

Clinical Study

Diaphragm Used with Replens Gel and Risk of Bacterial Vaginosis: Results from a Randomized Controlled Trial

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Received 2 July 2012; Accepted 26 September 2012

Academic Editor: Deborah B. Nelson

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Background. Bacterial vaginosis (BV) has been linked to female HIV acquisition and transmission. We investigated the effect of providing a latex diaphragm with Replens and condoms compared to condom only on BV prevalence among participants enrolled in an HIV prevention trial. **Methods.** We enrolled HIV-seronegative women and obtained a vaginal swab for diagnosis of BV using Nugent's criteria; women with BV (score 7–10) were compared to those with intermediate (score 4–6) and normal flora (score 0–3). During quarterly follow-up visits over 12–24 months a vaginal Gram stain was obtained. The primary outcome was serial point prevalence of BV during followup. **Results.** 528 participants were enrolled; 213 (40%) had BV at enrollment. Overall, BV prevalence declined after enrollment in women with BV at baseline (OR = 0.4, 95% CI 0.29–.56) but did not differ by intervention group. In the intention-to-treat analysis BV prevalence did not differ between the intervention and control groups for women who had BV (OR = 1.01, 95% CI 0.52–1.94) or for those who did not have BV (OR = 1.21, 95% CI 0.65–2.27) at enrollment. Only 2.1% of participants were treated for symptomatic BV and few women (5–16%) were reported using anything else but water to cleanse the vagina over the course of the trial. **Conclusions.** Provision of the diaphragm, Replens, and condoms did not change the risk of BV in comparison to the provision of condoms alone.

1. Introduction

Bacterial vaginosis (BV), the most common bacterial genital infection in women of reproductive age, has been linked to considerable gynecologic and obstetric morbidity. BV may be a cofactor in male-to-female and female-to-male human immunodeficiency virus type (HIV) transmission and other sexually transmitted infections (STIs) including herpes simplex virus type-2 (HSV-2) [1–4]. The highest prevalence of BV has been found in sub-Saharan Africa

where HIV infection is also common [3, 5]. It has been postulated that BV may increase susceptibility to HIV infection through a variety of mechanisms including altering the host's defense system, absence of the normal H₂O₂-producing vaginal lactobacilli (which maintain the natural acidic pH of the vagina), loss of the protective myeloperoxidase halide-hydrogen peroxide system [6], and by production of metabolic by-products that may increase HIV replication [7, 8] or activate target cells for HIV infection [9]. If any of these hypotheses are correct, reduction of BV prevalence could

significantly reduce female HIV acquisition. Furthermore, the high prevalence of BV in sub-Saharan Africa remains unexplained.

Though considerable evidence supports sexual transmission of BV, factors like clothing, climate, and hygiene may also influence vaginal flora [4]. Of the various risk factors associated with BV, the contribution of each to causing BV may vary depending on hygienic, sexual, and other practices [5]. BV treatment with metronidazole although effective in the initial clearing of symptoms and signs of BV [10] has since proven disappointing even in industrialized countries with recurrence rates reaching 40% within 3 to 6 months after treatment with oral or intravaginal preparations [11–13]. In Uganda, administration of metronidazole, azithromycin, and ciprofloxacin to large populations of adults of reproductive age every ten months did not reduce the prevalence of BV below 50% [14].

Barrier contraceptive devices, such as the diaphragm, lubricants, and microbicides have been associated with changes in vaginal flora [14–17]. Specifically, contraceptive use of the diaphragm with nonoxynol-9 (N-9-) based spermicide has been associated with altered vaginal bacterial microflora including decreased rates of *Lactobacillus* colonization and urinary tract infections (UTIs) [15, 16]. However, most have regarded alteration of vaginal flora and increased risk of UTI as a direct result of the N-9 spermicide and not related to the diaphragm alone [16]. Importantly, Buffergel a candidate microbicidal gel with the ability to maintain a low vaginal pH was associated with profound reductions in BV after a short period of use among participants in a phase 1 safety trial [17]. In several small trials, Miphil, a gel with acid-buffering properties, demonstrated effectiveness in treating women with BV [18]. Here, we studied the effect of the diaphragm used with Replens, a commercially available vaginal lubricant with weak pH buffering capacity, on the presence of BV for up to 24 months of followup among women enrolled in a large randomized controlled HIV prevention trial, the Methods for Improving Reproductive Health in Africa (MIRA) study.

