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Effects of Long-Term Use of Nonoxynol-9 on Vaginal Flora

Courtney A. Schreiber, MD, MPH, **Leslie A. Meyn, MS**, **Mitchell D. Creinin, MD**, **Kurt T. Barnhart, MD, MSCE**, and **Sharon L. Hillier, PhD**
From the Department of Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh School of Medicine and Magee-Womens Research Institute, Pittsburgh, Pennsylvania; and Penn Fertility Care and Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania Medical Center, Penn Fertility Care, Philadelphia, Pennsylvania.

Abstract

OBJECTIVE—Products containing nonoxynol-9 have been used as spermicidal contraceptives for many years, but limited data have been published describing the long-term effects of nonoxynol-9 use on the vaginal microbial ecosystem. This longitudinal study was conducted to examine the effects of nonoxynol-9 on the vaginal ecology.

METHODS—Vaginal swabs were obtained from 235 women enrolled in a randomized clinical trial before initiation of use of 1 of 5 different formulations of nonoxynol-9 for contraception, and up to 3 more samples were gathered over 7 months of use. The swab samples were evaluated in a single laboratory. The prevalence of several constituents of the normal vaginal flora was evaluated. The associations between nonoxynol-9 dosage, formulation, average product use per week, and number of sex acts per week were calculated.

RESULTS—The changes in prevalence of vaginal microbes after nonoxynol-9 use were minimal for each of the different nonoxynol-9 formulations. However, when both nonoxynol-9 concentration and number of product uses are taken into account, nonoxynol-9 did have dose-dependant effects on the increased prevalence of anaerobic gram-negative rods (odds ratio [OR] 2.4, 95% confidence interval [CI] 1.1–5.3), H_2O_2-negative lactobacilli (OR 2.0, 95% CI 1.0–4.1), and bacterial vaginosis (OR 2.3, 95% CI 1.1–4.7).

CONCLUSION—This study demonstrated that most nonoxynol-9 users experienced minimal disruptions in their vaginal ecology. There were no differences between the different formulations evaluated with respect to changes in vaginal microflora. However, independent of the nonoxynol-9 formulation, there was a dose-dependent effect with increased exposure to nonoxynol-9 on the risk of bacterial vaginosis and its associated flora.

LEVEL OF EVIDENCE—II-2

Products containing nonoxynol-9 have been used as vaginal spermicides for decades and are a widely used method of family planning. In the past several years, these products have also been evaluated as topical microbicides for prevention of sexually transmitted infections including human immunodeficiency virus (HIV).[1] Most of these studies were done in sex workers and did not demonstrate that women who used nonoxynol-9 had a decreased risk of sexually transmitted infection. Over the past decade, the importance of an intact vaginal ecosystem in resistance to urogenital infection has been highlighted. Most of the published data describing the effects of nonoxynol-9 use on the vaginal microbial ecosystem have assessed short-term nonoxynol-9 exposures, and their effects on the vaginal ecosystem have

Corresponding author: Courtney Schreiber, MD MPH, University of Pennsylvania, Department of Obstetrics and Gynecology, 3701 Market Street, Philadelphia, PA 19104; e-mail: corischreiber@yahoo.com..

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raised some concern. Women with vaginal colonization by lactobacilli are less likely to have[2] and acquire[3] sexually transmitted infections such as herpes simplex virus 2[4] and human papilloma virus[5] and are also less likely to acquire bacterial vaginosis.[3] Two cross-sectional studies and 1 longitudinal study have shown an association between the presence of vaginal lactobacilli and decreased prevalence or incidence of HIV.[6–8] Whether nonoxynol-9 affects lactobacilli colonization and the integrity of the vaginal ecosystem remains unclear.

Some in vitro studies have suggested that use of spermicides containing nonoxynol-9 would decrease colonization by lactobacilli,[9] whereas others have reported that most peroxide-producing strains of lactobacilli are resistant to nonoxynol-9.[10] The mechanism, however, by which nonoxynol-9 might alter the vaginal ecology in vivo is not clear, and whether these vaginal flora alterations are clinically relevant has not been elucidated. There are studies that have shown an adverse association between nonoxynol-9 use and the maintenance of vaginal flora,[9] although other clinical studies have not confirmed this association.[11] Some evidence suggests that nonoxynol-9 does not increase the risk of either vulvovaginal candidiasis[11,12] or bacterial vaginosis,[13] and that vaginal epithelia remain intact at lower doses of nonoxynol-9.[12] However, because certain doses of nonoxynol-9 seem to be at least transiently disruptive to vaginal ecology,[14,15] this study was designed to investigate the effect of long-term use and average weekly dose of 5 different formulations of nonoxynol-9 on vaginal flora in sexually active women in mutually monogamous relationships.

