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Hormonal contraception does not increase the risk of genital tract infections among a cohort of HIV serodiscordant couples in Lusaka, Zambia

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Abstract

Hormonal Contraception does not increase the risk of genital tract infections among a cohort of HIV serodiscordant couples in Lusaka, Zambia
By Katherine Tote

Background

There are growing concerns that contraceptive hormones, particularly Depot medroxyprogesterone acetate (DMPA), may alter the vaginal environment, contributing to the spread of HIV. Many local factors increase HIV transmission and could mediate the association between hormonal contraception and HIV risk, including the presence of sexually transmitted infections (STIs), vaginal *Candida,* associated vaginitis, and the divergence from healthy *Lactobaccili* dominant vaginal flora. The relationship between hormonal contraception and these genital outcomes is understudied, and previous research is inconclusive.

Methods

HIV-serodiscordant couples in Zambia were enrolled in a longitudinal cohort. From 1994 to 2002, both partners were seen quarterly and received physical exams, including genital examinations. Rates of three outcome infections of interest (bacterial vaginosis (BV), vaginal candidiasis, and trichomoniasis) were calculated. Bivariate associations between baseline and time-varying covariates and outcome infections of interest were evaluated via unadjusted Anderson-Gill survival models, and adjusted harzard ratios (aHRs) were generated using multivariable Anderson-Gill survival models including demographic and clinical factors found to be associated with both hormonal contraception and the outcome of interest. For each model, couple HIV serostatus was forced into the final multivariable models.

Results

There were 1558 cases of BV, 1529 cases of vaginal candida and 574 cases of trichomoniasis over 2139.39, 2121.85, and 2139.86 person-years of observation, respectively. DMPA users had significantly lower rates of trichomoniasis and BV. In adjusted models, DMPA was protective for BV (aHR=0.72) and trichomoniasis (aHR = 0.44) and oral contraceptive pills (OCPs) were protective for candidiasis (aHR=0.80).

Conclusions

Our results indicate that hormonal contraceptives are not risk factors for vaginal infections which may be associated with HIV acquisition. Further research is necessary to understand changes in the vaginal environment that may increase HIV risk.

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Introduction

Globally, nearly half of the 33.3 million people living with Human Immunodeficiency Virus (HIV) are women, many of whom are of reproductive age (1). Providing safe contraceptive care for women at risk or living with HIV is essential to adequately address their family planning needs and is crucial to the Prong II approach to reduce maternal to child transmission of HIV (2). For reproductive aged women, hormonal contraception is a central component for preventing unintended pregnancy. However, there are growing concerns that contraceptive hormones, particularly Depot medroxyprogesterone acetate (DMPA), may have an impact on immune function and/or alter the vaginal environment, contributing to the spread of HIV infection (3-6). While not all studies have demonstrated a consistent link, recent meta-analyses and systematic reviews have suggested between a 40-50% increase in HIV acquisition risk with DMPA (7-9). While data on combined hormonal contraceptive pills and HIV acquisition seem to show no increased risk, the data on other contraceptives are limited (7). The World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) have recognized this important knowledge gap and encourage research to clarify an association and explore mechanisms associated with hormonal contraceptives that may underlie an increase in HIV transmission risk (10).

The transmission of HIV infection is a multifaceted process of virus-host interactions. In addition to HIV viral load (11-13), many local factors increase HIV transmission and could mediate the association between hormonal contraception and HIV risk. These include the presence of other sexually transmitted infections (STIs), the presence of vaginal *Candida* and associated vaginitis

or vaginal inflammation, and divergence from healthy *Lactobaccili* dominant vaginal flora (14-23).

When the vaginal flora is dominated by *Lactobacillus* species, particularly *Lactobacillus crispatus*, studies have shown a decreased incidence of HIV, HPV, and trichomonas infection compared to women with bacterial vaginosis (BV), a polymicrobial anaerobic vaginal flora with low prevalence of *Lactobacilli* and elevated vaginal pH (24-26). Most studies have suggested that hormonal contraception does not alter the presence of BV or vaginal candidiasis and may even be protective for these infections. In a recent meta-analysis, all studies reviewed showed either statistically significant decrease in BV in hormonal contraceptive users or no significant difference when compared to non-hormonal contraceptive users (23, 27-31). Further, when including the three highest quality studies, they report a 10-20% reduction in BV in combined oral contraceptive users and a 18-30% reduction in DMPA users (32-34). Another meta-analysis including 55 studies reported an approximate 25% reduction in both incident and prevalent BV in hormonal contraceptive users compared with non-users, irrespective if the method of hormonal contraception was progestin-only or a combined estrogen-progestin method (35). While this evidence suggests that use of hormonal contraception either does not alter or *decreases* BV incidence (and thus BV is not responsible for increased susceptibility to STIs/HIV through this mechanism), many studies are limited due small sample sizes, combining different hormonal contraceptive methods, cross sectional study designs, and incomplete control of confounders such as condom use and sexual frequency. Further, as different populations may have different prevalence of vaginal infections or altered vaginal microenvironments, it is important to evaluate this potential association in high-risk couples in an area of high HIV

prevalence and among women with HIV, as several of these infections may modify risk of HIV transmission to uninfected partners.