2. Methods

2.1. Study Design and Participants. The MIRA trial recruited sexually active (an average of at least four sex acts per month), HIV-seronegative, women aged 18–49 years who were free of chlamydia and gonorrhea between September, 2003 and September, 2005 [19]. Women were recruited from family planning, well-baby, and general health clinics, and from community-based organizations, through printed media and radio. Two thousand four hundred and ninety-nine women were enrolled at the Zimbabwe site, where the MIRA-BV ancillary study was conducted (the main MIRA trial was also conducted in Johannesburg and Durban, South Africa). Participants who met protocol inclusion and exclusion criteria were then randomized into one of two groups: the intervention group, who received a clinician-fitted diaphragm (All-Flex Arcing Spring diaphragm; Ortho-McNeil Pharmaceutical, Raritan, NJ, USA), a supply of lubricant gel (Replens, Lil' Drug Store Products, Cedar

Rapids, IA, USA), and male condoms and the control group, who received male condoms only. All participants received a comprehensive HIV prevention package consisting of HIV/STI pretest and posttest counseling, treatment of curable, laboratory-diagnosed STIs, and intensive risk reduction counseling, which emphasized condom negotiation and was tailored to each participant's individual circumstances.

The randomization scheme was described in detail previously [19]. Participants were followed quarterly from September, 2003 to December, 2006. The follow-up period for the study was designed to be staggered, with the first enrolled participants were followed up for 24 months, the last enrolled were followed up for 12 months, and an overall expected average of 18 woman-months of followup per participant.

The study protocol was reviewed and approved by the University of California San Francisco Institutional Review Board Committee on Human Research and by the Medical Research Council of Zimbabwe Ethical Review Committee, the Medicines Control Authority of Zimbabwe and Western Institutional Review Board. An independent external audit, sponsored by Ibis Reproductive Health, was done by the Quintiles Corporation in November 2005, after study accrual was completed. This study is registered with ClinicalTrials.gov, number NCT00121459.

2.2. BV-Ancillary Study Procedures. The study was conducted as an ancillary study of the MIRA trial in two clinics within 30 km of Harare: Chitungwiza, a periurban municipality and Epworth, a slightly poorer and less developed suburb. BV study procedures followed almost identically procedures for the main MIRA trial, which have been published in detail [19]. Briefly, at screening, we obtained verbal consent to assess initial eligibility, followed by written informed consent for screening procedures, including diagnostic HIV and STI testing and answering an interviewer-administered questionnaire on demographics and sexual behavior. The enrolment visit was scheduled within 2 weeks and not more than 30 days after screening, for participants who met the initial eligibility criteria.

By design, approximately 500 participants from the two Zimbabwe clinics were enrolled serially into the BV-ancillary study. At their enrolment visit, participants provided written informed consent for the main trial and for the BV-ancillary study, women had to give consent to have an additional vaginal swab obtained at each visit. Women were reevaluated for study eligibility and had a pelvic examination that included collection of a vaginal swab for Gram stain evaluation of BV using Nugent criteria [20].

After randomization in to the main MIRA trial women in the intervention group were counseled to insert the diaphragm into their vagina at any time that was convenient to them before coitus and to leave it in place for at least 6 hours after sex. They were given detailed instructions on maintenance, cleaning, and storage of their diaphragm. Women were asked to empty an applicator of gel (about 2.5 g) into the dome of the diaphragm at the time of insertion, to spread gel onto the rim to facilitate insertion, and to insert another applicator of gel into the vagina before

each act of vaginal sex. At each visit, women received a 3-month supply of gel and were counseled that the effectiveness of the diaphragm and lubricant gel for the prevention of HIV infection was not known. To prevent HIV, they were asked to use condoms regardless of whether or not they used the diaphragm and gel. Participants were also told that they should not use the diaphragm and gel as a method of contraception. Women were advised to use other contraceptive methods and were provided with hormonal contraceptives through the clinic. They were encouraged to return to the clinic if they experienced any problems or needed more study products.