MATERIALS AND METHODS

This study was conducted at 3 sites between June 1998 and August 2002 as a planned substudy of a multicenter, randomized trial of the contraceptive effectiveness and safety of nonoxynol-9. The institutional review boards at each site approved the trial before commencement. All participants underwent an informed consent process. The details of the multicenter trial design and primary results have been published.[16] Briefly, sexually active women from 18–40 years of age with no history of subfertility and at low risk for sexually transmitted infections were eligible. At screening, all women underwent an interview, pelvic examination including Pap test, wet preparation, and urine pregnancy test. As a part of the larger multicenter trial, each participant was randomly assigned to use 1 of the following 5 nonoxynol-9 formulations as her only method of contraception for 7 months: a 52.5-mg gel, a 100-mg gel, a 150-mg gel, a 100-mg film, and a 100-mg suppository. Follow-up visits 2 through 4 were scheduled at weeks 4, 17, and 30. At each scheduled visit, the participant obtained 2 vaginal specimens by twice inserting the tip of a swab about one-half to 1 inch into her vagina and rotating it for 10 seconds. The swabs were placed into a specimen transport tube and sent by research personnel to a central laboratory for analysis.

Upon enrollment, the site study personnel telephoned a central randomization center to obtain an assignment to 1 of the 5 nonoxynol-9 formulations. The randomization scheme was computer-generated at Family Health International before the start of enrollment and was stratified by site with randomly ordered block sizes. Treatment assignment was not concealed from the participants or the clinical examiners. However, laboratory personnel were masked to the treatment assignment.

One swab was rolled across a glass slide and air dried for preparation of a Gram stain for assessment of vaginal flora. The slides were interpreted by a standard method for the diagnosis of bacterial vaginosis described by Nugent et al.[17] A score of 0 to 3 was interpreted as consistent with normal, *Lactobacillus*-predominant flora, a score of 4 to 6 corresponded to intermediate flora, and a score of 7–10 indicated bacterial vaginosis. The other swab was placed in Amies transport media and transported to the laboratory for culture of *Escherichia coli*, Lactobacillus, *Candida*, *Enterococcus*, and *Staphylococcus*. In addition to a 5% sheep blood

agar plate, a Rogosa agar plate was inoculated for recovery of lactobacilli and incubated in an anaerobic chamber. Lactobacilli were identified to the genus level by Gram stain morphology and production of lactic acid as assessed by gas chromatography. All lactobacilli were tested for the production of hydrogen peroxide in a qualitative assay using tetramethylbenzidine agar. Anaerobic gram-negative rods were detected on *Brucella* agar after 4–5 days of incubation under anaerobic conditions.

The sample size was estimated based on previous studies evaluating the effect of the diaphragm plus Conceptrol gel (Ortho-McNeil Pharmaceutical, Inc., Raritan, NJ) on vaginal microflora, which demonstrated an increase in the prevalence of *E. coli* from 15% before spermicide use to 48% after spermicide use.[18] Based on a 2-tailed McNemar's χ^2 test at the 0.05 significance level, a sample size of 40 women in each group, or a total of 200 women, would have 80% power to demonstrate similar increases in the prevalence of *E. coli* and other organisms between baseline and follow-up. The sample size was increased to a convenient number of 255 women, which allowed for a 22% discontinuation rate. A sample size of 47 in each group would have 80% power to detect a minimal between-group difference of 22% in the prevalence of *E. coli* after spermicide use.

All statistical analyses were performed using Stata statistical software, release 8.0 (StataCorp, College Station, TX), and all statistical tests were evaluated at the .05 significance level. Differences in the baseline characteristics of the women randomly assigned to the 5 nonoxynol-9 formulation groups were evaluated using analysis of variance or Pearson's χ^2, as appropriate. Vaginal colonization by hydrogen peroxide-producing and hydrogen peroxide-negative *Lactobacillus*, *Gardnerella vaginalis*, *E. coli*, *Enterococcus*, anaerobic gram-negative rods, and yeast was defined as detection of growth of the organism from agar culture. Differences in the prevalence of vaginal microbes and a Gram stain diagnosis of bacterial vaginosis at each visit among the nonoxynol-9 formulation groups were evaluated using Pearson's χ^2. Paired differences in prevalence between visits within an nonoxynol-9 formulation group were evaluated using McNemar's χ^2 test.