Trichomonas vaginalis, or trichomoniasis, is one of the most prevalent STIs worldwide, with an estimated 5% of reproductive aged women infected globally(36). *T. vaginalis* has a bidirectional relationship with HIV, meaning that trichomoniasis increases risk of HIV acquisition and HIV infection increases risk of trichomoniasis acquisition (37-39). Trichomoniasis is also often correlated with BV (40-42). The relationship between *T. vaginalis* infection and hormonal contraceptive use has been relatively understudied. DMPA use has been shown to be protective or have no association with trichomoniasis risk, while contraceptive implants and oral contraceptive pills (OCPs) have shown a negative association (33, 41, 43-45).

Vaginal *Candida* infection, or candidiasis, is the second most common cause of vaginitis and affects up to 20% of women worldwide annually (46). Like *T. vaginalis* and BV, vaginal *Candida* is associated with increased risk of HIV acquisition, and similarly, its relationship with hormonal contraceptive use is understudied. In some studies, hormonal IUDs and/or OCPs have been associated with increased risk of candidiasis, while others show no association (18, 23, 31, 47-51).

We sought to explore the association between different hormonal contraceptives and genital tract infections, including BV, trichomonas, and candidiasis within a longitudinal cohort of HIV serodiscordant couples in Zambia. Our goal was to confirm prior findings of no association or a protective effect of these contraceptives, add additional information on implants where there is

limited information in the literature, explore the impact of HIV infection on these associations, and contribute to the literature a high-quality evaluation controlling for important confounders that may modify observed associations.

Methods:

Study Design, Participants and Ethics: This study is a secondary analysis of a longitudinal cohort of HIV serodisordant couples (in which the man is HIV-positive and the woman HIV-negative (M+F-) or the man is HIV-negative and the woman HIV-positive (M-F+)) in Lusaka, Zambia. Married or cohabiting couples attended couples' voluntary HIV counseling and testing (CVCT) services either spontaneously or after receiving an invitation from a community promoter (52). CVCT services include group counseling, rapid HIV testing, and post-test couples counseling with mutual disclosure of results (53, 54). Heterosexual HIV serodiscordant couples were invited to enroll in an open longitudinal cohort study between 1994-2012. Follow-up visits included risk reduction counseling. The study intervention design, uptake of contraception immediately after the intervention , impact of informed consent on knowledge and concerns about contraceptive methods , demographics of the cohort, rates of unintended pregnancy and impact of contraceptive method on unintended pregnancy, impact of the intervention on incident pregnancy, unprotected sex, and STIs, patterns of contraceptive use and discontinuation, impact of hormonal contraceptive on HIV acquisition risk, HIV transmission to partners, and disease progression have been previously reported (55-62). This study was approved by the Institutional Review Boards at Emory University and the University of Zambia. Written informed consent was obtained from all participating couples.

Exposure of interest

Contraceptive method used since last visit (none/condoms only, combined OCPs (progesterone-only OCPs were prescribed to breastfeeding women until children were 6 months old), DMPA (150mg IM dosage), copper intrauterine device (IUD), contraceptive implant (Norplant, Jadelle), or permanent methods (hysterectomy/tubal ligation/vasectomy)) was recorded at baseline and three-monthly follow-up visits. In our primary analysis, contraceptive methods were categorized as implant, injectable, or OCP versus non-hormonal (none/condoms only). IUDs were excluded from this analysis.

Outcomes of interest

The three primary repeated outcomes of interest were BV, vaginal candidiasis and trichomoniasis. These time-varying outcomes were diagnosed by a wet-prep at baseline and at scheduled or client-initiated interim follow-up visits. BV was determined by a modified amsels criteria with vaginal discharge, >20% clue cells per high-power field, and + whiff test with KOH. Candida based on the presence of hyphae or budding yeast and trichomonas determined by the presence of trichomonads.

Baseline covariates

At enrollment, baseline demographic data was collected, including age, monthly income, and literacy in Nyanja. Clinical and behavioral characteristics included number of previous pregnancies, current pregnancy, couple HIV status (M+F- or M-F+), viral load (log10

copies/mL) of the positive partner, HIV stage of positive partner, and HSV-2 serology for both partners (categorized as positive, negative or discrepant).

Time-varying covariates

At scheduled quarterly (or client-initiated interim) follow-up visits, time-varying variables of interest collected included pregnancy, number of unprotected sexual acts since last visit, any unprotected sex act since last visit, sperm present on wet-prep, active genital or perianal ulcers for woman or male partner (by self-report or examination finding), positive RPR serology for syphilis(63), female genital discharge, male genital inflammation, and circumcision status of male partner. BV, vaginal candidiasis, and trichomoniasis were included as covariates in models where they were not the primary outcome of interest.

Longitudinal data collection

Data collection varied by type and frequency of data collected over 17 years of follow-up. This analysis is restricted to visits taking place between study initiation in 1994 and 2002, a period in which both partners were seen quarterly and received physical exams, including genital examinations and wet-prep. Plasma banking for VL testing was available beginning in 1999.

Data analysis

Rates with corresponding 95% confidence intervals for each outcome of interest were calculated as the number of incident infections per couple-year of follow-up, stratified by contraceptive method type. Couple years of follow-up were calculated from enrollment until the couple was

censored. Couples were censored when either partner died, the couples separated, the positive partner started antiretroviral treatment (ART), or if either partner was lost to follow-up.