At all visits, participants in both study groups received counseling on risk reduction and as many male condoms as desired. Counselors emphasized that condoms are the only known method to prevent HIV and STIs and that condoms should be used for every act of sex.

Participants returned 2 weeks after enrolment for resupply of products, for counseling, and to have any problems assessed. Thereafter, follow-up visits were scheduled quarterly to assess HIV and STI status and to obtain a vaginal swab for Gram stain evaluation of BV. Recent medical history, use of study products, and sexual behavior were ascertained through a face-to-face clinician-administered questionnaire and audio computer-assisted self-interview (ACASI). Use of intravaginal products including water and "other products" (e.g., soap, commercial douche, and natural products) during the past two-weeks was assessed through a face-to-face-administered questionnaire. Women were counted as having attended their quarterly visit if they visited during the period from 14 days before, to 73 days after, the scheduled date. Clinicians addressed any medical problems; a pelvic examination and urinalysis or wet mount were done when clinically indicated, and treatment was provided when appropriate.

2.3. Statistical Analyses. Data were managed at the Center for International Data Evaluation and Analysis at the University of California San Francisco. Data were electronically faxed from the clinic sites directly into a database system.

The primary outcome was BV detection by Nugent's criteria during followup. To minimize recall bias, per-protocol analyses were based on a priori product use at last sex as one of our two measures of diaphragm and condom use in all analyses. We also assessed a measure of cumulative use of the methods since last study visit (always, sometimes, or never). For calculation of sample size we assumed that BV detection during followup would be compared between arms in an intent-to-treat (ITT) analysis using generalized estimating equation (GEE) logistic regression model controlling for a within-participant correlation between repeated outcomes of 0.2, with an annual loss to followup of $\leq 5\%$. Based on these assumptions, 250 women per arm insured $\geq 80\%$ power to detect a $\pm 25\%$ difference in prevalence between the two study arms as significant at the 5% level (based on a two-sided test). This corresponded to a relative risk (odds ratio) of 0.75 (0.70).

Selected baseline characteristics were compared between the groups to examine the success of randomization. We

performed ITT and per-protocol analyses. All analyses were prespecified in an analytical plan finalized before analysis took place. The ITT analysis compared serial BV prevalence during followup between groups using a logistic regression model, including a binary indicator of group assignment as the primary predictor variable and accounting for possible within-participant correlation between repeated outcomes using generalized estimating equations (GEEs) methods. Results of the primary analysis were summarized by the estimated odds ratios comparing BV serial prevalence during followup in women in the intervention group to that in the control arm, with associated 95% confidence intervals (CIs). All results were stratified by the presence of BV at enrollment.

We used the same criteria for conducting the per-protocol analyses as were performed for the determination of the effect of the diaphragm on HIV acquisition [19]. In the per-protocol analyses the between-group comparison of the primary outcome, excluding follow-up periods where participants in the intervention group (diaphragm, Replens, and condom promotion) (1) did not report diaphragm use at last sex and (2) did not report use of the diaphragm consistently since the last follow-up visit, generally three months. Reported diaphragm use served as a proxy for gel use [21]. Person time in the control group was included even if no condom use was reported but was excluded if diaphragm use was reported.

2.4. Role of the Funding Source. The sponsor, The Bill & Melinda Gates Foundation maintained oversight of the trial through regular progress reports and meetings with investigators, and its program officer had input into key scientific decisions as a member of the Study Technical Advisory Committee. The sponsor had no other role in the data collection, data analysis, data interpretation, or writing of the report. The authors had access to all the data and shared final responsibility for the decision to submit for publication.

3. Results

Five hundred and forty-three women were enrolled in the BV ancillary study between February and October 2004; 9 subjects did not have baseline and 6 subjects did not have follow-up BV results leaving 528 (97%) evaluable subjects (Figure 1). Baseline sociodemographic characteristics, sexual behavior, and STIs were similar between the intervention and control arms (Table 1). At enrolment, 102 (39%) in the intervention arm and 111 (42%) in the control arm had BV. Since the prevalence of BV during followup differed predictably between women with and without BV at enrollment, we analyzed both groups separately.

