and percentages) and continuous variables (means and standard deviations) per couple (baseline variables, Table 1) or across all study intervals (time varying variables, Table 2) are displayed. Cox models were used in bivariate analysis of both baseline predictors (Table 3) and time-varying predictors (Table 4) to estimate crude hazard ratios (cHRs), 95% CIs, and p-values.

Exposures of interest significantly ($p<0.05$) associated with ART initiation in bivariate analyses were considered for inclusion in multivariable models. Proportional hazards of time-independent variables were assessed graphically and with log-log plots of Schoenfeld residuals. Multicollinearity was assessed for all candidate variables through examination of the proportion of variance of each predictor that was not explained by all of the other predictors (tolerance) and the review of the variance inflation factor. If multi-collinearity was present, variables with the greater attributable variance proportion and/or were not of interest a priori were dropped from the model. Multivariable Cox models were

stratified by the sex of the HIV-positive partner to evaluate predictors of time to ART initiation.

Goodness of model fit was assessed with log-likelihoods and using the Akaike information criterion (AIC) stepwise regression where entry criteria were set as 0.99 and staying in the model criteria 0.995. Adjusted hazard ratios (aHRs), 95% CIs, and p-values are reported (Table 5). All analyses were conducted with SAS v9.4 (Cary, NC).

**2.3.5** ***Ethics:*** The parent study was approved by the Office for Human Research Protections-registered Institutional Review Boards at Emory University and the Government of Rwanda. Secondary analyses were approved by the University of California, Los Angeles.

## 4.4 Results

Of the 1837 heterosexual HIV-1 serodiscordant couples included in the cohort (822 M+F- and 955 M-F+), 30% (n=544) had an HIV-1 positive partner initiate ART. Of the ART initiating couples, 39% had a seropositive male partner (M+F-) and 61% had a seropositive female partner (M-F+). Because of national ART scale-up and treatment guideline changes over time, we observed a constant increase in number of couples initiating ART from 2003-2007 in both M+F- and M-F+ couples (Figure 1). In Rwanda in 2007/2008, national treatment guidelines to begin ART at CD4 250 regardless of stage were implemented.

On average, couples with a male initiated ART tended to be older (male mean age 37.1 v. 36.5, female mean age 29.7 v. 29.2); have lived together longer (6.7 v. 6.6 years); have had a higher monthly income (mean USD $73 v. $62); both partner were more literate

in Kinyarwandan (women read easily  68% v. 63%, men read easily 76% v. 74%); fewer male (31% v. 36%) and female (3% v. 6%) partners were drunk in the year preceding baseline; female partners had fewer numbers of lifetime sex partners (2 v. 9) while male partners had more lifetime sex partners (12 v. 11); had fewer previous pregnancies (3.2 v. 3.4); were more likely for female partner to be pregnant (36% v. 32%) at baseline; female partner more likely to want a child (11% v. 8%) while male partner was less likely (7% v. 11%); and female partner had fewer STIs in past year (27% v. 38%) while male partners were more likely to have STI in past year (34% v. 26%) than those who did not initiate ART (Table 1).

Couples with an HIV positive female partner who initiated treatment tended to be older (mean male age 34.8 v. 33.9, mean female age 29.6 v. 28.5); had lived together longer (5.6 v. 4.8); had a higher monthly income (mean USD $67 v. $61); female partner more literate in Kinyarwandan (women read easily  66% v. 62%); fewer male (26% v. 32%) partners were drunk in the year preceding baseline; females (5.4 v. 4.5) had fewer numbers of lifetime sex partners while male partners had more lifetime sex partners (9.5 v. 8.5); had fewer previous pregnancies (3.2 v. 3.4); were less likely for both females (36% v. 32%) and males (11% v. 13%) to want a child; more female partner had STIs in past year (57% v. 54%) while fewer males (23% v. 31%) had an STI in past year, than their counterpart couples who did not initiate ART. (Table 1)

In time varying measures in couples with a positive male partner, fewer males that initiated ART had unprotected sex with study partner since last visit (any 26% v. 28%), more average number of outside partners (0.98 v. 0.97), and more male gential inflamation

(12% v. 10%), than those M+F- couples that never seroconverted (Table 2). Couples with a female positive partner that initated ART had fewer outside sexual partners since last visit (1.03 v. 0.97) and had fewer women that were breastfeeding (12% v. 17%) (Table 2).

In bivariate analysis, baseline factors associated with a faster time to ART initiation among HIV positive men included a higher monthly household income (cHR 1.00; 95% CI:1.00-1.01); having more sex partners in the past year (cHR 1.06; 95%CI: 1.01-1.10); female partner having fewer previous pregnancies (cHR 0.91; 95%CI: 0.85-0.98); being WHO stage II (cHR 1.38; 95% CI:1.01-1.88), or stage IV (cHR 1.67; 95% CI:1.13-2.46); and having a higher viral load (log10 copies/ml) (cHR 1.67; 95% CI:1.13-2.46) (Table 1). Time-varying covariates independently associated with time to ART initiation include only use of OCPs as method of contraception in most recent period (2.73; 95%CI:1.20-6.20) (Table 2).

In bivariate analysis of couples with an HIV positive female partner that initiated ART, a higher monthly household income (cHR 1.01; 95% CI:1.00-1.01), more lifetime sex partners of male partner (cHR 1.00; 95%CI: 1.00-1.01); WHO Stage II (cHR 1.61; 95% CI:1.20-2.17); WHO stage III (cHR 1.8; 95% CI:1.06-2.27); WHO stage IV (cHR 1.9; 95% CI:1.14-3.34); less likely to have a male partner that had an STI in the previous year (cHR 0.69; 95% CI:0.50-0.94); and and having a higher viral load (log10 copies/ml) (cHR 1.66; 95% CI:1.23-2.23) (Table 1). Being pregnant (cHR 2.18 95%CI: 1.52-3.12) and male genital inflammation (cHR 3.37; 95%CI: 1.19-5.93) were independent time-varying predictors of earlier time to ART.

After adjusting for calendar time in the final multivariable models, time to ART initiation in M+F- couples was only predicted by baseline viral load (aHR 1.54; 95%CI: 1.01-2.34). In our final M-F+ multivariable model, baseline VL (aHR 1.43; 95%CI: 1.02-2.02) and WHO stage IV compared to WHO stage I (aHR 4.85; 95%CI: 1.45-16.26) predicted time to ART initiation of positive women in serodiscordant couples (Table 3).

## 4.5 Discussion

Our results show both expected and unexpected predictors of ART initiation. As expected, WHO stage and baseline log10 viral load were important covariates in estimating time to ART initiation, but surprisingly only stage IV remained in the final M-F+ model. Although couples and individual characteristics ended up predicting time to ART in bivariate models, none of these remained significant in our final multivariable models in either M+F- or M-F+ couples. Based on findings from other studies [88] of ART initiation in serodiscordant couples in sub-Saharan Africa, we expected socioeconomic factors to predict earlier ART initiation, yet this was not observed. It may be that wealthier serodiscordant couples opted not to enter our study after their counseling and testing or wealthier individuals in Kigali experienced greater stigma around serostatus and treatment.