Descriptive analyses of baseline and time-varying measures were stratified by intervals where outcome infections were detected. Counts and percentages (calculated over all study intervals for baseline or time-varying variables) described categorical variables while means and standard deviations described continuous variables.

Bivariate associations between baseline and time-varying covariates and outcome infections of interest were evaluated via unadjusted Anderson-Gill survival models to generate crude hazard ratios (HRs) and 95% confidence intervals (CIs). Anderson-Gill survival models estimated the total effect of time-varying contraceptive method type on time to repeated outcome infection. Covariates significantly ($p<0{\cdot}05$) associated with the exposure and outcome of interest were considered as potential confounders. Variable multi-collinearity was assessed and was not determined to be present, using condition indices of 30 and variance decomposition proportions of 0·05 as cutoff criteria. Adjusted HRs (aHRs) and 95% CIs are presented for covariates in the final multivariate models. Analyses were initially stratified by couple HIV status, but couple HIV status was not found to be an effect measure modifier. For each model, couple HIV serostatus were forced into the final multivariable models.

Sensitivity analyses

Though HSV-2 infection may be a confounder of the relationship between hormonal contraceptives and outcome infections of interest, it was excluded from adjusted models due to

high levels missingness. A sensitivity analysis including both male and female HSV-2 status was conducted for BV and trichomoniasis outcomes. Male partner HSV-2 status was not found to be a significant confounder with *Candida,* so the sensitivity analysis includes only female HSV-2 serostatus.

Women using copper IUDs were excluded from the non-hormonal group. We conducted a sensitivity analysis to assess the impact of their inclusion on our results. Additionally, to address concerns that presence of vaginal discharge may overlap with diagnoses of trichomoniasis, we conducted a sensitivity analysis in which all covariates were included in multivariable models, excluding vaginal discharge, for all three outcomes.

Analyses were conducted with SAS v9.3 (Cary, NC).

Results:

Rates of infection by contraceptive method (Table 1): There were 1558 cases of BV, 1529 cases of vaginal candida and 574 cases of trichomoniasis over 2139.39, 2121.85, and 2139.86 person-years of observation, respectively. DMPA users had significantly lower rates of trichomoniasis and BV. There were no other significant differences in outcome rates by contraceptive method.

Unadjusted and multivariable evaluation of BV (Table 2): In the bivariable analysis, woman's younger age, being HIV positive, being HSV positive, having active genital ulcers, trichomoniasis and vaginal discharge all increased risk of BV, while DMPA use, breastfeeding, and having more pregnancies at baseline was protective for BV. Reporting unprotected sex and

having sperm noted on wet-prep increased risk of BV. Male partner HSV positivity was protective for BV while foreskin smegma increased BV risk. Significant ($p<0.05$) confounders with contraceptives included in the multivariable model included woman's age, couple HIV status, breastfeeding, trichomoniasis, and vaginal discharge. In the final model, DMPA was protective for BV.

Unadjusted and multivariable evaluation of vaginal candidiasis (Table 3): In the bivariable analysis, woman's younger age, reporting any unprotective sex since last visit, being pregnant, sperm on a wet prep, and vaginal discharge all increased risk of candida, while having more prior pregnancies, being HSV positive, and breastfeeding were protective. No male partner factors altered risk. In these bivariable analyses, DMPA and OCPs were significantly associated with decreased candida risk. Significant ($p<0.05$) confounders with contraceptives that were included in the multivariable model included woman's age, current pregnancy, and vaginal discharge. In this final model, OCPs decreased the risk of candida.

Unadjusted and multivariable evaluation of trichomoniasis (Table 4): In bivariable analysis, DMPA use was protective against trichomoniasis. Younger age, having fewer prior pregnancies, being HIV positive, pregnancy, vaginal discharge, active genital ulcers, BV, and male partner foreskin smegma were associated with increased risk of Trichomonas, while breastfeeding, candida, and male partner HSV positivity were protective. Significant ($p<0.05$) confounders of contraceptive use included in the multivariable model were HIV status, active genital ulcers, BV, *Candida*, and vaginal discharge. In this final model, DMPA reduced risk of trichomoniasis.

Sensitivity analyses (Tables 5-7): When couple HSV-2 status is included in multivariable models, there is no indication that hormonal contraceptive methods alter risk for any of the outcome infections, but positive female HSV-2 status is associated with increased risk of BV (p=.02) and decreased risk of candidiasis (p=.01), while male HSV-2 positivity is associated with decreased risk of BV (p=.01). When women using copper IUDs are included within the non-hormonal contraceptive referent group, the adjusted HRs from multivariable models do not differ from initial models. Adjusted HRs for the relationship between contraceptive method and genital outcome after excluding vaginal discharge from the models did not differ from initial models.

Discussion:

In this longitudinal cohort of HIV-serodiscordant couples in Lusaka, Zambia, use of DMPA decreased risk of BV and trichomoniasis, and use of OCPs was associated with reduced risk of vaginal candidiasis. No method of hormonal contraception was associated with significant increased risk of any of the outcome infections. This concurs with previous findings that contraceptive use does not increase risk of adverse vaginal infections and thus implies that any increased risk of HIV associated with hormonal contraceptive use is generated via alternative mechanisms (29-31).