Over the course of the study, participants randomized to the intervention arm reported consistent (always) diaphragm use during the previous three months at 904 (60.1%) out of 1505 visits. Women also reported always using gel at 908 (60.3%) visits indicating that in most instances when the diaphragm was used, gel was used as well. At enrollment, 29% of women reported always use a condom during the previous three months while 74% of women reported condom use at last sex with no significant

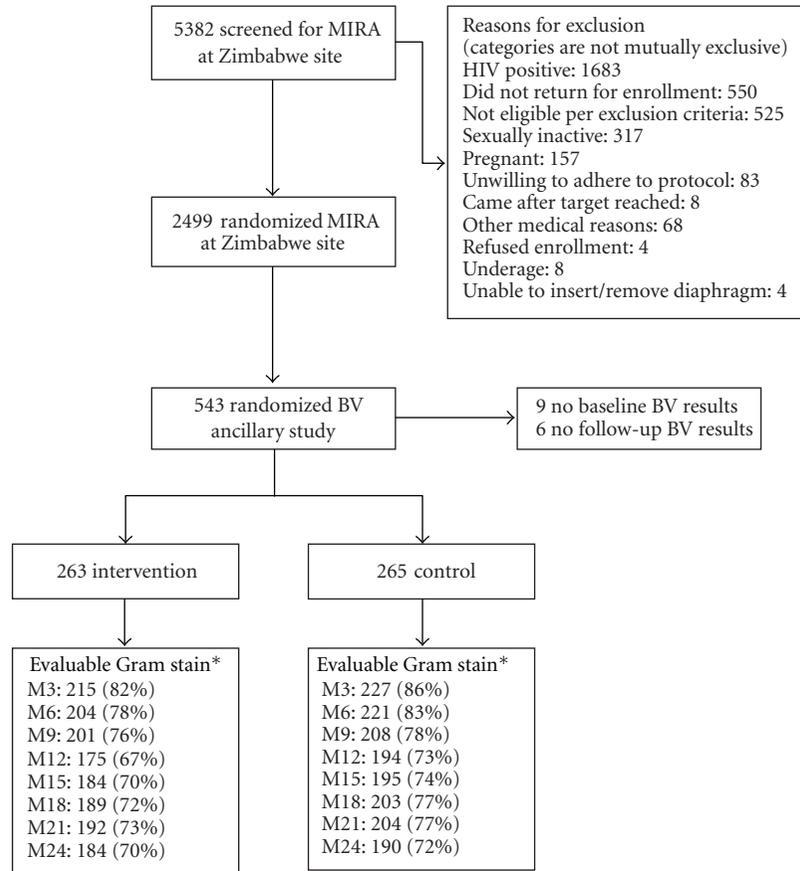


FIGURE 1: Bacterial vaginosis (BV) ancillary study trial profile: * number with an evaluable vaginal Gram stain at each follow-up visit by arm and percent evaluable over total number enrolled in each arm (number evaluable is less than those presenting for followup mainly due to menses a time when specimens for vaginal Gram stain were not obtained) †M: month of follow-up visit.

differences found between study arms (Table 1). During followup a greater proportion of women enrolled in the control arm reported condom use at last sex (range per visit: 78%–88%) than in the intervention arm (range per visit: 45%–60%).

For participants with BV at baseline, the odds of prevalent BV decreased an average of 12% per visit with a 60% overall decline from baseline during followup (OR = 0.40, 95% CI 0.25–0.65); this decline was similar between arms ($P = 0.99$; Figure 2). For those without BV at enrollment, the odds of prevalent BV increased nonsignificantly during the study in comparison to baseline an average of 6% per visit with a 50% overall increase for participants in the intervention arm (OR = 1.5, 95% CI 0.99–2.25) in comparison to a 3% per visit and a 24% overall increase from baseline for women in the control arm (OR = 1.24, 95% CI 0.77–1.99); this increase was not significantly different between arms ($P = 0.55$; Figure 2).