In the final M+F- and M-F+ multivariable models, many of the predictors of ART that were significant in bivariate analysis, but not ultimately in the multivariable models. This may be an artifact of other sociological and cultural gender roles and nuances in Rwandan couples we were not able to capture, but it does highlight the important of couples studies and how an individual's characteristics can have large influences on important health outcomes of both partners. Facilitated disclosure of serostatus within a

stable partnership through couples counseling and testing has been found to promote family support, associated with improved engagement in HIV care for infected partners and high levels of adherence to ART [110,111]. A study in Kenya assessing the willingness of partners in serodiscordant couples to start PrEP gave their participants a hypothetical choice of starting either early ART or PrEP for HIV-1 prevention. They found 52.5% of HIV-1 infected participants preferred to initiate ART early and 56.9% of HIV-1 uninfected participants would preferred to use PrEP. [105] Partners in serodiscordant partnerships may differ in their preferences of HIV preventions strategies and these preferences and should be incorporated into the positive partners' ART care for targeted and streamlined service delivery.

Interestingly, neither prevalent nor incident pregnancy nor desire to have a child were associated with time to ART initiation, which was not what we expected given scaled-up PMTCT program in Rwanda during the study timeframe [22] and observations in other studies. [9,11,66] One longitudinal study of HIV serodiscordant couples in Kenya found evidence that women chose to avoid becoming pregnant following ART initiation [86], indicating that perhaps women may try to fulfill their fertility desires before their own or their partners ART initiation. Stigma of HIV and early beliefs about the effects of ART on women's reproductive systems could have been responsible for these couples not initiating ART early.

Broader sociological influences at the time such as HIV stigma and cultural or familiar expectations were unmeasured and may have had larger influences than our measured predictors. The availability of ART treatment (waitlists and clinical criteria) were

also factors that impacted the ability to initiate ART that we tried to control for, yet the fact that couples were in the study and remained in the study allowed these couples to be checked frequently and have access to referrals to necessary services.

Limitations to our model include the potential misclassification of exposures of interest as well as the potential for incomplete or inaccurate reporting of our outcome. This was an ART naïve cohort with initiation of ART of the positive partner as criteria for censoring. As ART began scaling up nationally during study follow-up time, it may have effected couples differentially and created dependent censoring. Bias analyses on the censoring using inverse probability of treatment weighting in marginal structural models should be conducted in further analyses.

Although the Rwandan government policy on changing ART criteria specific to HIV serodiscordant couples was only drafted during the end of our study and not yet implemented, it is possible some clinics and physicians used their discretion to starting some HIV positive partners in serodiscordant couples earlier than others, potentially introducing bias.

## 4.6 Conclusions

Time to ART initiation among heterosexual HIV-1 discordant couples did not vary greatly based on the sex of the seropositive partner. Clinical characteristics of the positive partner were the most influential predictor of shorter time to ART initiation among both M+F- /M-F+ couples. Risk and protective factors associated with ART initiation differed by sex of positive partner, indicating the importance of partner characteristics that may influence behaviors and decisions beyond clinical criteria. Important lessons in developing

ART programs targeting serodiscordant couples to both initiate ART and for long-term adherence to achieve viral load suppression may want to focus on the couple rather than the individual.

## 4.6 Tables and Figures

**Figure 1. Distribution of HIV-1 positive partners in discordant couples initiating ART by sex and calendar year in a Rwandan cohort study, 2002-2011**

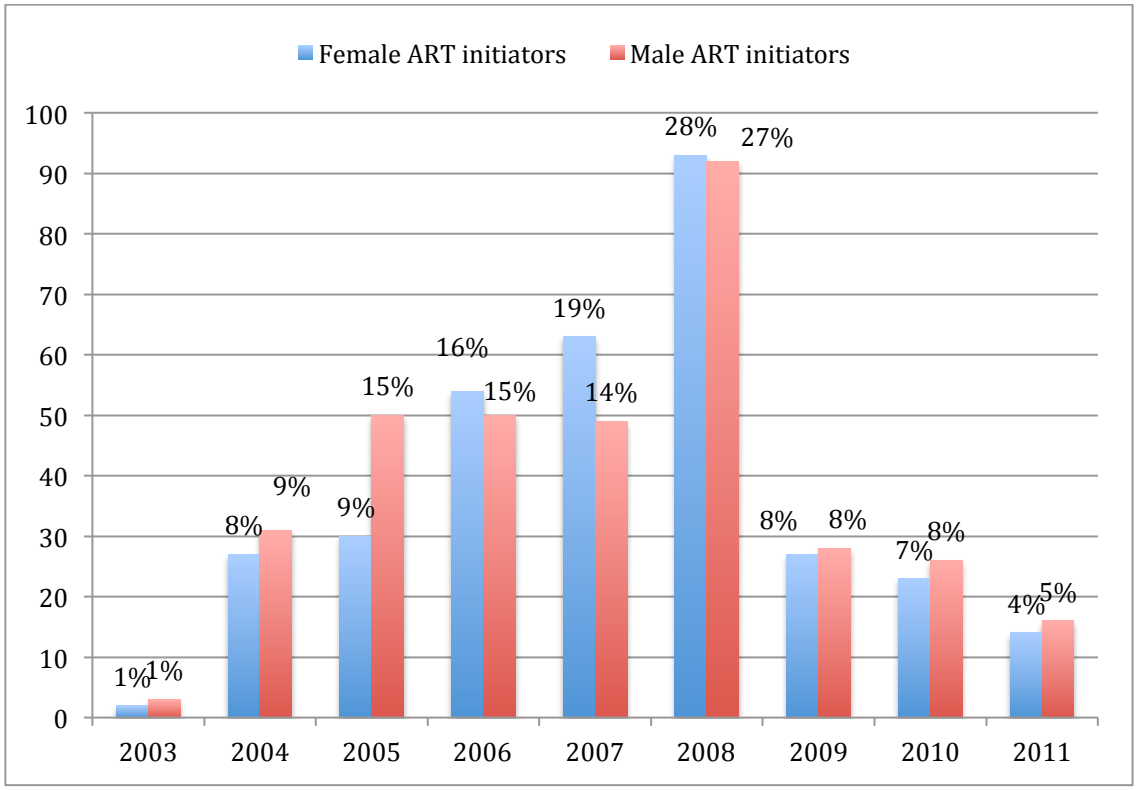


**Table 1. Baseline covariate descriptive statistics and bivariate associations of time to ART initiation by sex of HIV positive partner among Rwandan HIV discordant couples**