Though we were unable to control for HSV-2 serostatus in our adjusted models due to a high degree of missingness, it is an important HIV risk factor to consider (64, 65). HSV-2 is highly prevalent in sub-Saharan Africa, with estimates ranging from 70 to 83 percent , and some studies indicate that hormonal contraceptive use, DMPA in particular, may increase infection incidence (66-70) via the diminishing of the genital mucosal barrier. The relationship between HSV-2,

hormonal contraception, and HIV acquisition is complex, and the small body of research has produced conflicting results. A study of women recruited from family planning clinics in Zimbabwe, Uganda, and Thailand found that hormonal contraceptives increased risk of HIV acquisition only among HSV-2 seronegative women and posited that the strong effect of the herpes virus overwhelms the effect of contraceptives among seropositive participants (71). Conversely, in a study of high risk Kenyan women, both hormonal contraception and HSV-2 were associated with increased risk of HIV acquisition, but risk associated with contraceptive use was unrelated to HSV serostatus (72). In our analysis, when we controlled for male and female HSV-2 status in a sensitivity analysis, all significant associations between hormonal contraceptive methods and genital outcomes were erased.

In our unadjusted model and sensitivity analysis including both male and female HSV-2 serostatus, female HSV-2 positivity was associated ($p=.025$) with increased risk of bacterial vaginosis. Concurrent with these findings, prior research indicates a bidirectional relationship between BV and HSV-2 (73-75). As both infections are associated with increased risk of HIV acquisition, it is important to take this interaction into account when examining causal pathways and HIV transmission mechanisms.

While it was not included in our final model as it was not found to be a confounder with hormonal contraceptives, another mechanism for increased HIV risk observed in our cohort is the association between incidence of unprotected sex and vaginal candidiasis ($p=.02$). Unprotected sex is clearly a risk factor for HIV acquisition within serodiscordant couples and the observed increased risk of candida may amplify that risk (76).

In our cohort, trichomoniasis and vaginal discharge were associated with increased risk of BV ($p<.001$), vaginal discharge was associated with increased risk of *Candida* ($p<.001$), and vaginal discharge, Candida, and BV increased risk of trichomoniasis ($p<.001$). The strong correlations between these conditions implies that these factors cannot be examined alone when exploring mechanisms of HIV acquisition. The interrelationship and co-occurrence of these conditions is also associated with increased risk of HIV transmission and vaginal shedding of HIV RNA (77-79).

While we did not find evidence of increased incidence of vaginal infections associated with hormonal contraceptive use, these results may not be applicable to intravaginal hormonal contraceptive methods, such as the vaginal ring and hormonal IUDs. Both hormonal IUDs and intravaginal rings have been shown to aid in the formation of *Candida* biofilm, particularly among women with BV infection (80, 81). However, recent studies have found no evidence of impact on the vaginal microbiome caused by hormonal IUDs or sustained vaginal ring usage (82-84).

The observed protective effect of DMPA on BV and of OCPs on vaginal candidiasis may be related to alterations to the menstrual cycle. Menses is an important factor in shifting vaginal microbiota, and the variations in cycle length or frequency resulting from hormonal contraception may have an impact on these shifts (85, 86). The mechanism by which DMPA use protects against *T. vaginalis* infection is unclear, but it has been suggested that exogenous hormones interfere with binding to androgen and estrogen receptors present on the parasite (87).

Our study is limited by self-report of many indices. Though outcome infections have been confirmed by laboratory diagnosis, the sensitivity and specificity of techniques in diagnosing infections limit our ability to make firm conclusions. Future evaluation with more sensitive techniques, such as 16srRNA analyses, may identify shifts in vaginal microbiota and its diversity associated with hormonal contraceptive use, with alternative changes in the vaginal environment potentially part of the mechanistic pathway associated with HIV. Our study benefits from a longitudinal design and our ability to control for changes in contraceptive use and the impact of time-varying clinical and behavioral characteristics.

Conclusions

Our results indicate that vaginal infections do not act as a mediator in the relationship between hormonal contraceptives and HIV acquisition. Further research is necessary to illuminate causal pathways and the mechanism by which this association occurs.

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Tables

TABLE 1. Rates of infection by contraceptive method

	Number of events	Couple-years of follow-up time	Rate per 100 couple-years	95%CI	
BV	**1558**	**2139.39**	**72.82**	**69.28**	**76.51**
Non-hormonal*	**1265**	1692.24	74.75	70.72	78.96
DMPA	**89**	168.14	52.93	42.76	64.83
Implant	**13**	11.96	108.71	60.45	181.20
OCPs	**191**	267.05	71.52	61.91	82.22
Candida	**1529**	**2121.85**	**72.06**	**68.52**	**75.74**
Non-hormonal*	**1255**	1675.37	74.91	70.85	79.14
DMPA	**101**	167.89	60.16	49.25	72.79
Implant	**9**	11.96	75.26	36.70	138.10
OCPs	**164**	266.63	61.51	52.62	71.48
Trichomonas	**574**	**2139.26**	**26.83**	**24.70**	**29.09**
Non-hormonal*	**489**	1692.12	28.90	26.42	31.55
DMPA	**18**	168.14	10.71	6.54	16.59
Implant	**2**	11.96	16.72	2.80	55.25
OCPs	**65**	267.05	24.34	18.94	30.83