Average weekly nonoxynol-9 dose was calculated by multiplying the number of times the participant used the product, as obtained from the subjects' coital diaries, by the dose of nonoxynol-9 in that product and then dividing by the number of weeks since the prior visit. Generalized estimating equations were used to identify factors that were associated with the prevalence of vaginal microbial colonization and Gram stain diagnosis of bacterial vaginosis after spermicide use. Generalized estimating equations were also used to identify organisms associated with the prevalence of self-reported symptoms after spermicide use. Generalized estimating equations are a regression method that is able to model the marginal expectation of repeated, correlated outcomes. An exchangeable working correlation matrix was specified that assumes a common within-group correlation, and modified sandwich estimates of the variance were calculated, which produces valid standard errors even if the within-group correlation structure is not correctly specified. Sandwich estimates of the variance combine the variance estimate for the specified model with the variance matrix constructed from the data. The sandwich estimate is then modified to consider the sums of the observations for each individual. Statistical inference was based on the generalized Wald test statistic.[19] Multivariate generalized estimating equations models were developed using forward stepwise regression. Variables were retained in the model if the Wald test statistic had a *P* value of 0.05 or less. Models for the prevalence of each organism after spermicide use were adjusted for baseline colonization status. The population averaged odds ratios derived from the generalized estimating equations models are presented along with the 95% confidence intervals and *P* values.

RESULTS

A total of 269 women were enrolled and 235 (87%) completed between 2 and 4 visits. The median time between the enrollment visit and visits 2, 3, and 4 were 4, 17, and 30 weeks, respectively. Of the 235 women with follow-up, 46 (20%) were assigned to use the 52.5-mg gel, 43 (18%) the 100-mg gel, 46 (20%) the 150-mg gel, 51 (22%) the 100-mg film, and 49 (21%) the 100-mg suppository. Sixty-four percent of the participants used their assigned spermicide with every coital act. The demographic characteristics of the 235 participants with follow-up are displayed in Table 1. The most common contraceptive method used by the women in all groups was the oral contraceptive pill (used by 75% of study participants at some point in the past). However, a range of contraceptive methods were used by these women, including implants, depomedroxyprogesterone acetate, condoms (male and female), intrauterine devices, spermicides, diaphragm, periodic abstinence, and withdrawal. There was no statistically significant difference in the types of contraceptives used across the 5 groups. Reports of urogenital symptoms and infections were not statistically significantly different among the groups, and few (1–3%) had symptoms within the week before enrollment.

The proportion of women with colonization by the individual microorganisms at baseline and subsequent visits are listed in Table 2. There was an overall difference at visit 2 in the percentage of women with an increase the prevalence of yeast, and there was a difference in the presence of H_2O_2-negative lactobacilli at visit 3 as well with *Enterococcus* at visit 4. In the 52.5-mg gel group, there was a statistically significant increase in *Enterococcus* from the baseline visit to visit 2, but this resolved by visit 3. In the 100-mg film group, the number of H_2O_2-negative lactobacilli decreased significantly between visits 2 and 3, and this effect was persistent.

Symptomatology was uncommon in this study. Thirty women were colonized with *Candida* spp after nonoxynol-9 use, and a statistically significant proportion of these women complained of vaginal burning ($P = .002$), vaginal itching ($P = .004$), and vulvar burning ($P = .04$). Pelvic pain and vaginal bleeding were not associated with vaginal flora disturbances, but dyspareunia was associated with the presence of *Gardnerella vaginalis* ($P = .03$). There were no significant complaints that were associated with a diagnosis of bacterial vaginosis.

There was a decrease in the prevalence of yeast in those women who used the film and suppository nonoxynol-9 formulation, even after controlling for baseline colonization status. The prevalence of anaerobic gram-negative rods was increased in those who used the 100-mg gel, but the prevalence of *Enterococcus* was decreased in this same group.

