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Increased Risk of HIV-1 Transmission in Pregnancy: A Prospective Study among African HIV-1 Serodiscordant Couples

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Abstract

Background—Physiologic and behavioral changes during pregnancy may alter HIV-1 susceptibility and infectiousness. Prospective studies exploring pregnancy and HIV-1 acquisition risk in women have found inconsistent results. No study has explored the effect of pregnancy on HIV-1 transmission risk from HIV-1 infected women to male partners.

Methods—In a prospective study of African HIV-1 serodiscordant couples, we evaluated the relationship between pregnancy and the risk of 1) HIV-1 acquisition among women and 2) HIV-1 transmission from women to men.

Results—3321 HIV-1 serodiscordant couples were enrolled, 1085 (32.7%) with HIV-1 susceptible female partners and 2236 (67.3%) with susceptible male partners. HIV-1 incidence in women was 7.35 versus 3.01 per 100 person-years during pregnant and non-pregnant periods (hazard ratio [HR] 2.34, 95% confidence interval [CI] 1.33–4.09). This effect was attenuated and not statistically significant after adjusting for sexual behavior and other confounding factors (adjusted HR 1.71, 95% CI 0.93–3.12). HIV-1 incidence in male partners of infected women was 3.46 versus 1.58 per 100 person-years when their partners were pregnant versus not pregnant (HR

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Contributors

NRM, RH, DD, AW, and JMB designed the study, and RH, DD, and JMB performed the analysis. All investigators contributed to data collection and writing of the manuscript, and all approved the final draft. NRM, RH, and JMB wrote the initial manuscript draft and vouch for the data, analysis, interpretation and manuscript submission.

2.31, 95% CI 1.22–4.39). This effect was not attenuated in adjusted analysis (adjusted HR 2.47, 95% CI 1.26–4.85).

Conclusions—HIV-1 risk increased two-fold during pregnancy. Elevated risk of HIV-1 acquisition in pregnant women appeared in part to be explained by behavioral and other factors. This is the first study to show pregnancy increased the risk of female-to-male HIV-1 transmission, which may reflect biological changes of pregnancy that could increase HIV-1 infectiousness.

Keywords

pregnancy; HIV-1 transmission; Africa; heterosexual; serodiscordant

Introduction

In sub-Saharan Africa, women account for 60% of HIV-1 infected adults [1]. Many African countries with high HIV-1 prevalence also have high fertility rates, and women are pregnant for a substantial portion of their adult years. Physiologic changes during gestation – including high levels of progesterone that could induce systemic or genital mucosal immunologic changes [2, 3] – may increase the risk for women to acquire HIV-1 during pregnancy and pregnant HIV-1 infected women to transmit to their sexual partners. Unprotected sex associated with efforts to conceive and continued during pregnancy may also increase HIV-1 risk.

High HIV-1 incidence during pregnancy has been reported among women from several populations [4–7], but prospective studies exploring pregnancy and HIV-1 acquisition risk in women have found inconsistent results: one documented a 2-fold increased risk [8] and two found no increased risk [9, 10]. Acute infection of women during pregnancy carries a high rate of HIV-1 transmission to infants [11]; thus, clear evidence of increased HIV-1 acquisition in women during pregnancy may promote policies for repeat HIV-1 testing in late pregnancy in high prevalence settings [12]. Among HIV-1 infected women, one study found increased HIV-1 shedding in genital secretions during pregnancy, suggesting increased infectiousness [13]; however, no prospective study has directly explored pregnancy as a risk factor for female-to-male HIV-1 transmission.

We evaluated the relationship between pregnancy and risk of HIV-1 acquisition in women and HIV-1 transmission from women to men in a prospective study of African HIV-1 serodiscordant couples.

Methods

Population and procedures

Between November 2004 and April 2007, 3408 HIV-1 serodiscordant couples from seven African countries (Botswana, Kenya, Rwanda, South Africa, Tanzania, Uganda, Zambia) were enrolled in the Partners in Prevention HSV/HIV Transmission Study, a randomized, placebo-controlled, clinical trial of acyclovir HSV-2 suppressive therapy for the prevention of HIV-1 transmission.[15] Acyclovir did not decrease HIV-1 risk within the couples (hazard ratio [HR] versus placebo 0.92, 95% confidence interval [CI], 0.60–1.41) [16]. This report is a secondary analysis of data from this prospective study.