*no method, condoms, permanent (excludes IUDs)

Table 2. Unadjusted and multivariable evaluation of BV

	Non-BV intervals		BV intervals		cHR	95%CI		p-value	aHR	95% CI		p-value
	N intervals	%	N intervals	%								
EXPOSURE OF INTEREST												
Contraceptive method (time-varying)												
Non-hormonal^	4851	67%	1265	81%	***Ref***				***Ref***			
DMPA	588	8%	89	6%	0.674	0.497	0.913	0.0109	0.723	0.548	0.955	0.0225
Implant	34	0%	13	1%	1.694	0.744	3.857	0.2089	1.654	0.86	3.182	0.1317
OCPs	1808	25%	191	12%	0.997	0.809	1.228	0.9747	0.996	0.816	1.215	0.9671
WOMAN BASELINE VARIABLES												
Demographics												
Woman age (per year increase)*	27.8	7.4	26.7	7.3	0.984	0.973	0.996	0.0090	0.981	0.971	0.992	0.001
Monthly family income (per USD increase)*	55.1	65.7	54.8	56.1	1.000	0.999	1.001	0.9127				
Woman reads Nyanja												
Yes, easily	1523	24%	336	21%	***Ref***							
With difficulty/not at all	4785	76%	1229	79%	1.101	0.905	1.339	0.3378				
Number of previous pregnancies (per pregnancy	3.8	2.6	3.3	2.6	0.947	0.912	0.983	0.0041				
Clinical												
Couple HIV Status (Cjbase)												
M+F-	3499	55%	647	42%	***Ref***							
M-F+	2836	45%	911	58%	1.537	1.315	1.796	0.0001	1.392	1.192	1.624	0.0001
HIV stage of positive partner												
Stage I	1692	27%	426	27%	1.249	0.874	1.785	0.2215				
Stage II	2418	38%	580	37%	1.226	0.864	1.741	0.2540				
Stage III	1704	27%	460	30%	1.370	0.962	1.951	0.0806				
Stage IV	520	8%	92	6%	***Ref***							
Log viral load (per log10 copies/ml increase)*	4.7	0.8	4.7	0.8	0.982	0.876	1.100	0.7506				
HSV-2 (prevalent)												
Positive	3089	82%	756	87%	1.494	1.053	2.119	0.0245				
Negative	475	13%	70	8%	***Ref***							
Discrepant	213	6%	45	5%	1.246	0.740	2.097	0.4075				
WOMAN TIME-VARYING VARIABLES												
Sexual behavior and family planning characteristics												
No. unprotected sex acts with study partner since last visit*	3.4	11.1	5.6	16.4	0.998	0.993	1.001	0.3168				
Any self-reported unprotected sex with study partner since last visit												
Yes	2516	40%	736	47%	1.269	1.136	1.417	0.0001				
No	3815	60%	822	53%	***Ref***							
Sperm present on wet prep												
Yes	877	17%	306	25%	1.504	1.315	1.720	0.0001				
No	4281	83%	934	75%	***Ref***							
Pregnant												
Yes	629	10%	154	10%	1.028	0.858	1.233	0.7623				
No	5630	90%	1394	90%	***Ref***							
Breastfeeding												
Yes	1751	28%	317	20%	0.690	0.592	0.805	0.0001	0.696	0.601	0.806	0.0001
No	4584	72%	1241	80%	***Ref***				***Ref***			
Syphilis (RPR)												
Yes	49	1%	22	2%	1.358	0.855	2.158	0.1954				
No	5654	99%	1378	98%	***Ref***							
Active genital ulcer (acute or chronic)												
Yes	891	14%	271	17%	1.299	1.116	1.512	0.0008	1.076	0.926	1.251	0.3365
No	5444	86%	1287	83%	***Ref***				***Ref***			
Candida												
Yes	1210	19%	319	20%	1.053	0.926	1.199	0.4298				
No	5047	81%	1239	80%	***Ref***							
Trichomonas Vaginalis												
Yes	368	6%	207	13%	2.001	1.719	2.330	0.0001	1.706	1.455	2.001	0.0001
No	5966	94%	1351	87%	***Ref***				***Ref***			
Vaginal discharge												
Yes	629	10%	258	17%	1.582	1.380	1.814	0.0001	1.351	1.183	1.544	0.0001
No	5625	90%	1299	83%	***Ref***				***Ref***			
MAN BASELINE AND TIME-VARYING VARIABLES												
Circumcised male partner												
Yes	872	14%	252	16%	***Ref***							
No	5462	86%	1306	84%	0.861	0.684	1.084	0.2020				
HSV-2 status of man												
Positive	2450	64%	493	57%	0.714	0.570	0.895	0.0035				
Negative	918	24%	272	32%	***Ref***							
Discrepant	431	11%	95	11%	0.739	0.527	1.038	0.0812				
Genital inflammation of man												
Yes	3532	56%	797	51%	0.975	0.857	1.110	0.7021				
No	2784	44%	759	49%	***Ref***							
Genital ulcer of man												
Yes	1104	17%	249	16%	0.940	0.817	1.082	0.3876				
No	5220	83%	1307	84%	***Ref***							
Foreskin smegma												
Yes	563	9%	158	10%	1.209	1.007	1.450	0.0414				
No	5737	91%	1391	90%	***Ref***							