Only 2.1% of participants were treated for symptomatic BV, and antibiotic use during the last four weeks for any indication was reported infrequently during the course of the study (range per visit: 0.2%–0.7%). Use of water to clean the vagina in the past two weeks was common during followup and did not significantly differ by study arm; intervention

arm range per visit: 67%–77%; control arm range per visit: 64%–76%. Use of other products to clean the vagina in the past two weeks was less commonly reported overall and did not differ by study arm during the course of the study either; intervention arm range per visit: 6%–12%; control arm range per visit: 5%–12%.

In the ITT analysis, BV prevalence over time did not differ between the intervention and control groups for women who had BV at enrollment (OR = 1.01, 95% CI 0.52–1.94, $P = 1.0$) and did not have BV at enrollment (OR = 1.21, 95% CI 0.65–2.27, $P = 0.5$) (Figure 2, Table 2). In the per-protocol analysis, limited to women reporting diaphragm use at last sex, women in the intervention arm with and without BV at enrollment did not have an altered odds of BV (OR = 0.90, 95% CI 0.46–1.76; and OR = 1.34, 95% CI 0.68–2.62, resp.) in comparison to the control arm (Table 2). We performed a second per-protocol analysis limited to women reporting consistent diaphragm use since the last visit in the intervention arm; women without BV at enrollment had a nonsignificant increased odds of BV (OR = 1.83, 95% CI 0.90–3.71) compared to those in the control arm; the intervention was not associated with an altered odds of BV in women with BV at enrollment (OR = 1.17, 95% CI 0.56–2.45) (Table 2).

TABLE 1: Baseline demographics, clinical and exam findings, and initial laboratory findings for trial participants by group assignment.

Baseline characteristics	Intervention (N = 263)	Control (N = 265)
Sociodemographic variables		
Age (mean ± SD)	28.1 (7.4)	28.1 (6.9)
Married	249 (95%)	251 (95%)
Years of education (mean ± SD)	9.6 (2.1)	9.9 (1.9)
How many live births		
Number of live births (mean ± SD)	2.3 (1.6)	2.3 (1.6)
Current contraceptive method (multiple methods reported)		
Long term (i.e., tubal ligation)	4 (2%)	3 (1%)
Injectable hormones (i.e., depomedroxyprogesterone)	36 (14%)	42 (16%)
Combined oral contraceptives	160 (61%)	171 (65%)
Barrier (i.e., condom)	36 (14%)	33 (12%)
Other/none	27 (10%)	16 (6%)
Number of sexual partners in life (median, range)	1 (1–10)	1 (1–5)
Number of times sex in last 3 months (mean ± SD)	4.4 (3.3)	4.5 (3.5)
Reported condom use at last sex	190 (72%)	200 (75%)
Reported condom use in past 3 months		
Never	79 (30%)	70 (26%)
Sometimes	114 (43%)	113 (43%)
Always	69 (26%)	82 (31%)
Clinical history and exam at enrollment		
History of abnormal discharge during past 3 months	8 (3%)	6 (2%)
History of abnormal discharge ongoing	1 (0.4%)	0 (0%)
Abnormal vaginal discharge on exam	18 (7%)	22 (8%)
Screening laboratory results		
BV diagnosed by Gram stain criteria		
Normal (Nugent's score: 0–3)	120 (46%)	120 (45%)
Intermediate (Nugent's score: 4–6)	41 (16%)	34 (13%)
BV (Nugent's score: 7–10)	102 (39%)	111 (42%)
<i>Lactobacillus</i> by Gram stain (median, range)		
	2 (0–4)	2 (0–4)
<i>Lactobacillus</i> Gram stain score abnormal (2–4)		
	144 (55%)	139 (52%)
Vaginal pH ≥ 4.7		
	109 (43%)	109 (43%)
<i>Chlamydia trachomatis</i>		
	4 (1.5%)	3 (1.1%)
<i>Neisseria gonorrhoeae</i>		
	3 (1.1%)	1 (0.4%)
<i>Trichomonas vaginalis</i>		
	8 (3.0%)	7 (2.6%)
HSV-2 seropositive		
	128 (48.7%)	128 (48.3%)

TABLE 2: The odds of prevalent bacterial vaginosis (BV) during followup in participants randomized to intervention compared to control arms, stratified by presence and absence of BV, normal vaginal flora, and normal lactobacillus at enrollment.