Eligible couples were ≥18 years of age, reported ≥3 episodes of vaginal intercourse during the three months prior to screening, and intended to remain as a couple. HIV-1 infected partners were seropositive for HSV-2, had a CD4 count ≥250 cells/mm^3, and were not taking antiretroviral therapy (ART). HIV-1 infected women who were pregnant at screening

were excluded from the study and enrolled women who became pregnant during follow-up interrupted study medication use until the completion of pregnancy. Pregnant HIV-1 uninfected women were eligible for enrollment, as they did not receive study medication, and those who became pregnant during follow-up continued usual study procedures.

Participants were followed for up to 24 months, with all participants completing the study by October 2008. HIV-1 infected partners were seen monthly and HIV-1 uninfected partners quarterly. Data on sexual behavior – specifically, number of sexual acts with and without condoms within the partnership and with external partners – were collected at each visit on standardized case report forms using face-to-face interviews in local languages. Plasma for HIV-1 RNA quantification was collected at baseline and months 3, 6, 12, and study exit, and CD4 counts were performed every 6 months. HIV-1 infected persons who met national guidelines for initiation of ART, as a result of CD4 decline or clinical status, were referred to local HIV-1 care clinics. HIV-1 infected women who became pregnant were referred for prevention of mother-to-child transmission services. HIV-1 uninfected partners were seen quarterly for HIV-1 serologic testing. Contraceptive use was recorded at each study visit.

Participants received comprehensive HIV-1 prevention services including HIV-1 risk-reduction counseling (both individual and as a couple), quarterly syndromic sexually transmitted infection (STI) treatment, and provision of free condoms. The study protocol was approved by the University of Washington Human Subjects Review Committee and ethics review boards at each study site. Participants provided written informed consent.

Laboratory procedures

HIV-1 serologic testing was by rapid HIV-1 antibody tests, with positive results confirmed by HIV-1 Western blot [16]. For initially HIV-1 uninfected partners who seroconverted to HIV-1, analysis of HIV-1 *env* and *gag* gene sequences from both members of the couple was used to determine whether transmission was genetically linked within the partnership [16, 17]. Serologic testing for HSV-2 and nucleic acid amplification testing for STIs (*Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis*) was done at study enrollment [15]. CD4 quantification was performed using standard flow cytometry. Plasma HIV-1 RNA was quantified using the COBAS TaqMan real-time HIV-1 RNA assay, version 1.0 (Roche Diagnostics, Indianapolis, IN).

Pregnancy procedures and definition of pregnancy exposure

Urine pregnancy tests were performed at screening and quarterly follow-up visits for HIV-1 infected women. For HIV-1 uninfected women, urine pregnancy tests were done based on report of missed menses. Date of last menstrual period (LMP), estimated date of delivery, and pregnancy outcome were collected for all pregnancies. We defined the start of pregnancy as the date of LMP and the end of pregnancy as the date of delivery or pregnancy loss. Complete data on LMP and date of delivery were available for 96% of pregnancies observed during the study; for the remaining 4% of pregnancies that were missing either of these parameters, we estimated start and end dates using the reported duration of the pregnancy (based on maternal history) and either LMP or date of delivery. We defined HIV-1 uninfected women and male partners of HIV-1 infected women as "*pregnancy exposed*" during the time period between LMP and 6 weeks following the end of pregnancy, thus including the early post-partum period as pregnancy-exposed time; inclusion of the early post-partum period is consistent with other studies of pregnancy as a risk factor for HIV-1 acquisition and reflects physiologic changes of pregnancy that persist in the early post-partum period [18–20].

Statistical analysis

Twenty-seven couples in which the HIV-1 infected partner's baseline serology did not confirm both HIV-1 and HSV-2 infection were excluded from the analysis, as were 60 couples in which the HIV-1 uninfected participant did not complete any follow-up visits for assessment of HIV-1 seroconversion.

Pregnancy was assessed as a time-varying exposure, with each participant's time divided into quarterly intervals corresponding to a period between study visits and each time interval coded as pregnancy exposed or unexposed. We compared participant characteristics during pregnancy-exposed versus unexposed intervals using generalized estimating equations. We calculated pregnancy incidence by dividing the number of incident pregnancies by follow-up time when women were at risk to become pregnant.