USD: United States Dollar; OCP: oral contraceptive pill; IUD: copper intrauterine device; STI: sexually transmitted infection, VL collected from 1999, *Indicates a continuous variable, mean and standard deviation reported, ^no method, condoms, permanent (excludes IUDs

Table 2. Unadjusted and multivariable evaluation of *Candida*

	Non-Candida intervals		Candida intervals		cHR	95%CI		p-value	aHR	95% CI		p-value
	N intervals	%	N intervals	%								
EXPOSURE OF INTEREST												
Contraceptive method (time-varying)												
Non-hormonal^	4794	76%	1255	82%	***Ref***				***Ref***			
DMPA	574	9%	101	7%	0.722	0.542	0.961	0.0254	0.767	0.579	1.015	0.0639
Implant	38	1%	9	1%	0.964	0.544	1.707	0.8994	1.102	0.562	1.822	0.969
OCPs	880	14%	164	11%	0.772	0.632	0.942	0.0110	0.798	0.654	0.974	0.0263
WOMAN BASELINE VARIABLES												
Demographics												
Woman age (per year increase)*	27.8	7.5	26.5	6.8	0.981	0.971	0.991	0.0003	0.983	0.873	0.993	0.0011
Monthly family income (per USD increase)*	55.1	63.9	55.0	64.9	1.000	0.999	1.001	0.8006				
Woman reads Nyanja												
Yes, easily	1512	24%	330	22%	***Ref***							
With difficulty/not at all	4750	76%	1194	78%	1.091	0.930	1.279	0.2859				
Number of previous pregnancies (per pregnancy increase	3.8	2.7	3.3	2.5	0.941	0.915	0.968	0.0001				
Clinical												
Couple HIV Status (Cjbase)												
M+F-	3346	53%	767	50%	***Ref***				***Ref***			
M-F+	2941	47%	762	50%	1..087	0.943	1.253	0.2504	1.063	0.923	1.224	0.3986
HIV stage												
Stage I	1690	27%	404	26%	1.100	0.808	1.498	0.3692				
Stage II	2426	39%	548	36%	1.054	1.078	1.466	0.6341				
Stage III	1665	26%	475	31%	***1.278***	0.939	1.739	0.1182				
Stage IV	505	8%	102	7%	***Ref***							
Log viral load (per log10 copies/ml increase)*	4.7	0.9	4.7	0.8	0.960	0.857	1.075	0.4800				
HSV-2 (prevalent)												
Positive	3110	84%	686	79%	0.696	0.545	0.888	0.0036				
Negative	399	11%	136	16%	***Ref***							
Discrepant	212	6%	41	5%	***0.605***	0.355	1.033	0.0657				
WOMAN TIME-VARYING VARIABLES												
Sexual behavior and family planning characteristics												
No. unprotected sex acts with study partner since last visit*	3.6	11.5	5.0	15.3	1.001	0.998	1.004	0.4658				
Any self-reported unprotected sex with study partner since last visit												
Yes	2540	40%	694	45%	1.135	1.020	1.263	0.0201				
No	3744	60%	834	55%	***Ref***							
Sperm present on wet prep												
Yes	916	18%	267	21%	1.230	1.062	1.424	0.0057				
No	4190	82%	1021	79%	***Ref***							
Pregnant												
Yes	512	8%	258	17%	***1.934***	1.670	2.239	0.0001	1.632	1.39	1.914	0.0001
No	5712	92%	1260	83%	***Ref***				***Ref***			
Breastfeeding												
Yes	1695	27%	359	23%	***0.843***	0.728	0.976	0.0224	0.886	0.762	1.030	0.1150
No	4591	73%	1170	77%	***Ref***				***Ref***			
Syphilis (RPR)												
Yes	61	1%	9	1%	***0.575***	0.286	1.157	0.1208				
No	5601	99%	1376	99%	***Ref***							
Active genital ulcer (acute or chronic)												
Yes	918	15%	239	16%	1.000	0.929	1.253	0.3200				
No	5368	85%	1290	84%	***Ref***							
Bacterial Vaginosis												
Yes	1210	19%	319	20%	***1.006***	0.880	1.149	0.9321				
No	5047	81%	1239	80%	***Ref***							
Trichomonas Vaginalis												
Yes	487	8%	87	6%	***0.792***	0.621	1.009	0.0590				
No	5798	92%	1442	94%	***Ref***							
Vaginal discharge												
Yes	619	10%	268	18%	***1.637***	1.412	1.896	0.0001	1.480	1.278	1.712	0.0001
No	5652	90%	1260	82%	***Ref***				***Ref***			
MAN BASELINE AND TIME-VARYING VARIABLES												
Circumcised male partner												
Yes	885	14%	228	15%	***Ref***							
No	5400	86%	1301	85%	0.955	0.777	1.174	0.6626				
HSV-2 status of man												
Positive	2365	64%	540	62%	0.882	0.718	1.084	0.1344				
Negative	928	25%	245	28%	***Ref***							
Discrepant	426	11%	91	10%	0.795	0.588	1.074	0.2341				
Genital inflammation of man												
Yes	3469	55%	816	54%	0.972	0.864	1.092	0.6300				
No	2803	45%	707	46%	***Ref***							
Genital ulcer of man												
Yes	1089	17%	254	17%	0.940	0.817	1.111	0.5379				
No	5190	83%	1269	83%	***Ref***							
Foreskin smegma												
Yes	564	9%	145	10%	1.073	0.893	1.290	0.4521				
No	5688	91%	1376	90%	***Ref***							