	Vaginal gram stain findings at enrollment			
	BV present N = 213	BV absent N = 315	Normal vaginal flora* N = 240	Normal <i>Lactobacillus</i> † N = 259
Intent-to-treat analysis (OR, 95% CI)	1.01 (0.52–1.94)	1.21 (0.65–2.27)	1.37 (0.62–3.07)	1.06 (0.63–1.79)
Per-protocol analysis (reported diaphragm used at last sex) (OR, 95% CI)	0.90 (0.46–1.76)	1.34 (0.68–2.62)	1.64 (0.71–3.82)	0.96 (0.56–1.65)
Per-protocol analysis (reported diaphragm always used since last visit‡) (OR, 95% CI)	1.17 (0.56–2.45)	1.83 (0.90–3.71)	2.52 (1.04–6.12)	1.23 (0.70–2.20)

*Nugent's score = 0–3.

†*Lactobacillus* score = 0–1 per Nugent's score.

‡Generally three months.

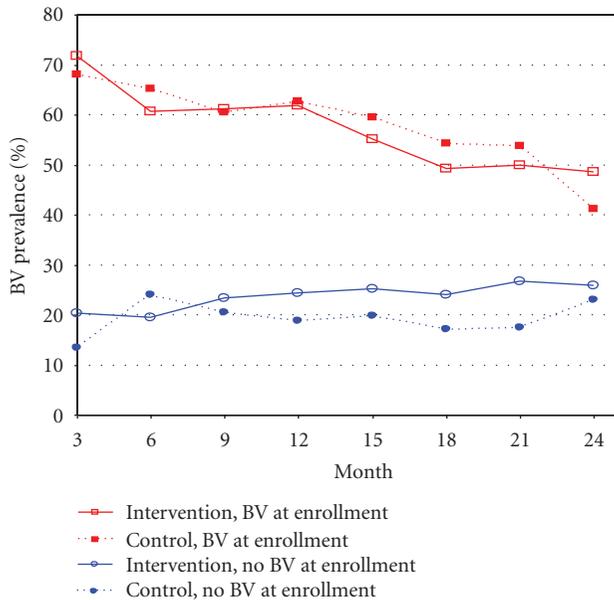


FIGURE 2: Intent-to-treat (ITT) analysis of percent of subjects diagnosed with bacterial vaginosis (BV) during followup among participants randomized to the intervention and control arms stratified by presence or absence of BV at enrolment.

Two further *a priori* analyses were performed. First, we compared the prevalence of BV between groups at followup, restricted to women with normal vaginal flora (Nugent's score 0–3) at enrollment. Participants in the intervention group with normal vaginal flora at enrollment did not have an altered odds of BV during followup in comparison to the control group in ITT (OR = 1.37, 95% CI 0.62–3.07) and in per-protocol analysis limited to women who reported diaphragm use at last sex (OR = 1.64, 95% CI 0.71–3.82). However, women with normal vaginal flora at enrollment reporting consistent diaphragm use since their last visit had an increased odds of BV during followup (OR = 2.52, 95% CI 1.02–6.22) in comparison to the control arm (Table 2). Next, we compared the prevalence of BV among participants with normal *Lactobacillus* (score: 0–1) found on Gram stain at enrollment. Among women with normal *Lactobacillus* on Gram stain at enrollment, being in the intervention arm was not associated with an altered odds of BV during followup in ITT or in the two per-protocol analyses (Table 2) in comparison to the control arm.

4. Discussion

Our study demonstrated no difference in the risk of BV (as measured by Nugent score) for women provided with the diaphragm plus Replens gel in addition to male condoms compared to those provided male condoms alone. However, in the analysis limited to women with normal vaginal flora at enrollment, participants who reported consistent diaphragm use over the preceding three months had a significantly increased odds of BV during followup compared to the control arm. This result did not seem to stem

from differential vaginal cleansing practices or antibiotic use including treatment for symptomatic BV between study arms. It is worth noting the statistical power was reduced following stratification of the results by presence versus absence of BV at enrollment. Thus, we cannot rule out smaller differences between arms in regards to BV prevalence at followup as was our original intention. Furthermore, while we believe that our data on use of the diaphragm and gel are valid, we could not confirm their use in this trial.