| | M+F- Couples | | | | | | M-F+ Couples | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Non-ART initiators (N = 671) | | ART initiators (N= 211) | | cHR | 95%CI | Non-ART initiators (N = 622) | | ART initiators (N= 333) | | cHR | 95%CI |
| | N | % | N | % | | | N | % | N | % | | |
| Demographics | | | | | | | | | | | | |
| Age of male* | 36.5 | 7.7 | 37.1 | 7.3 | 1.02 | .99-1.03 | 33.9 | 8.6 | 34.8 | 8.7 | 1.00 | 0.99-1.02 |
| Age of female* | 29.2 | 6.3 | 29.7 | 6.2 | 1.01 | 0.99-1.03 | 28.5 | 6 | 29.6 | 6.1 | 1.01 | 0.99-1.03 |
| Age difference* | 7.8 | 6.3 | 7.8 | 5.8 | 1.01 | 0.98-1.03 | 6.7 | 6.3 | 6.7 | 6.3 | 1.00 | 0.98-1.02 |
| Years cohabiting* | 6.6 | 5.5 | 6.7 | 5.8 | 0.99 | 0.97-1.02 | 4.8 | 4.3 | 5.6 | 4.9 | 1.01 | 0.97-1.03 |
| Monthly household income (USD)* | 62.2 | 67.6 | 73.1 | 94.6 | 1.00 | 1.00-1.01 | 60.7 | 61.6 | 66.7 | 67.8 | 1.01 | 1.00-1.01 |
| Woman reads Kinyarwanda | | | | | | | | | | | | |
| Yes, easily | 420 | 63 | 144 | 68 | 1.32 | 0.99-1.76 | 382 | 62 | 145 | 66 | 1.2 | 0.98-1.55 |
| With difficulty/no | 247 | 37 | 67 | 32 | ref | | 239 | 38 | 75 | 34 | ref | |
| Man reads Kinyarwanda | | | | | | | | | | | | |
| Yes, easily | 495 | 74 | 160 | 76 | 0.95 | 0.69-1.30 | 443 | 71 | 154 | 70 | 0.85 | 0.68-1.07 |
| With difficulty/no | 172 | 26 | 51 | 24 | ref | | 178 | 29 | 66 | 30 | ref | |
| Female drunk in past year | | | | | | | | | | | | |
| Yes | 39 | 6 | 6 | 3 | 0.58 | 0.26-1.29 | 39 | 6 | 14 | 6 | 0.91 | 0.53-1.57 |
| No | 626 | 94 | 205 | 97 | ref | | 582 | 94 | 206 | 94 | ref | |
| Male drunk in past year | | | | | | | | | | | | |
| Yes | 241 | 36 | 66 | 31 | 0.76 | 0.57-1.02 | 201 | 32 | 58 | 26 | 0.76 | 0.56-1.02 |
| No | 426 | 64 | 145 | 69 | ref | | 420 | 68 | 162 | 74 | ref | |
| Number of years in Kigali* | | | | | | | | | | | | |
| Male | 15.8 | 12.5 | 15.4 | 12 | 1.00 | 0.99-1.01 | 13.2 | 11.7 | 12.4 | 10.6 | 0.99 | 0.98-1.01 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Female | 12.4 | 10.9 | 11.6 | 10.3 | 0.99 | 0.98-1.01 | 12.4 | 10.1 | 14 | 11.5 | 1.01 | .99-1.02 |
| **Sexual Behavior** | | | | | | | | | | | | |
| Female number lifetime sex partners* | 6.8 | 116.9 | 2 | 1.5 | 0.98 | 0.8-1.10 | 5.4 | 27.8 | 4.5 | 12.2 | 1.00 | 0.99-1.01 |
| Male number lifetime sex partners* | 10.9 | 27.7 | 11.6 | 27.8 | 1.00 | 1.00-1.01 | 8.5 | 16.2 | 9.5 | 16.5 | 1.00 | 1.00-1.01 |
| Female number sex partners last year* | 1.1 | 0.5 | 1 | 0.15 | 0.83 | 0.38-1.78 | 1.2 | 1.1 | 1.1 | 0.4 | 0.98 | 0.80-1.19 |
| Male number sex partners last year* | 1.5 | 1 | 1.6 | 3.5 | 1.06 | 1.01-1.10 | 1.4 | 0.9 | 1.4 | 1 | 1.00 | 0.86-1.17 |
| **Family Planning** | | | | | | | | | | | | |
| Number of previous pregnancies* | 3.4 | 2.2 | 3.1 | 2.1 | 0.91 | 0.85-0.98 | 2.9 | 1.8 | 3 | 1.9 | 0.94 | 0.87-1.00 |
| Pregnant at baseline | | | | | | | | | | | | |
| Yes | 205 | 32 | 76 | 36 | 1.39 | 1.00-1.84 | 151 | 25 | 60 | 28 | 1.21 | 0.90-1.63 |
| No | 445 | 68 | 133 | 64 | ref | | 458 | 75 | 158 | 72 | ref | |
| Female want child | | | | | | | | | | | | |
| Yes | 40 | 8 | 12 | 11 | 1.78 | 0.97-3.24 | 47 | 11 | 7 | 7 | 0.96 | 0.45-2.08 |
| Don't know/No | 452 | 92 | 97 | 89 | ref | | 392 | 89 | 97 | 93 | ref | |
| Male want child | | | | | | | | | | | | |
| Yes | 51 | 11 | 7 | 7 | 0.82 | 0.38-1.77 | 54 | 13 | 10 | 11 | 0.91 | 0.47-1.76 |
| Don't know/No | 418 | 89 | 94 | 93 | ref | | 352 | 87 | 85 | 89 | ref | |
| **Clinical** | | | | | | | | | | | | |
| HIV stage of positive partner | | | | | | | | | | | | |
| Stage I | 232 | 34 | 65 | 31 | ref | | 301 | 48 | 79 | 36 | ref | |
| Stage II | 268 | 40 | 103 | 49 | 1.38 | 1.01-1.88 | 214 | 34 | 96 | 44 | 1.61 | 1.20-2.17 |
| Stage III | 139 | 21 | 37 | 17 | 1.05 | 0.70-1.58 | 90 | 15 | 40 | 18 | 1.55 | 1.06-2.27 |
| Stage IV | 32 | 5 | 6 | 3 | 1.17 | 0.51-2.71 | 17 | 3 | 5 | 2 | 0.99 | 0.40-2.44 |
| VL of positive partner* (log10 copies/ml) | 4.2 | 1 | 4.3 | 1 | 1.67 | 1.13-2.46 | 3.7 | 1.1 | 3.9 | 0.8 | 1.66 | 1.23-2.23 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Circumcised male partner | | | | | | | | | | | | |
| Yes | 117 | 17 | 33 | 16 | 0.92 | 0.63-1.33 | 143 | 23 | 46 | 21 | 0.85 | 0.61-1.18 |
| No | 554 | 83 | 178 | 84 | ref | | 479 | 77 | 171 | 79 | ref | |
| Female had STI in last year | | | | | | | | | | | | |
| Yes | 246 | 38 | 56 | 27 | 0.59 | 0.44-0.81 | 331 | 54 | 124 | 57 | 1.02 | 0.78-1.34 |
| No | 404 | 62 | 153 | 73 | ref | | 278 | 46 | 94 | 43 | ref | |
| Male had STI in last year | | | | | | | | | | | | |
| Yes | 137 | 26 | 72 | 34 | 0.78 | 0.6-1.03 | 191 | 31 | 50 | 23 | 0.69 | 0.50-0.94 |
| No | 388 | 74 | 137 | 66 | ref | | 418 | 69 | 168 | 77 | ref | |
| Female positive HSV-2 | | | | | | | | | | | | |
| Yes | 404 | 69 | 143 | 68 | 0.91 | 0.68-1.22 | 434 | 75 | 172 | 78 | 1.37 | 0.99-1.89 |
| No | 181 | 31 | 67 | 32 | ref | | 142 | 25 | 48 | 22 | ref | |
| Male positive HSV-2 | | | | | | | | | | | | |
| Yes | 394 | 63 | 123 | 59 | 1.1 | 0.83-1.45 | 290 | 52 | 107 | 49 | 0.9 | 0.70-1.19 |
| No | 229 | 37 | 84 | 41 | ref | | 262 | 48 | 111 | 51 | ref | |