Colonization at baseline was the most significant risk factor for colonization with the same microbe later at follow-up ($P < .001$ for each). African-American women had a statistically significantly increased risk for *Gardnerella vaginalis* colonization (odds ratio [OR] 2.7, 95% confidence interval [CI] 1.6–4.5), anaerobic gram-negative colonization (OR 2.5, 95% CI 1.2–4.9), and bacterial vaginosis (OR 3.6, 95% CI 1.9–6.7) when compared with women of other races. African-American race was also associated with a decreased risk of colonization by H_2O_2-producing lactobacilli (OR 0.5, 95% CI 0.3–0.8). Vaginal micro-flora did differ between sites, but these differences were attributable to differences in ethnic distribution by site. Thus, after statistical adjustment for race, site-specific differences in vaginal microflora were no longer significant. Similarly, the type of nonoxynol-9 delivery system had limited impact on microbe colonization (Table 3) after accounting for baseline microbe colonization.

To test the hypothesis that increased weekly exposure to nonoxynol-9 accounted for changes in the vagina microflora, average weekly nonoxynol-9 exposures were categorized based on quartiles as< 100 mg, 100–174 mg, 175–281 mg, and > 281 mg. There was a dose-dependant relationship between average weekly nonoxynol-9 exposure and an increase in the prevalence

of anaerobic gram-negative rods and bacterial vaginosis, even after adjustment for baseline colonization (Table 4). The increased risk for bacterial vaginosis and anaerobic gram-negative rods associated with nonoxynol-9 exposure was present even after adjustment for race (OR 2.2, 95% CI 1.1–4.4; OR 2.4, 95% CI 1.1–5.3, respectively).

The mean number of coital acts, product usages, and average milligram dose of nonoxynol-9 per week are shown in Table 5. With the exception of those participants in the 100-mg gel group, there was no difference in number of coital acts per week among the groups. This group also accounts for the difference found in product use per week. Average milligram dose per week was predictably increased with the higher-dose nonoxynol-9 formulations and also increased in the group who used the 100-mg nonoxynol-9 gel (because that group had more coital acts per week).

DISCUSSION

This longitudinal investigation did not show dramatic effects of nonoxynol-9 on the vaginal ecologic system. The most significant risk factor for being colonized with an organism after spermicide use was baseline colonization, not nonoxynol-9 exposure. However, even after adjustment for colonization status at baseline and for race, an increase in nonoxynol-9 exposure was associated with a statistically significant increase in colonization by anaerobic gram-negative rods, H_2O_2-negative lactobacilli, and bacterial vaginosis. Furthermore, the vehicle itself, gel, film, and suppository, was not generally associated with changes in the vaginal ecology independent of nonoxynol-9 dose. Rather, it was the average nonoxynol-9 exposure per week, which reflects the number of sex acts with spermicide, as well as the dose of each application used that increased the risk for vaginal flora disturbances.

The clinical significance of this dose-dependant relationship is less clear than its statistical significance; symptomatology was uncommon in this trial. The laboratory diagnosis of bacterial vaginosis, as opposed to the clinical diagnosis, was used in this study because this testing can be standardized across sites. Not all of the women with a Gram stain consistent with bacterial vaginosis would have fulfilled the clinical criteria for the diagnosis of bacterial vaginosis. Nevertheless, an increase in the dose of nonoxynol-9 was associated with vaginal ecologic disruptions, and whether these changes predispose women to HIV or other virulent sexually transmitted infections remains an important question. Even if the dose-dependant changes in the vaginal flora did not produce symptoms in the study population, it is possible that the changes themselves create an environment that facilitates infection with sexually transmitted infections. Nonoxynol-9 use would clearly be limited if this were the case.

The population studied here is representative of people who are in long-standing, mutually monogamous relationships. In this sample, nonoxynol-9 was not generally disruptive to the vaginal flora. Because of the low incidence of sexually transmitted infections in the study population, associations between nonoxynol-9, vaginal ecologic disruption, and sexually transmitted infection transmissibility could not be delineated. However, studies have demonstrated an association of nonoxynol-9 use with increased HIV acquisition in the sex worker population,[20,21] where increased weekly dose may have been a factor. An association with the presence of bacterial vaginosis and increased HIV prevalence has been described.[6] It is possible that the increase in bacterial vaginosis that is found with increased nonoxynol-9 dose in this study is an intermediate state for the increased HIV incidence that has been found on other studies. Further research in this area is necessary to test this hypothesis.

Eighty-seven percent of the participants completed 75% of the planned follow-up visits. Weaknesses of the study include that the both product use and frequency of coital acts were dependant upon information extracted from participant diaries. There was no objective way to

verify this information. Another weakness of the analysis is the lack of control group for comparison.