The primary outcome was HIV-1 seroconversion. We analyzed the association between pregnancy and HIV-1 using Cox proportional hazards regression. For female-to-male HIV-1 transmission, we restricted HIV-1 seroconversions to those events that were genetically linked within the partnership, to reduce misclassification bias; men who acquired HIV-1 from a different partner contributed follow-up until HIV-1 seroconversion and were censored thereafter. For HIV-1 acquisition in women, our primary analysis did not restrict to linked transmissions since the exposure (pregnancy) and outcome (HIV-1 seroconversion) were present in the same individuals (i.e., HIV-1 at-risk women); however, we performed a sensitivity analysis restricting to linked transmissions. We performed analyses classifying the pregnancy-exposure intervals as "early" (defined as up until the first HIV-1 test occurring during a pregnancy, thus capturing HIV-1 transmission at or close to the time of conception) or "late" (all HIV-1 tests occurring after the first test during pregnancy).

To assess potential confounding of the relationship between pregnancy and HIV-1 risk, we performed multivariate Cox proportional hazards analysis controlling for demographic, clinical, and behavioral factors. Variables assessed for potential confounding included the following baseline variables: randomization arm of the HIV-1 infected partner (acyclovir versus placebo), age of the HIV-1 uninfected partner, region (East versus southern Africa), number of living children, STIs (in either partner), HSV-2 serostatus in the HIV-1 uninfected partner, and circumcision status of the male partner, as well as the following time-dependent variables: highly-effective contraceptive use during past month (any oral, injectable, or implantable method, intrauterine device, or surgical sterilization), any unprotected sex with the study partner in the past month, any additional sex partners during the past month, total number of sex acts and unprotected sex acts during the past month, CD4 count of the HIV-1 infected partner, plasma HIV-1 RNA concentrations in the HIV-1 infected partner, and ART use by the HIV-1 infected partner during the past 3 months. Confounding factors that altered the HR of the effect of pregnancy on HIV-1 risk by ≥10% were included in the final multivariate model. Age of the HIV-1 uninfected partner, recent contraceptive use by the female partner, and any unprotected sex during the prior month were the only factors that confounded the relationship between pregnancy and HIV-1 and thus these were included in the final multivariate models.

We also analyzed our data using a case-crossover approach [21]. The case-crossover design minimizes unmeasured confounding by restricting the analysis to those who experience the study outcome (i.e., HIV-1 seroconversion). For this analysis, we included only those participants who acquired HIV-1, and we compared the probability of pregnancy exposure in follow-up intervals during which HIV-1 seroconversion occurred (the case period) to time intervals before HIV-1 transmission occurred (control periods). Thus, each seroconverter in the case-crossover analysis served as her/his own control. Separate analyses were performed for male-to-female and female-to-male HIV-1 transmission. Conditional logistic regression

was used, and adjusted models controlled for contraceptive use and unprotected sex as time-varying covariates.

Data were analyzed using SAS version 9.2.

Results

Population

A total of 3321 couples were included in this analysis: 1085 (32.7%) in which the man was infected with HIV-1 and 2236 (67.3%) in which the woman was HIV-1 infected (Table 1). Most couples were married and cohabitating. Among HIV-1 infected participants, the median CD4 count was 461 cells/mm^3 and median plasma HIV-1 RNA concentration was 4.1 $\log_{10}$ copies/mL.

Follow-up and HIV-1 incidence

Median follow-up for HIV-1 seropositive and HIV-1 seronegative partners was 20.9 (IQR 15.6–24.1) and 19.9 months (IQR 14.3–23.9), respectively. For HIV-1 seronegative partners, 5141 person-years of follow-up (1695 among HIV-1 seronegative women and 3446 among HIV-1 seronegative men) were accrued for assessment of HIV-1 seroincidence, during which 151 HIV-1 seroconversions occurred (61 in women and 90 in men). Among women, overall HIV-1 incidence was 3.60 cases per 100 person-years. Of the 90 incident HIV-1 infections in men, 58 (64.4%) were determined by viral sequencing to be genetically linked within the partnership, for an incidence of linked transmission of 1.68 cases per 100 person-years.