cHR: crude Hazard Ratio, CI: Confidence Interval, VL: Viral Load

*mean,SD reported for continuous variables

**Table 2. Descriptive statistics and bivariate associations of time-varying covariates by ART initiation, Rwandan serodiscordant couples**

| | M+F- Couples | | | | | | M-F+ Couples | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Non-ART initiators (N =6658) | | ART initiators (N= 2015) | | cHR | 95%CI | Non-ART initiators (N = 5653) | | ART initiators (N= 1554) | | cHR | 95%CI |
| | n intervals | % | n intervals | % | | | n intervals | % | n intervals | % | | |
| **Sexual History** | | | | | | | | | | | | |
| Any protected sex with study partner since last visit | | | | | | | | | | | | |
| Yes | 6480 | 93 | 1693 | 94 | 0.7 | 0.34-1.45 | 4593 | 93 | 1788 | 94 | 1.66 | 0.61-4.52 |
| No | 492 | 7 | 107 | 6 | ref | | 351 | 7 | 117 | 6 | ref | |
| Any unprotected sex with study partner since last visit | | | | | | | | | | | | |
| Yes | 1931 | 28 | 482 | 25 | 0.85 | 0.59-1.20 | 1577 | 32 | 609 | 31 | 1.17 | 0.85-1.62 |
| No | 5043 | 72 | 1414 | 75 | ref | | 3365 | 68 | 1390 | 69 | ref | |
| Number of outside sex partners since last visit* | 0.97 | 0.51 | 0.98 | 0.17 | 0.62 | 0.32-1.21 | 0.97 | 0.2 | 1.03 | 1.53 | 1.03 | 0.99-1.08 |
| **Family Planning** | | | | | | | | | | | | |
| Pregnant during interval | | | | | | | | | | | | |
| Yes | 635 | 13 | 181 | 13 | 0.8 | 0.47-1.33 | 524 | 13 | 219 | 14 | 2.18 | 1.52-3.12 |
| No | 4326 | 87 | 1263 | 87 | ref | | 3622 | 87 | 1305 | 86 | ref | |
| Method of contraception since last visit | | | | | | | | | | | | |
| Implant | 414 | 6 | 91 | 4 | 1.22 | 0.70-2.10 | 272 | 5 | 75 | 5 | 0.56 | 0.31-0.99 |
| Injection | 490 | 7 | 114 | 6 | 0.9 | 0.54-1.51 | 693 | 12 | 144 | 12 | 0.62 | 0.40-0.96 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| OCPs | 60 | 1 | 32 | 2 | 2.73 | 1.20-6.20 | 105 | 2 | 24 | 2 | 0.64 | 0.24-1.74 |
| non-hormonal** | 6485 | 87 | 1769 | 88 | ref | | 4497 | 81 | 4497 | 81 | ref | |
| Breastfeeding during interval | | | | | | | | | | | | |
| Yes | 2931 | 50 | 774 | 48 | 0.87 | 0.65-1.14 | 841 | 17 | 234 | 12 | 0.97 | 0.66-1.44 |
| No | 2921 | 50 | 851 | 52 | ref | | 4060 | 83 | 1768 | 88 | ref | |
| **Clinical** | | | | | | | | | | | | |
| Female GI | | | | | | | | | | | | |
| Yes | 691 | 9 | 174 | 9 | 0.72 | 0.35-1.46 | 816 | 15 | 397 | 18 | 1.32 | 0.85-2.04 |
| No | 6761 | 91 | 1837 | 91 | ref | | 4800 | 85 | 1829 | 82 | ref | |
| Female GU | | | | | | | | | | | | |
| Yes | 185 | 2 | 45 | 2 | 0.6 | 0.15-2.42 | 292 | 6 | 155 | 7 | 0.8 | 0.40-2.03 |
| No | 7111 | 98 | 1913 | 98 | ref | | 4948 | 94 | 1972 | 93 | ref | |
| Male GI | | | | | | | | | | | | |
| Yes | 450 | 10 | 143 | 12 | 1.22 | 0.40-3.90 | 305 | 7 | 133 | 7 | 3.37 | 1.19-5.93 |
| No | 3946 | 90 | 1042 | 88 | ref | | 4160 | 93 | 1732 | 24 | ref | |
| Male GU | | | | | | | | | | | | |
| Yes | 266 | 6 | 76 | 6 | 0.84 | 0.31-2.28 | 172 | 4 | 66 | 4 | 1.25 | 0.56-2.83 |
| No | 4126 | 94 | 1109 | 94 | ref | | 4292 | 96 | 1798 | 96 | ref | |

cHR: crude Hazard Ratio, CI: Confidence Interval, GI: Genital Inflammation, GU: Genital Ulceration