Overall, this trial supports the safety of nonoxynol-9 as a contraceptive with respect to its effects on the vaginal ecosystem. Raymond et al[16] recently showed that these 5 nonoxynol-9 formulations are safe and effective forms of contraception, with increased dose being associated with increased efficacy. Although we found that there was clearly an effect of increased dose of nonoxynol-9 on the vaginal ecosystem, this effect was present only among the most frequent users.

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Table 1

Participant Characteristics

Characteristic	Gel A (n = 46)	Gel B (n = 43)	Gel C (n = 46)	Film (n = 51)	Suppository (n = 49)	*P*
Mean age ± standard deviation (y)	28.3 ± 5.7	27.7 ± 5.4	28.0 ± 5.1	27.1 ± 5.0	27.2 ± 5.1	.8
Race/ethnicity						.7
White, non-Hispanic	28 (61)	21 (49)	26 (57)	30 (59)	26 (53)	
African-American, non-Hispanic	13 (28)	20 (47)	16 (35)	20 (39)	18 (37)	
Hispanic	3 (7)	1 (2)	1 (2)	1 (2)	2 (4)	
Other	2 (4)	1 (2)	3 (7)	0	3 (6)	
Education						.96
No degree/high school/GED	13 (28)	14 (32)	11 (24)	17 (33)	12 (24)	
Associate degree (< 4 y college)	14 (30)	16 (37)	17 (37)	17 (33)	16 (33)	
College (4 y)	13 (28)	11 (26)	11 (24)	11 (22)	14 (29)	
Postgraduate	6 (13)	2 (5)	7 (15)	6 (12)	7 (14)	
Marital status						.5
Married, living with partner	15 (33)	13 (30)	23 (50)	16 (31)	18 (37)	
Single, living with partner	11 (24)	15 (35)	10 (22)	12 (24)	12 (24)	
Single, not living with partner	20 (43)	15 (35)	13 (28)	23 (45)	19 (39)	
Current smoking						.7
No	32 (70)	32 (74)	36 (78)	40 (78)	40 (82)	
Yes	14 (30)	11 (26)	10 (22)	11 (22)	9 (18)	
Gravidity						.3
0	15 (33)	15 (35)	16 (35)	18 (35)	22 (45)	
1	15 (33)	5 (12)	7 (15)	14 (27)	9 (18)	
2	7 (15)	8 (19)	9 (20)	6 (12)	10 (20)	
≥3	9 (20)	15 (35)	14 (30)	13 (25)	8 (16)	
Parity						.08
0	23 (50)	19 (44)	19 (41)	25 (49)	34 (69)	
1	13 (28)	7 (16)	10 (22)	12 (24)	8 (16)	
≥2	10 (22)	17 (40)	17 (37)	14 (27)	7 (14)	

GED, general equivalency diploma.

Values are n (%) unless otherwise specified.