Pregnancy during follow-up

At enrollment, 94 (8.7%) HIV-1 uninfected women and no HIV-1 infected women were pregnant, as pregnancy in HIV-1 infected women was a study exclusion criterion. An additional 226 pregnancies occurred among HIV-1 uninfected women after enrollment (incidence of 15.3 per 100 person-years, with1480 person-years of follow-up at risk for pregnancy), and 503 pregnancies (all detected after enrollment) occurred among HIV-1 infected women (incidence 16.0 per 100 person-years, with 3147 person-years of follow-up at risk for pregnancy). Twenty-seven HIV-1 uninfected and 27 infected women experienced two pregnancies; one HIV-1 infected woman was pregnant three times. Of the 320 total pregnancies in HIV-1 uninfected women, the median duration of pregnancy was 40 weeks (IQR 37–40), and 191 (59.7%) ended in live births, 32 (10.0%) in pregnancy losses, and 13 (4.1%) in unknown outcomes; 84 women (26.3%) were pregnant at study exit and pregnancy outcome information was not available. Of 503 pregnancies in HIV-1 infected women, the median duration of pregnancy was 38 weeks (IQR 21–40), and 216 (42.9%) ended in live births, 143 (28.4%) in pregnancy losses (119 of these 143 [83.2%] before 20 weeks' gestation, in part reflecting "chemical pregnancies" that were detected because of scheduled quarterly pregnancy testing in the study protocol), 14 (2.8%) in unknown outcomes, and 128 (25.5%) were ongoing at study exit. Overall, 13.6% of total person-years among HIV-1 uninfected women (988 of 7591 quarterly follow-up intervals) and 10.1% among HIV-1 uninfected men were defined as "pregnancy exposed." Couples who became pregnant were younger and more likely to report unprotected sex during pregnancy-exposed follow-up intervals (Table 2). Plasma HIV-1 concentrations and CD4 counts in HIV-1 infected partners were not significantly different during pregnant versus non-pregnant periods.

Effect of pregnancy on HIV-1 acquisition in women—Of the 61 HIV-1 seroconversions among women, 17 (27.9%) occurred during pregnancy (Table 3). The

incidence of HIV-1 during pregnancy was 7.35 per 100 person-years compared with 3.01 per 100 person-years during non-pregnant intervals (HR 2.34, p=0.003). HIV-1 risk was elevated during both early (HR 3.55, p=0.007) and late pregnancy (HR 2.10, p=0.02). In multivariate analysis, the effect of pregnancy on HIV-1 risk was attenuated and was no longer statistically significant (adjusted HR 1.71, p=0.08). In a sensitivity analysis of the 49 HIV-1 seroconversions in women that were genetically linked to their male partner, the results were similar to those for all seroconversions in women: HR 2.44 (95% CI 1.31–4.53, p=0.005) and adjusted HR 1.81 (95% CI 0.93–3.52, p=0.08).

Effect of pregnancy on female-to-male HIV-1 transmission—Of the 58 HIV-1 transmissions to men, 12 (20.7%) occurred during pregnancy. The incidence of female-to-male HIV-1 transmission was 3.46 per 100 person-years during pregnancy compared to 1.58 per 100 person-years when the female partner was not pregnant. This effect was statistically significant (HR 2.31, p=0.01), and remained statistically significant after adjustment for confounding factors (adjusted HR 2.47, p=0.01). Subgroup analysis suggested increased HIV-1 transmission risk during both early (adjusted HR 2.64, p=0.05) and late pregnancy (adjusted HR 2.37, p=0.04), although there were small numbers of seroconversions in these subcategories (N=5 and 7, respectively).

For the 216 pregnancies among HIV-1 infected women that ended in live births, 176 (81.5%) received antiretroviral medications, either short-course single or dual-agent therapy at the time of delivery (n=102) or combination ART during the pregnancy (n=74). For the 12 female-to-male HIV-1 transmissions that occurred during pregnancy, 9 (75.0%) women received antiretrovirals during pregnancy, although only two when HIV-1 transmission occurred: one was in a couple for whom transmission coincided with the HIV-1 infected woman's use of short-course zidovudine during labor and the other, as previously reported [22], was in a couple in which the HIV-1 infected woman initiated combination ART early in the pregnancy and her partner seroconverted shortly thereafter. Further adjustment of the relationship between pregnancy and male-to-female HIV-1 transmission risk for antiretroviral use during pregnancy did not substantially change the risk estimate in Table 3 (adjusted HR 2.30, 95% confidence interval 1.15–4.61, p=0.02).