*mean,SD reported for continuous variables

**IUD;tubal ligation; hysterectomy; condoms only; none

## Table 3. Multivariable Cox models of time to ART initiation by sex of positive partner in HIV serodiscordant couples

| | ART Initiation | | | |
|---|---|---|---|---|
| | M+F- Couples | | | |
| | aHR* | 95%CI | | p-value |
| Monthly income (USD) | 1.00 | 0.99 | 1.01 | 0.54 |
| Number of previous pregnancies | 0.84 | 0.69 | 1.02 | 0.08 |
| Male number sex partners last year | 1.29 | 0.82 | 2.02 | 0.26 |
| Female STI in the past year | 0.74 | 0.34 | 1.59 | 0.44 |
| VL of positive partner (log10 copies/ml) | 1.54 | 1.01 | 2.34 | 0.04 |
| | M-F+ Couples | | | |
| | aHR* | 95%CI | | p-value |
| *Baseline* | | | | |
| Monthly income (USD) | 1.00 | 0.99 | 1.01 | 0.83 |
| Male had STI in past year | 1.16 | 0.56 | 2.39 | 0.69 |
| VL of positive partner (log10 copies/ml) | 1.45 | 1.03 | 2.04 | 0.03 |
| WHO stage | | | | |
| II v. I | 1.85 | 0.77 | 4.43 | 0.17 |
| III v. I | 1.32 | 0.45 | 3.86 | 0.61 |
| IV v. I | 5.56 | 1.58 | 19.60 | <.01 |
| *Time-varying* | | | | |
| Pregnant during interval | 1.57 | 0.46 | 5.39 | 0.47 |
| Male genital ulcer | 0.59 | 0.08 | 4.50 | 0.61 |
| Contraception method during interval | | | | |
| implant v. non-hormonal | 0.73 | 0.09 | 5.67 | 0.76 |
| injection v. non-hormonal | 1.11 | 0.38 | 3.31 | 0.84 |
| OCP v. non-hormonal | - | - | - | - |

*adjusted for calendar time
aHR: adjusted hazard ratio; CI: confidence interval

## Chapter V. Public health Implications

Although our studies were of ART naïve HIV-1 serodiscordant couples before the current environment of prevalent uptake of ART by HIV positive individuals, HIV incidence among HIV serodiscordant couples remains high in sub-Saharan Africa [4,5]. Overall we found that partnerships characteristics, not just individual, are influential in predicting HIV transmission, hormonal contraceptive use, and ART initiation in heterosexual HIV-1 serodiscordant couples in Rwanda.

Among serodiscordant couples, both individual and partner characteristics play important roles in predicting linked HIV transmission. Ensuring further incorporation of partner behavioral and clinical risk factors in risk factor modeling and clinical prediction tools as well as into practical couples counseling is important. Our findings support evidence that couples testing and counseling help couples understand risk and limit risk behaviors as the linked HIV transmission incidence declined after the initial 3month follow-up period. Both partners in heterosexual HIV discordant partnerships should be regularly screened for STIs and other non-sexually transmitted causes of genital inflammation in women, such as BV or candida, regardless of HIV status. Screening and prophylactic treatment will help reduce inflammation and ulceration thus reducing the risk of HIV acquisition and transmission.

Our second study described predictors of women ever using HC during the study, correlates of HC use over time, and described pregnancy by fertility desires and HC use by serostatus of the woman within the HIV-1 serodiscordant cohort. We found any exposure to HC use was similar across HIV+ and HIV- women, but less common among HIV- women that seroconverted. On average, the use of condoms only or no method across all time intervals remained the most prevalent manner of contraception. Given this study provided routine

counseling and free HC contraception methods and any medical care necessary such as the implantation of an IUD or delivery of an injection, this low uptake is informative in the face of the removal of "structural barriers." More than 20% of women in each HIV status group had an incident pregnancy, and some reported using HC methods during the time of their conception indicating these pregnancies may have been unwanted. In light of the widespread uptake of ART and the effect that has on women's ability to conceive and couple's family planning, the need to understand barriers to HC adoption in order to prevent unwanted pregnancies is essential. Safer conception counseling should both be highlighted in couples counseling and testing services but also integrated into routine ART treatment counseling for HIV serodiscordant couples. Methods to assist in actually practicing safe conception need to be made available to these couples in an affordable and easily accessible manner.

Our final study found that time to ART, depending on the sex of the positive partner, was largely driven by clinical criteria in our final models, but in bivariate models found many behavioral influences as well. Even in light of vastly different ART climate now than during the study, the predictors of early ART initiation in serodiscordant couples can teach us about important partnership factors and can potentially be used to create targeted programs to ensure partners in serodiscordant relationships adhere to treatment to achieve viral load suppression. Our study also demonstrated that some of our widely held assumptions such as women initiate ART earlier, may not hold for all sub-populations such as serodiscordant couples. The success of ART achieving viral load suppression among serodiscordant couples depends on key factors such as early detection of the infected partner so that they can promptly initiate ART, and sustained adherence, minimizing drug resistance, and reducing rates of treatment failure. Thus ART for prevention necessarily relies on important complimentary dyad interventions such as

couples testing and counseling and the acceptability and willingness of HIV infected partners to initiate ART even if they are asymptomatic.

Our findings demonstrated the importance of couple-based interventions in protecting individuals and partners from the risk of HIV transmission. Collecting and using partner risk factor data has shown to play a significant role in contributing to individual risk. Public health programs focused on HIV prevention among serodiscordant couples should be streamlined into couples-base treatment programs and focus on early identification of serostatus of both partners.

## Chapter VI. References


1. World Health Organization. (2013). Global update on HIV treatment 2013: results, impact and opportunities.

2. Dunkle, K.L., et al. New heterosexually transmitted HIV infections in married or cohabiting couples in urban Zambia and Rwanda: an analysis of survey and clinical data. The Lancet, 2008, 371(9631): 2183-91.

3. Eyawo O et al. HIV status in discordant couples in sub-Saharan Africa: a systematic review and meta-analysis. The Lancet Infectious Diseases, 2011,11(4):263–264.

4. Lingappa JR et al. Regional differences in prevalence of HIV-1 discordance in Africa and enrollment of HIV-discordant couples into an HIV-1 prevention trial. PLoS One, 2008, 3(1):e1411.

5. World Health Organization. (2012). Guidance on couples HIV testing and counselling including antiretroviral therapy for treatment and prevention in serodiscordant couples: recommendations for a public health approach.

6. Kaiser R, Bunnell R, Hightower A, et al. Factors associated with HIV infection in married or cohabitating couples in Kenya: results from a nationally representative study. PLoS One.2011;6(3):e17842.

7. Bunnell R, Opio A, Musinguzi J, et al. HIV transmission risk behavior among HIV-infected adults in Uganda: results of a nationally representative survey. AIDS. 2008 Mar 12; 22(5):617-24.

8. Were WA, Mermin JH, Wamai N, et al. Undiagnosed HIV infection and couple HIV discordance among household members of HIV-infected people receiving antiretroviral therapy in Uganda. J Acquir Immune Defic Syndr. 2006; 43(1): 91–5.

9. Gray R, Ssempiija V, Shelton J, et al. The contribution of HIV discordant relationships to new HIV infections in Rakai, Uganda. AIDS. 2011; 25(6):863–5.