USD: United States Dollar; OCP: oral contraceptive pill; IUD: copper intrauterine device; STI: sexually transmitted infection, VL collected from 1999, *Indicates a continuous variable, mean and standard deviation reported, ^no method, condoms, permanent (excludes IUDs)

Table 4. Unadjusted and multivariable evaluation of *T. vaginalis*

	Non-Trich intervals		Trich intervals		cHR	95%CI		p-value	cOR p-value	aHR	95% CI		p-value
	N intervals	%	N intervals	%									
EXPOSURE OF INTEREST													
Contraceptive method (time-varying)													
Non-hormonal^	5626	77%	489	85%	***Ref***					***Ref***			
DMPA	659	9%	18	3%	0.378	0.221	0.645	0.0004		0.437	0.252	0.756	0.0031
Implant	45	1%	2	0%	0.620	0.103	3.733	0.6016		0.504	0.075	3.396	0.4811
OCPs	981	13%	65	11%	0.873	0.634	1.203	0.4067		0.95	0.692	1.305	0.7517
WOMAN BASELINE VARIABLES													
Demographics													
Woman age (per year increase)*	27.6	7.3	26.9	7.6	0.986	0.967	1.006	0.1699					
Monthly family income (per USD increase)*	55.3	64.3	52.4	59.3	0.999	0.996	1.001	0.3482					
Woman reads Nyanja													
Yes, easily	1731	24%	127	22%	***Ref***								
With difficulty/not at all	5560	76%	445	78%	1.073	0.755	1.526	0.6944					
Number of previous pregnancies (per pregnancy increase	3.4	2.7	3.1	2.3	0.911	0.864	0.960	0.0005	0.5334				
Clinical													
Couple HIV Status (Cjbase)													
M+F-	3919	54%	227	39%	***Ref***					***Ref***			
M-F+	3398	46%	348	61%	1.742	1.352	2.246	0.0001	0.0001	1.493	1.148	1.94	0.0028
HIV stage													
Stage I	1.986	0%	131	23%	1.086	0.638	1.849	0.7624					
Stage II	2776	52%	222	39%	1.339	0.797	2.247	0.2698					
Stage III	1977	37%	188	33%	***1.506***	0.882	2.571	0.1134					
Stage IV	578	11%	34	6%	***Ref***								
Log viral load (per log10 copies/ml increase)*	4.7	0.8	4.6	0.8	0.888	0.727	1.083	0.2412					
HSV-2 (prevalent)													
Positive	3516	82%	328	89%	0.368	0.103	1.315	0.1239					
Negative	512	12%	33	9%	***Ref***								
Discrepant	252	6%	6	2%	***1.440***	0.924	2.243	0.1074					
WOMAN TIME-VARYING VARIABLES													
Sexual behavior and family planning characteristics													
No. unprotected sex acts with study partner since last visit*	3.8	12.2	5.2	13.8	1.002	0.997	1.007	0.3642					
Any self-reported unprotected sex with study partner since last visit													
Yes	3002	41%	249	43%	1.044	856.000	1.274	0.6688					
No	4311	59%	326	57%	***Ref***								
Sperm present on wet prep													
Yes	1104	18%	79	20%	1.067	0.832	1.368	0.6107					
No	4895	82%	320	80%	***Ref***								
Pregnant													
Yes	708	10%	74	13%	***1.321***	1.016	1.717	0.0375	0.0001	1.208	0.906	1.609	0.1976
No	6530	90%	494	87%	***Ref***					***Ref***			
Breastfeeding													
Yes	1953	27%	115	20%	***0.648***	0.498	0.868	0.0030	0.0001	0.786	0.587	1.051	0.1045
No	5364	73%	460	80%	***Ref***					***Ref***			
Syphilis (RPR)													
Yes	64	1%	7	1%	***1.255***	0.592	2.655	0.5535					
No	6536	99%	496	99%	***Ref***								
Active genital ulcer (acute or chronic)													
Yes	1019	14%	143	25%	1.815	1.453	2.268	0.0001	0.0001	1.298	1.038	1.622	0.022
No	6298	86%	432	75%	***Ref***					***Ref***			
Bacterial Vaginosis													
Yes	1351	18%	207	36%	***2.207***	1.807	2.696	0.0001	0.0001	1.889	1.535	2.326	0.0001
No	5966	82%	368	64%	***Ref***					***Ref***			
Candida													
Yes	1442	20%	87	15%	***0.747***	0.574	0.971	0.0295	0.0001	0.653	0.500	0.852	0.0017
No	5798	80%	487	85%	***Ref***					***Ref***			
Vaginal discharge													
Yes	755	10%	132	23%	***2.286***	1.843	2.837	0.0001	0.0052	1.942	1.568	2.404	0.0001
No	6483	90%	441	77%	***Ref***					***Ref***			
MAN BASELINE AND TIME-VARYING VARIABLES													
Circumcised male partner													
Yes	1025	14%	99	17%	***Ref***								
No	6291	86%	476	83%	0.761	0.531	1.089	0.1356					
HSV-2 status of man													
Positive	2734	64%	209	57%	0.697	0.491	0.989	0.0433	0.0006				
Negative	1073	25%	117	32%	***Ref***								
Discrepant	488	11%	38	10%	***0.694***	0.420	1.123	0.1372	0.0013				
Genital inflammation of man													
Yes	3992	55%	336	59%	1.056	0.863	1.291	0.2798					
No	3306	45%	237	41%	***Ref***								
Genital ulcer of man													
Yes	1250	17%	103	18%	1.004	0.803	1.255	0.9745					
No	6055	83%	471	82%	***Ref***								
Foreskin smegma													
Yes	642	9%	79	14%	1.508	1.169	1.944	0.0015	0.7500				
No	6637	91%	490	86%	***Ref***								