Case-crossover analysis

To further explore unmeasured confounding between women who experienced versus did not experience pregnancy we performed case-crossover analyses, which were restricted to persons who acquired HIV-1 during follow-up. The association between pregnancy and HIV-1 acquisition in women was statistically significant in unadjusted (odds ratio [OR] 1.40, 95% CI 0.45–4.38, p=0.56) and adjusted analysis (adjusted OR 1.33, 95% CI 0.42–4.25, p=0.63). In contrast, the association between pregnancy and female-to-male HIV-1 transmission was statistically significant in both unadjusted analysis (OR 5.62, 95% CI 1.18–26.89, p=0.03) and after adjusting for unprotected sex and contraceptive use (adjusted OR 13.63, 95% CI 1.60–116.22, p=0.02). Thus, the case-crossover results were consistent with those obtained in multivariate Cox regression analysis.

Discussion

In this prospective study of more than 3300 African heterosexual HIV-1 serodiscordant couples, pregnancy was associated with increased incidence of HIV-1 acquisition in women and HIV-1 transmission from women to men. Elevated risk of HIV-1 acquisition in pregnant women appeared to be in part explained by behavioral and other factors, as the relationship between pregnancy and HIV-1 acquisition risk was not statistically significant after controlling for sexual behavior and other confounding variables. The finding that pregnancy

increases the risk of female-to-male HIV-1 transmission two-fold is novel and has important public health implications for reducing HIV-1 transmission in high HIV-1 prevalence settings. These findings should stimulate new strategies to strengthen family planning and maternal health services for women with and at risk for HIV-1, in order to reduce unwanted pregnancies and avert HIV-1 transmission to pregnant women and from pregnant HIV-1 infected women to their infants and partners. In addition, our results strengthen the argument for initiation of combination ART in HIV-1 infected women during early pregnancy [12], to suppress maternal HIV-1 viral load and thereby reduce the risk of both perinatal and sexual transmission of HIV-1.

Increased HIV-1 risk during pregnancy could reflect a higher frequency of unprotected sexual activity among women who become pregnant versus those who do not become pregnant or could be caused by biologic changes in pregnancy and the postpartum period, such as immunologic changes affecting both the adaptive and innate immune systems [20, 23], that may facilitate HIV-1 transmission. We found the risk for female-to-male transmission was statistically significant after adjustment for confounding factors and may thus primarily reflect biological changes of pregnancy that may increase HIV-1 infectiousness. High progesterone states, like pregnancy, increase shedding of HIV-1 in female genital secretions and increase levels of HIV-1 co-receptors (e.g., CCR-5 expressing cells) in the genital mucosa, suggesting a possible mechanism for pregnancy to increase the infectiousness of women with HIV-1 [2, 3, 24]. One prior study found a 4.5-fold increased shedding of HIV-1 infected cells in genital secretions in pregnant compared with non-pregnant HIV-1 infected women [13].

In contrast to our female-to-male HIV-1 transmission results, we found the effect of pregnancy on HIV-1 acquisition risk in women was attenuated in multivariate analysis that controlled for age, contraceptive use, and unprotected sex and was no longer statistically significant, suggesting these factors in part accounted for increased HIV-1 risk for pregnant women. Both HIV-1 acquisition and pregnancy require unprotected sex, and elevated risk of HIV-1 acquisition during pregnancy may thus be largely due to behavioral risk differences of women who become pregnant versus not.

In our subset analysis, HIV-1 transmission risks were elevated during both early and late pregnancy, with slightly higher risk estimates during early pregnancy, perhaps reflecting unprotected sex leading to pregnancy and HIV-1 acquisition. Socio-cultural pressure for a couple to conceive may outweigh the desire to prevent HIV-1 transmission within HIV-1 serodiscordant couples, and in the absence of techniques for conception that reduce HIV-1 transmission potential, couples may choose to risk unprotected sex [25]. We observed high incidences of pregnancy among both HIV-1 infected and uninfected women in this cohort.

Our study had several strengths. Our large sample size and multinational cohort make this the largest, most diverse sampling of HIV-1 serodiscordant couples reported. An important aspect of our study is that we determined genetic viral linkage of transmitted HIV-1 within partnerships, which minimized misclassification in our analysis of female-to-male HIV-1 transmission [16]. We performed additional analyses using a case-cross over approach, which controls for unmeasured confounders between persons, which yielded similar results to our proportional hazards analysis, lending further credibility to our findings. All HIV-1 infected participants in our study were co-infected with HSV-2, which is true of approximately 80% of HIV-1 infected persons in sub-Saharan Africa [26], and thus this is unlikely to limit the generality of our findings.