10. Curran, K., Baeten, J. M., Coates, T. J., Kurth, A., Mugo, N. R., & Celum, C. (2012). HIV-1 Prevention for HIV-1 Serodiscordant Couples. Current HIV/AIDS Reports, 9(2), 160–170.

11. 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.

12. United Nations Department of Economic and Social Affairs. The World Statistics Pocketbook, 2014 ed. New York. Series V, No. 38, 2014.

13. National Institute of Statistics of Rwanda and MEASURE DHS. Rwanda Demographic and Health Survey. Calverton: ICF International; 2012. Available from: http://dhsprogram.com/publications/publication-FR259-DHS-Final-Reports.cfm

14. Center for Treatment and Research on AIDS, Malaria, Tuberculosis and Other Epidemics. Rwanda Triangulation Project. San Francisco, UCSF: 2008.

15. Rwanda Biomedical Centre. Rwanda HIV and AIDS National Strategic Plan July 2013-June 2018. Institute of HIV/AIDS, Disease Prevention & Control, 2014.

16. Rwanda Ministry of Health. Behavioral and biological surveillance survey among female sex workers. Center for Treatment and Research on AIDS, Malaria, Tuberculosis and Other Epidemics: December 2010.

17. Chemaitelly, H., S.F. Awad, and L.J. Abu-Raddad, The risk of HIV transmission within HIV-1 sero-discordant couples appears to vary across sub-Saharan Africa. Epidemics, 2014. 6:1-9.
18. Chemaitelly, H., et al., Sources of HIV incidence among stable couples in sub-Saharan Africa. J Int AIDS Soc, 2014. 17:18765.

19. Nuwagaba-Biribonwoha H, Jakubowski A, Mugisha V, Basinga P, Asiimwe A, Nash D, and Batya Elul. Low risk of attrition among adults on antiretroviral therapy in the Rwandan national program: a retrospective cohort analysis of 6, 12, and 18-month outcomes. BMC Public Health. 2014; 14: 889.

20. Republic of Rwanda. Country Progress Report January 2008 - December 2009, United Nations General Assembly Special Session on HIV and AIDS. Kigali: National AIDS Control Commission (CNLS); 2010.

21. Republic of Rwanda. Ministry of Health Republic of Rwanda. Annual Report 2011–2012. Kigali: Ministry of Health; 2012.

22. WHO, UNAIDS, UNICEF: Global HIV/AIDS Response. Towards Universal Access. 2011. Epidemic update and health sector progress towards Universal Access Progress Report 2011.

23. Auvert B, Taljaard D, Lagarde E, et al. Randomized, controlled intervention trial of male circumcision for reduction of HIV infection risk: the ANRS 1265 Trial. PLoS Med. 2005; 2(11): e298.

24. Bailey RC, Moses S, Parker CB, et al. Male circumcision for HIV prevention in young men in Kisumu, Kenya: a randomised controlled trial. Lancet. 2007; 369(9562):643–56.

25. Gray RH, Kigozi G, Serwadda D, et al. Male circumcision for HIV prevention in men in Rakai, Uganda: a randomised trial. Lancet. 2007; 369(9562):657–66.

26. Baeten JM, Celum C, Coates TJ. Male circumcision and HIV risks and benefits for women. Lancet. 2009; 374(9685):182–4.

27. Mayer, K. H., & Venkatesh, K. K. (2011). Interactions of HIV, other sexually transmitted diseases, and genital tract inflammation facilitating local pathogen transmission and acquisition. *American Journal of Reproductive Immunology*,*65*(3), 308-316.

28. Ward H, Ronn M. Contribution of sexually transmitted infections to the sexual transmission of HIV. Current opinion in HIV and AIDS. 2010 Jul;5(4):305-10.

29. Gray RH, Wawer MJ, Brookmeyer R, et al. Probability of HIV-1 transmission per coital act in monogamous, heterosexual, HIV-1- discordant couples in Rakai, Uganda. Lancet. 2001; 357 (9263):1149–53.

30. Ndase P, Celum C, Thomas K, et al. 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):65–71.

31. Mah, Timothy L., and Daniel T. Halperin. "Concurrent sexual partnerships and the HIV epidemics in Africa: evidence to move forward." *AIDS and Behavior*14.1 (2010): 11-16.

32. Carter, M. W., Kraft, J. M., Koppenhaver, T., Galavotti, C., Roels, T. H., Kilmarx, P. H., et al. (2007). 'A bull cannot be contained in a single kraal': Concurrent sexual partnerships in Botswana. *AIDS and Behavior, 11*(6), 822–830.

33. Matthews LT, Baeten JM, Celum C, et al. Periconception preexposure prophylaxis to prevent HIV transmission: benefits, risks, and challenges to implementation. AIDS. 2010;24 (13):1975–82.

34. Hughes, James P., et al. "Determinants of per-coital-act HIV-1 infectivity among African HIV-1–serodiscordant couples." *Journal of Infectious Diseases* (2012): jir747.

35. Allen C, Mbonye M, Seeley J, et al. ABC for people with HIV: responses to sexual behaviour recommendations among people receiving antiretroviral therapy in Jinja, Uganda. Cult Health Sex.2011;13(5):529–43.

36. 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.

37. Heffron R, Were E, Celum C, et al. A prospective study of contraceptive use among African women in HIV-1 serodiscordant partnerships. Sexually Transmitted Diseases. 2010;37 (10):621–8.

38. Guthrie BL, Choi RY, Bosire R, et al. Predicting pregnancy in HIV-1-discordant couples. AIDS and Behavior. 2010;14(5):1066–71.

39. Heffron, R., Donnell, D., Rees, H., Celum, C., Mugo, N., Were, E., ... & Coombs, R. W. (2012). Use of hormonal contraceptives and risk of HIV-1 transmission: a prospective cohort study. *The Lancet infectious diseases*,*12*(1), 19-26.

40. 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.

41. 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.

42. Haddad, L.B., et al., *Contraceptive Methods and Risk of HIV Acquisition or Female-to-Male Transmission.* Curr HIV/AIDS Rep, 2014.

43. Kinuthia, J., et al., *Cofactors for HIV-1 incidence during pregnancy and postpartum period.* Curr HIV Res, 2010. 8(7): p. 510-4.

44. 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.

45. 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.

46. Harling, G., et al., *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.

47. 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.

48. Biraro, S., et al., *HIV-1 transmission within marriage in rural Uganda: a longitudinal study.* PLoS One, 2013. 8(2): p. e55060.

49. Seeley, J., et al., *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.

50. 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.

51. Coldiron, M.E., et al., *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.

52. Ruzagira, E., et al., *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.

53. Royce RA, Sena A, Cates W Jr, Cohen MS (1997) Sexual transmission of HIV. N Engl J Med 336: 1072–1078.

54. Powers KA, Poole C, Pettifor AE, Cohen MS (2008) Rethinking the heterosexual infectivity of HIV-1: a systematic review and meta-analysis. Lancet Infect Dis 8: 553–563.