USD: United States Dollar; OCP: oral contraceptive pill; IUD: copper intrauterine device; STI: sexually transmitted infection, VL collected from 1999, *Indicates a continuous variable, mean and standard deviation reported, ^no method, condoms, permanent (excludes IUDs)

Table 5. Sensitivity analysis including HSV-2 status in multivariable models

OUTCOME: BV	aHR	95% CI:		p-value
Contraceptive method (time-varying)				
Non-hormonal^	***Ref***			
DMPA	0.793	0.556	1.13	0.1995
Implant	1.82	0.397	8.346	0.4411
OCPs	1.014	0.802	1.291	0.9073
Female HSV-2 Status				
Positive	1.52	1.077	2.147	0.0174
Negative	***Ref***			
Discrepant	1.245	0.746	2.077	0.4014
Male HSV-2 Status				
Positive	0.757	0.609	0.941	0.0122
Negative	***Ref***			
Discrepant	0.8	0.585	1.094	0.162
OUTCOME: Candida	aHR	95% CI:		p-value
Contraceptive method (time-varying)				
Non-hormonal^	***Ref***			
DMPA	0.935	0.656	1.333	0.7108
Implant	1.333	0.334	5.322	0.6844
OCPs	0.828	0.654	1.049	0.118
Female HSV-2 Status				
Positive	0.731	0.569	0.94	0.0147
Negative	***Ref***			
Discrepant	0.616	0.373	1.019	0.0591
OUTCOME: Trich	aHR	95% CI:		p-value
Contraceptive method (time-varying)				
Non-hormonal^	***Ref***			
DMPA	0.508	0.244	1.056	0.0698
Implant	1.6	0.291	8.81	0.5892
OCPs	1.012	0.696	1.47	0.9512
Female HSV-2 Status				
Positive	1.386	0.87	2.211	0.1699
Negative	***Ref***			
Discrepant	0.425	0.12	1.512	0.1865
Male HSV-2 Status				
Positive	0.802	0.554	1.161	0.2426
Negative	***Ref***			
Discrepant	0.806	0.512	1.276	0.3609

Table 6. Sensitivity analysis, including copper IUDs with non-hormonal methods

OUTCOME: BV	aHR	95% CI:		p-value
Contraceptive method (time-varying)				
Non-hormonal^	***Ref***			
DMPA	0.727	0.55	0.96	0.0244
Implant	1.662	0.864	3.195	0.1279
OCPs	1	0.82	1.22	0.9994
OUTCOME: Candida	aHR	95% CI:		p-value
Contraceptive method (time-varying)				
Non-hormonal^	***Ref***			
DMPA	0.773	0.584	1.024	0.0724
Implant	1.018	0.567	1.83	0.9519
OCPs	0.804	0.659	0.98	0.0312
OUTCOME: Trich	aHR	95% CI:		p-value
Contraceptive method (time-varying)				
Non-hormonal^	***Ref***			
DMPA	0.436	0.252	0.754	0.003
Implant	0.501	0.074	3.383	0.5023
OCPs	0.948	0.691	1.301	0.7422

Table 7. Sensitivity analysis, excluding vaginal discharge from multivariable models

OUTCOME: BV	aHR	95% CI:		p-value
Contraceptive method (time-varying)				
Non-hormonal^	***Ref***			
DMPA	0.783	0.529	1.16	0.2227
Implant	1.338	0.272	6.571	0.7201
OCPs	0.977	0.745	1.283	0.8693
OUTCOME: Candida	aHR	95% CI:		p-value
Contraceptive method (time-varying)				
Non-hormonal^	***Ref***			
DMPA	0.852	0.602	1.207	0.3674
Implant	0.498	0.103	2.408	0.3858
OCPs	0.823	0.643	1.054	0.122
OUTCOME: Trich	aHR	95% CI:		p-value
Contraceptive method (time-varying)				
Non-hormonal^	***Ref***			
DMPA	0.668	0.387	1.155	0.1485
Implant	1.721	0.27	10.976	0.5656
OCPs	1.011	0.687	1.486	0.9569