To reduce risk for HIV-1 acquisition and transmission during pregnancy, couples HIV-1 testing with targeted risk reduction for HIV-1 serodiscordant couples, promotion of condom

use, and family planning to prevent unwanted pregnancy should be prioritized. For HIV-1 serodiscordant couples who desire fertility, pre-conception counseling, timed unprotected sex, combination ART use by the infected partner [22, 27], and potentially antiretroviral pre-exposure prophylaxis (PrEP) may offer complementary options for meeting reproductive goals [28].

Recent studies have recognized high HIV-1 incidence in women during pregnancy and the early post-partum period [4–7]. However, in many antenatal clinics focused on prevention of mother-to-child HIV-1 transmission, little attention is paid to counseling of women found to be HIV-1 seronegative during routine antenatal testing. Our results render further support for wider implementation of repeat HIV-1 testing in pregnancy [12].

In many settings, antiretroviral treatment for pregnant HIV-1 infected women is limited to short-course, mono- or dual-agent therapy given only during the peri-partum period to reduce mother-to-child HIV-1 transmission; this approach results in residual HIV-1 risk for infants and likely has limited effects for reducing transmission to male partners during pregnancy. Early initiation of combination ART to maximally suppress HIV-1 levels for all HIV-1 infected pregnant women should be considered as a public health intervention to decrease HIV-1 transmission risk [12], not only to infants but also to sexual partners [27, 29, 30]. Male involvement in antenatal care settings is often very low [31], but male participation has been associated with increased uptake of perinatal prevention strategies and reduced infant HIV-1 transmission [14, 32].

This study provides strong evidence that pregnancy is a high risk period for HIV-1 transmission, both to women and from HIV-1 infected women to their male partners. Increased HIV-1 risk from pregnant HIV-1 infected women to male partners is novel and requires further studies to understand the possible biologic mechanisms that many explain this finding. Similarly, behavioral factors related to pregnancy appear important in increasing HIV-1 acquisition risk for women. Prenatal couples HIV-1 counseling and testing, implementation of repeat HIV-1 testing in pregnancy, and earlier initiation of combination ART should become part of routine antenatal care to protect mothers, infants, and male partners from HIV-1.

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Table 1

Enrollment characteristics, prospective study of 3321 African heterosexual HIV-1 serodiscordant couples

	Median (interquartile range) or n (%)			
	Male-to-female transmission analysis (N=1085 couples)		Female-to-male transmission analysis (N=2236 couples)	
	HIV-1 infected male	HIV-1 uninfected female	HIV-1 infected female	HIV-1 uninfected male
Individual characteristics				
Age, years	37.4 (32.0–45.0)	30.6 (25.4–37.8)	30.0 (25.3–34.8)	35.1 (29.6–42.1)
Education, years	8 (7–11)	8 (6–10)	8 (6–10)	9 (7–12)
Contraceptive use**	N/A	234 (21.6)	530 (23.7)	N/A
Circumcised (men only)	366 (33.8)	N/A	N/A	1228 (54.9)
Couple characteristics*				
East Africa (vs. southern Africa)	743 (68.5)		1452 (64.9)	
Married	874 (80.6)		1647 (73.7)	
Living together	1012 (93.3)		1997 (89.3)	
Years lived together	6 (3–13)		5 (2–9)	
# children with study partner	2 (1–3)		1 (0–2)	
Sexual behavior*				
# sex acts with study partner in prior month	4 (2–8)		4 (2–8)	
Any unprotected sex with study partner in prior month	289 (26.6)		682 (30.5)	
Any sex with outside partner in prior month	87 (8.0)	5 (0.9)	33 (1.5)	96 (9.1)
HIV-1 seropositive partner characteristics				
Plasma HIV-1 RNA,$\log_{10}$ copies/mL	4.3 (3.7–4.9)	N/A	4.0 (3.2–4.5)	N/A
CD4 count, cells/mm^3	426 (334–571)	N/A	481 (354–663)	N/A
Randomized to acyclovir (vs. placebo)	558 (51.4)	N/A	1108 (49.6)	N/A

*Couple demographic and behavior characteristics al as reported by the HIV-1 uninfected partner

**Any contraceptive use includes: oral, injectable and implantable contraceptives, intrauterine device, hysterectomy and bilateral tubal ligation

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Table 2

Participant characteristics during pregnant and non-pregnant exposed study quarterly follow-up intervals