55. Greenblatt RMLS, Plummer FA, Quinn TC, Critchlow CW, Ashley RL, et al. (1988) Genital ulceration as a risk factor for human immunodeficiency virus infection. AIDS 2: 47–50.

56. Baeten JM, Kahle E, Lingappa JR, Coombs RW, et al. Genital HIV-1 RNA predicts risk of heterosexual HIV-1 transmission. Sci Transl 2011;Med 3:77ra29.

57. Quinn TC, Wawer MJ, Sewankambo N, et al. Viral load and heterosexual transmission of human immunodeficiency virus type 1. Rakai Project Study Group. N Engl J Med. 2000;342(13):921–9.

58. Weller S, Davis K (2002) Condom effectiveness in reducing heterosexual HIV transmission. Cochrane Database Syst Rev: CD003255.

59. Allen S, Tice J, Van de Perre P, et al. Effect of serotesting with counselling on condom use and seroconversion among HIV discordant couples in Africa. Bmj. 1992; 304(6842):1605–9.

60. Allen S, Meinzen-Derr J, Kautzman M, et al. Sexual behavior of HIV discordant couples after HIV counseling and testing. AIDS. 2003;17(5):733–40.

61. Burton J, Darbes LA, Operario D. Couples-focused behavioral interventions for prevention of HIV: systematic review of the state of evidence. AIDS Behav. 2010;14(1):1–10.

62. Baeten JM, Donnell D, Ndase P, Mugo NR, Campbell JD, Wangisi J, et al. Antiretroviral prophylaxis for HIV prevention in heterosexual men and women. N Engl J Med. 2012;367:399–410.

63. Thigpen MC, Kebaabetswe PM, Paxton LA, Smith DK, Rose CE, Segolodi TM, et al. Antiretroviral preexposure prophylaxis for heterosexual HIV transmission in Botswana. N Engl J Med. 2012;367:423–434.

64. World Health Organization, Hormonal contraceptive methods for women at high risk of HIV and living with HIV: 2014 Guidance statement. 2014: Geneva, Switzerland.
65. Celum, C., et al., Acyclovir and transmission of HIV-1 from persons infected with HIV-1 and HSV-2. N Engl J Med, 2010. 362(5): 427-39.

66. Hallett TB, Baeten JM, Heffron R, et al. Optimal uses of antiretrovirals for prevention in HIV-1 serodiscordant heterosexual couples in South Africa: A modelling study. PLoS Med. 2011;8(11).

67. 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.

68. 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.

69. Petruney T, Robinson E, Reynolds H, Wilcher R, Cates W (2008) Contraception is the best kept secret for prevention of mother-to-child HIV transmission. Bull World Health Organ 86: B.

70. UNFPA (2013) State of the World Population Report. Motherhood in Childhood: Facing the challenge of adolescent pregnancy. [cited 20 February 2015]: Available from: http://www.unfpa.org/publications/state-world-population-2013.

71. Basinga P et al., Unintended Pregnancy and Induced Abortion in Rwanda: Causes and Consequences, New York: Guttmacher Institute, 2012.

72. Morrison CS, Chen P-L, Kwok C, Baeten JM, Brown J, et al. Hormonal Contraception and the Risk of HIV Acquisition: An Individual Participant Data Meta-analysis. PLoS Med. 2015 Jan 22;12(1):e1001778.

73. Ralph, L.J., McCoy S.I., Shiu K., Padian N.S., Hormonal contraceptive use and women's risk of HIV acquisition: a meta-analysis of observational studies. Lancet Infect Dis. 2015 Jan 8. doi: 10.1016/S1473-3099(14)71052-7.

74. Wall Kristin M., Kilembe William, Vwalika Bellington, Khu Naw Htee, Brill Ilene, Chomba Elwyn, Johnson Brent A., Haddad Lisa, Tichacek Amanda, Allen Susan, Hormonal contraception does not increase women's HIV acquisition risk in Zambian discordant couples, 1994–2012, Contraception (2015).

75. Campbell M, Sahin Hodoglugil NN, Potts M. Barriers to fertility regulation: a review of the literature. Stud Fam Plann 2006;37(2):87–97.

76. Cooper D, Moodley J, Zweigenthal V, et al. Fertility intentions and reproductive health care needs of people living with HIV in Cape Town, South Africa: implications for integrating reproductive health and HIV care services. AIDS Behav. 2009;13 Suppl1:38–46.

77. Laher F, Todd CS, Stibich MA, et al. A qualitative assessment of decisions affecting contraceptive utilization and fertility intentions among HIV-positive women in Soweto, South Africa. AIDS Behav. 2009;13 Suppl 1:47–54.

78. Grabbe K, Stephenson R, Vwalika B, et al. Knowledge, use, and concerns about contraceptive methods among sero-discordant couples in Rwanda and Zambia. Journal of Women's Health.2009;18(9):1449–56.

79. Muhoza, ND, et al., Measuring the Success of Family Planning Initiatives in Rwanda: A Multivariate Decomposition Analysis. DHS Working Papers, Calverton, Maryland: Feb 2013.

80. Matthews LT, Mukherjee JS. Strategies for harm reduction among HIV-affected couples who want to conceive. AIDS Behav. 2009;13 Suppl 1:5–11.

81. Barreiro P, Castilla JA, Labarga P, et al. Is natural conception a valid option for HIV-serodiscordant couples? Hum Reprod. 2007;22(9):2353–8.

82. Gray R, Serwadda D, Wawer M. Empirical observations underestimate the proportion of HIV infections attributable to sexually transmitted diseases in Mwanza and Rakai STD treatment trials: simulation results. Sexually Transmitted Diseases. 2007; 34(1):61.

83. Lyles, C. M., Kay, L. S., Crepaz, N., Herbst, J. H., Passin, W. F., Kim, A. S., ... & Mullins, M. M. (2007). Best-evidence interventions: Findings from a systematic review of HIV behavioral interventions for US populations at high risk, 2000-2004. *American journal of public health*, *97*(1), 133-143.

84. Guthrie BL, Choi RY, Liu AY, et al. Barriers to antiretroviral initiation in HIV-1-discordant couples. J Acquir Immune Defic Syndr. 2011;58(3):e87–93.

85. Curran, K., Ngure, K., Shell-Duncan, B., Vusha, S., Mugo, N. R., Heffron, R., ... & Baeten, J. M. (2014). If I am given antiretrovirals I will think I am nearing the grave': Kenyan HIV serodiscordant couples' attitudes regarding early initiation of antiretroviral therapy. *AIDS (London, England)*, *28*(2), 227.

86. Anand A, Shiraishi RW, Bunnell RE, et al. Knowledge of HIV status, sexual risk behaviors and contraceptive need among people living with HIV in Kenya and Malawi. AIDS. 2009;23 (12):1565–73.