As has previously been reported, the study attained a high rate of condom use that was maintained over time [19]. However, as in the multisite MIRA HIV prevention trial, women enrolled in this ancillary study and randomized to the control arm reported higher condom use than those in the intervention arm, suggesting that diaphragm use may have been compensatory, that is, that women provided with the diaphragm were less likely to negotiate condom use with their partners [22]. Sexual intercourse without a condom has been associated with prevalent and recurrent BV [23, 24], providing further evidence that a sexually transmitted factor plays a role in BV pathogenesis [5]. Thus, we hypothesize that increased condom use in both arms led to the significant decline in BV over time among those with BV at enrollment and that differential condom use between arms and/or diaphragm use in the intervention arm may have contributed at least partially to the lower prevalence of BV among those with normal vaginal flora at enrollment randomized to the control arm in our per-protocol analysis.

Alternatively, we cannot rule out that the device itself could predispose to abnormal vaginal flora. van der Straten et al. studied the safety of the diaphragm used with KY personal lubricant and cellulose sulfate (CS) gel and CS gel alone over a six-month period in a similar population [25]. Although they did not report any statistically significant difference between treatment groups, BV appeared to be most common among women in the diaphragm and CS arm (34.2% by Amsel's and 39.5% by Nugent's score) and least common among those in the CS-alone group (15.0% by Amsel's and 22.5% by Nugent's score; $P = 0.07$ and $P = 0.14$, resp.). It is possible that even intermittent use of the diaphragm could physically affect the vaginal distribution of immune factors such as immunoglobulin (Ig) A, secretory leukocyte protease inhibitor (SLPI), cytokines, and chemokines originating from the endocervix and endometrium; evidence suggests that these factors inhibit growth of abnormal vaginal flora while promoting colonization of the vagina by lactobacilli [26, 27].

A prior investigation of young women including university students in the USA demonstrated an increased risk of BV in women using the diaphragm for contraception, an association that had previously been thought to be due to the use of N-9 [15, 16]. Among women initiating new contraceptive methods, the diaphragm used with N-9 was associated with increased vaginal colonization by *Escherichia coli*, *Enterococcus* species, anaerobic gram-negative rods, and BV [15]. While data related to diaphragm use without a spermicide is not available, recent studies have investigated the effects of Replens on vaginal microflora. Replens contains carbopol and polycarbophil, negatively charged polymers

that maintain the vaginal pH in the physiologic range, that may have microbicidal properties and have some activity against BV-associated bacteria [18, 28]. Although we did not directly assess use of Replens, we believe there was very little diaphragm use reported without gel. Thus, when used with a diaphragm, Replens gel did not appear to protect against BV.

Following the HIV results of the MIRA trial [19], widespread use of the diaphragm for HIV prevention is unlikely at this time. However, women who choose to use the diaphragm for pregnancy prevention should be counseled about the potential increased risk of BV. With the development of multipurpose technologies to prevent HIV and pregnancy underway, this investigation provides additional insight regarding BV pathogenesis with important implications for microbicide, cervical barrier, and combination product research.

Conflict of Interests

No author has a conflict of interests to report.

Acknowledgments

The authors would first and foremost like to thank the women who participated in this study. They also thank the administrative and data coordination staff at the University of California, San Francisco; the entire MIRA study teams at Ibis Reproductive Health; the University of Zimbabwe-University of California, San Francisco Collaborative Research Programme; the laboratory staff at the Anaerobe laboratory; Elizabeth Bukusi at the Centre for Microbiology Research, Kenya Medical Research Institute. The authors thank our Community Advisory Board who provided valuable input before and during the study. The MIRA Trial Technical Advisory Committee had a critical role in advising the investigators on important scientific decisions during the trial. They thank Barbara van der Pol and Carol Spiegel for providing invaluable laboratory consultation. They would like to acknowledge the time and oversight provided by the members of the MIRA data safety monitoring board. The MIRA trial was funded through a Grant from the Bill & Melinda Gates Foundation (no. 21082).

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