	Median (interquartile range) or n (%)					
	Male-to-female HIV-1 transmission analysis (HIV-1 uninfected female participants)			Female-to-male HIV-1 transmission analysis (HIV-1 uninfected male participants)		
	Pregnant intervals (n=988)	Non-pregnant intervals (n=6603)	p-value*	Pregnant intervals (n=1331)	Non-pregnant intervals (n=13,948)	p-value*
Demographic characteristics						
Age of HIV-1 uninfected partner						
<25	397 (40.2%)	1068 (16.2%)	<0.001	75 (5.7%)	577 (4.3%)	<0.001
25-34	505 (51.1%)	2882 (43.6%)		730 (55.0%)	5260 (39.7%)	
≥35	86 (8.7%)	2653 (40.2%)		522 (39.3%)	7429 (56.0%)	
Children with study partner						
None	264 (26.7%)	1545 (23.4%)	<0.001	386 (29.0%)	4339 (32.7%)	0.03
One	297 (30.1%)	1448 (21.9%)		436 (32.8%)	3544 (26.7%)	
Two or more	427 (43.2%)	3610 (54.7%)		509 (38.2%)	5385 (40.6%)	
Sexual behavior						
Unprotected sex with study partner	161 (16.4%)	610 (9.2%)	<0.001	224 (16.8%)	1777 (13.4%)	0.005
Any sex with additional partner	48 (4.9%)	137 (2.3%)	<0.001	175 (13.2%)	1358 (11.2%)	0.16
Clinical characteristics						
Contraceptive use, female partner**	72 (7.3%)	1476 (22.4%)	<0.001	173 (13.8%)	3935 (30.8%)	<0.001
Plasma HIV-1 RNA , $\log_{10}$ copies/mL, HIV-1 infected partner	4.4 (3.6–5.0)	4.3 (3.5–4.9)	0.42	4.0 (3.1–4.6)	3.9 (3.2–4.6)	0.76
CD4 count, cells/ mm^3, HIV-1 infected partner	438 (310–611)	410 (309–564)	0.10	455 (327–638)	459 (334–636)	0.36

*Comparisons among pregnancy exposure groups are adjusted for correlation by multiple measures from the same woman using generalized estimating equations.

**Contraceptive use during pregnancy intervals was either contraceptive failures documented at the time of pregnancy detection or during the early postpartum period.

Table 3

Pregnancy and HIV-1 risk

	HIV-1 cases/ person-years of follow up	HIV-1 incidence (per 100 person/ years)	Hazard ratio	95% CI	p-value	Adjusted hazard ratio*	95% CI	p-value
Male-to-female HIV-1 transmission								
All seroconversions	61/1695	3.60						
Not during pregnancy	44/1464	3.01	1.0	REF	REF	1.0	REF	REF
During pregnancy	17/231	7.35	2.34	1.33–4.09	0.003	1.71	0.93–3.12	0.08
During early pregnancy	5/47	10.59	3.55	1.41–8.97	0.007	1.95	0.73–5.21	0.18
During late pregnancy	12/184	6.52	2.10	1.11–3.99	0.02	1.69	0.87–3.31	0.12
Female-to-male HIV-1 transmission (confirmed as linked within the couple by viral sequence analysis)								
All seroconversions	58/3446	1.68						
Not during pregnancy	46/2905	1.58	1.0	REF	REF	1.0	REF	REF
During pregnancy	12/347	3.46	2.31	1.22–4.39	0.01	2.47	1.26–4.85	0.01
During early pregnancy	5/113	4.41	2.69	1.07–6.78	0.04	2.64	1.02–6.84	0.05
During late pregnancy	7/234	3.00	2.09	0.93–4.69	0.08	2.37	1.03–5.46	0.04

*Adjusted for age of the HIV-1 uninfected partner, contraceptive use by the female partner, and any unprotected sex during the prior month. Further adjustment for randomization arm of the HIV-1 infected partner (acyclovir vs. placebo), region (East versus southern Africa), number of living children, STIs at study enrollment (in either partner), enrollment HSV-2 serostatus in the HIV-1 uninfected partner, circumcision status (of male HIV-1 uninfected partners), any additional sex partners during the past month, total number of unprotected sex acts during the past month, CD4 count of the HIV-1 infected partner, plasma HIV-1 RNA concentrations in the HIV-1 infected partner, and antiretroviral therapy use by the HIV-1 infected partner did not substantially change (by ≥10%) these hazard ratios.