87. De Walque D. Sero-discordant couples in five African countries: implications for prevention strategies. Popul Dev Rev. 2007;33 (3):501–23.

88. World Health Organization. (2013). Global update on HIV treatment 2013: results, impact and opportunities. Geneva, Switzerland.

89. Rosen, S., & Fox, M. P. (2011). Retention in HIV care between testing and treatment in sub-Saharan Africa: a systematic review. *PLoS Med*, *8*(7), e1001056.

90. Bassett IV, Regan S, Chetty S, et al. Who starts antiretroviral therapy in Durban, South Africa?….not everyone who should. AIDS. 2010;24(Suppl 1):S37–44.

91. Bassett IV, Wang B, Chetty S, et al. Loss to care and death before antiretroviral therapy in Durban, South Africa. J Acquir Immune Defic Syndr. 2009;51:135–139.

92. Larson BA, Brennan A, McNamara L, et al. Early loss to follow up after enrolment in pre-ART care at a large public clinic in Johannesburg, South Africa. Trop Med Int Health. 2010;15(Suppl 1):43–47.

93. Dahab M, Charalambous S, Karstaedt AS, et al. Contrasting predictors of poor antiretroviral therapy outcomes in two South African HIV programmes: a cohort study. BMC Public Health. 2010;10:430.

94. Gorbach P, Holmes K. Transmission of STIs/HIV at the partnership level: beyond individual-level analyses. Journal of Urban Health. 2003; 80(4):15-25.

95. Ewart C. Social action theory for a public health psychology. American Psychologist. 1991; 46(9): 931-46.

96. Crankshaw, T. L., et al. A conceptual framework for understanding HIV risk behavior in the context of supporting fertility goals among HIV serodiscordant couples. Reprod Health Matters.2012.December; 20(39 Suppl): 50–60.

97. Albarracin D, Rothman AJ, Di Clemente R, et al. Wanted: a theoretical roadmap to research and practice across individual, interpersonal, and structural levels of analysis. AIDS and Behavior. 2010 Dec; 14(Suppl 2):185–88.

98. Karney BR, Hops H, Redding CA, et al. A framework for incorporating dyads in models of HIV prevention. AIDS and Behavior. 2010 Dec; 14(Suppl 2):189–203.

99. Boeras DI, Luisi N, Karita E, McKinney S, Sharkey T, Keeling M, et al. Indeterminate and discrepant rapid HIV test results in couples' HIV testing and counselling centres in Africa. J Int AIDS Soc 2011;14:14–8.

100. Trask SA, Derdeyn CA, Fideli U, Chen Y, Meleth S, Kasolo F, et al. Molecular

epidemiology of human immunodeficiency virus type 1 transmission in a heterosexual cohort of discordant couples in Zambia. J Virol 2002;76(1):397–405.

101. Campbell MS, Mullins JI, Hughes JP, Celum C, Wong KG, Raugi DN, et al. Viral linkage in HIV-1 seroconverters and their partners in an HIV-1 prevention clinical trial. PLoS One 2011;6(3):e16986.

102. Dionne-Odom J, Karita E, Kilembe W, Henderson F, Vwalika B, Bayingana R, et al. Syphilis treatment response among HIV-discordant couples in Zambia and Rwanda. Clin Infect Dis 2013;56(12):1829–37.

103. 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.

104. Kanters, S., Nansubuga, M., Mwehire, D., Odiit, M., Kasirye, M., Musoke, W., ... & Mills, E. J. (2013). Increased mortality among HIV-positive men on antiretroviral therapy: survival differences between sexes explained by late initiation in Uganda. *HIV/AIDS–Research and Palliative Care*, *5*, 111-119.

105. Heffron, R., Ngure, K., Mugo, N., Celum, C., Kurth, A., Curran, K., & Baeten, J. M. (2012). Willingness of Kenyan HIV-1 serodiscordant couples to use antiretroviral based HIV-1 prevention strategies. *Journal of acquired immune deficiency syndromes (1999)*, *61*(1), 116.

106. Batani J, Brum T, Calvet G, Dusara P, Dhayarkar S, Gamble T, et al. 20th Conference on Retroviruses and Opportunistic Infections. Atlanta, GA: 2013. Acceptance of ART in the delay DRM after notification of interim study results: data from HPTN 052.

107. Sterne, Jonathan AC, et al. "Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls." *Bmj* 338 (2009): b2393.

108. Liamputtong, Pranee. *Childrearing and infant care issues: A cross-cultural perspective*. Nova Publishers, 2007.

109. Bonte, M., and H. Van Balen. "Prolonged lactation and family spacing in Rwanda." *Journal of biosocial science* 1.02 (1969): 97-100.

110. Ware NC, Idoko J, Kaaya S, et al. Explaining adherence success in sub-Saharan Africa: an ethnographic study. PLoS Med. 2009;6(1):e11.

111. Stirratt MJ, Remien RH, Smith A, et al. The role of HIV serostatus disclosure in antiretroviral medication adherence. AIDS and Behavior. 2006;10(5):483–93.

112. Ngure K, Heffron R, Mugo N, et al. Successful increase in contraceptive uptake

among Kenyan HIV-1-serodiscordant couples enrolled in an HIV-1 prevention trial. AIDS. 2009;23 Suppl 1:S89–95.

113. Medley, A., Baggaley, R., Bachanas, P., Cohen, M., Shaffer, N., & Lo, Y.-R. (2013). Maximizing the impact of HIV prevention efforts: Interventions for couples. *AIDS Care*, *25*(12), 1569–1580.

114. Ministry of Health. *Guide de Prise en Charge des Personnes Infectees par le VIH au Rwanda*. Kigali, Rwanda: Ministry of Health, 2003.

115. Ministry of Health of Rwanda, National HIV Care and Treatment Guidelines, Ministry of Health of Rwanda, Kigali, Rwanda, 2007.

116. Ministry of Health of Rwanda, National HIV Treatment and Care Guidelines, Ministry of Health of Rwanda, Kigali, Rwanda, 2009.

117. World Health Organization, Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection: Recommendations for a Public Health Approach, World Health Organization, Geneva, Switzerland, 2013.

118. Lowrance, D. W., Ndamage, F., Kayirangwa, E., Ndagije, F., Lo, W., Hoover, D. R., ... & Rukundo, A. (2009). Adult clinical and immunologic outcomes of the national antiretroviral treatment program in Rwanda during 2004-2005. JAIDS Journal of Acquired Immune Deficiency Syndromes, 52(1), 49-55.

119. Fideli US, Allen SA, Musonda R, et al. Virologic and immunologic determinants of heterosexual transmission of human immunodeficiency virus type 1 in Africa. AIDS Res Hum Retroviruses 2001;17:901-910.