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Table 2

Microflora Prevalence by Visit Number and Product Formulation

Microflora	n	52.5-mg Gel	n	100-mg Gel	n	150-mg Gel	n	100-mg Film	n	100-mg Suppository	*P*
H_2O_2 + *Lactobacillus*											
Baseline	46	29 (63)	43	24 (56)	46	27 (59)	51	29 (57)	49	31 (63)	.9
Visit 2	46	35 (76)	43	24 (56)	46	29 (63)	51	27 (53)	49	30 (61)	.2
Visit 3	35	24 (69)	32	22 (69)	34	22 (65)	38	24 (63)	37	27 (73)	.9
Visit 4	21	15 (71)	21	17 (81)	26	17 (65)	22	15 (68)	22	15 (68)	.8
H_2O_2– *Lactobacillus*											
Baseline	46	8 (17)	43	7 (16)	46	6 (13)	51	9 (18)	49	6 (12)	.9
Visit 2	46	8 (17)	43	5 (12)	46	5 (11)	51	12 (24)*	49	8 (16)	.4
Visit 3	35	6 (17)	32	6 (19)	34	11 (32)	38	1 (3)*	37	8 (22)	.03
Visit 4	21	3 (14)	21	3 (14)	26	4 (15)	22	1 (5)	22	4 (18)	.7
Gardnerella vaginalis											
Baseline	46	14 (30)	43	22 (51)	46	15 (33)	51	23 (45)	49	24 (49)	.1
Visit 2	46	13 (28)	43	18 (42)	46	13 (28)	51	24 (47)	49	20 (41)	.2
Visit 3	35	11 (31)	32	14 (44)	34	10 (29)	38	17 (45)	37	14 (38)	.6
Visit 4	21	7 (33)	21	8 (38)	26	7 (27)	22	9 (39)	22	8 (36)	.9
Escherichia coli											
Baseline	46	17 (37)	43	21 (49)	46	13 (28)	51	18 (35)	49	16 (33)	.3
Visit 2	46	22 (48)	43	18 (42)	46	21 (46)	51	21 (41)	49	24 (49)	.9
Visit 3	35	13 (37)	32	10 (31)	34	10 (29)	38	9 (24)	37	18 (49)	.2
Visit 4	21	4 (19)	21	6 (29)	26	8 (31)	22	8 (35)	22	8 (36)	.8
Enterococcus											
Baseline	46	24 (52)*	43	25 (58)	46	23 (50)	51	28 (55)	49	26 (53)	.95
Visit 2	46	33 (72)*	43	24 (56)	46	26 (57)	51	26 (51)	49	31 (63)	.3
Visit 3	35	18 (51)	32	16 (50)	34	17 (50)	38	22 (58)	37	22 (59)	.9
Visit 4	21	15 (71)	21	8 (38)	26	12 (46)	22	12 (52)	22	17 (77)	.04
Anaerobic gram-negative rods											
Baseline	46	37 (80)	43	37 (86)	46	32 (70)	51	39 (76)	49	38 (78)	.4
Visit 2	46	35 (76)	43	41 (95)	46	36 (78)	51	40 (78)	49	43 (88)	.08
Visit 3	35	28 (80)	32	29 (91)	34	28 (82)	38	27 (71)	37	30 (81)	.4
Visit 4	21	13 (62)	21	18 (86)	26	21 (81)	22	16 (70)	22	20 (91)	.1
Yeast											
Baseline	46	6 (13)	43	6 (14)	46	6 (13)	51	5 (10)	49	7 (14)	.97
Visit 2	46	10 (22)	43	8 (19)	46	3 (7)	51	2 (4)	49	5 (10)	.03
Visit 3	35	5 (14)	32	6 (19)	34	2 (6)	38	1 (3)	37	4 (11)	.2
Visit 4	21	4 (19)	21	3 (14)	26	4 (15)	22	0	22	1 (5)	.2
Staphylococcus aureus											
Baseline	46	0	43	1 (2)	46	1 (2)	51	0	49	3 (6)	.2
Visit 2	46	1 (2)	43	1 (2)	46	1 (2)	51	1 (2)	49	1 (2)	1.0
Visit 3	35	0	32	1 (3)	34	0	38	1 (3)	37	0	.5
Visit 4	21	0	21	0	26	0	22	0	22	1 (5)	.4
Nugent score											
Baseline	45		42		45		51		47		.6
Normal (0–3)		24 (53)		16 (38)		26 (58)		24 (47)		25 (53)	
Intermediate (4–6)		11 (24)		16 (38)		10 (22)		12 (24)		9 (19)	
Bacterial vaginosis (7–10)		10 (22)		10 (24)		9 (20)		15 (29)		13 (28)	
Visit 2	46		43		45		51		48		.4
Normal (0–3)		27 (59)		19 (44)		23 (51)		19 (37)		26 (54)	
Intermediate (4–6)		11 (24)		10 (23)		14 (31)		18 (35)		13 (27)	
Bacterial vaginosis (7–10)		8 (17)		14 (33)		8 (18)		14 (27)		9 (19)	
Nugent score											

Microflora	n	52.5-mg Gel	n	100-mg Gel	n	150-mg Gel	n	100-mg Film	n	100-mg Suppository	*P*
Baseline	45		42		45		51		47		.6
Normal (0–3)		24 (53)		16 (38)		26 (58)		24 (47)		25 (53)	
Intermediate (4–6)		11 (24)		16 (38)		10 (22)		12 (24)		9 (19)	
Bacterial vaginosis (7–10)		10 (22)		10 (24)		9 (20)		15 (29)		13 (28)	
Visit 2	46		43		45		51		48		.4
Normal (0–3)		27 (59)		19 (44)		23 (51)		19 (37)		26 (54)	
Intermediate (4–6)		11 (24)		10 (23)		14 (31)		18 (35)		13 (27)	
Bacterial vaginosis (7–10)		8 (17)		14 (33)		8 (18)		14 (27)		9 (19)	
Visit 3	34		33		32		37		36		.9
Normal (0–3)		17 (50)		15 (45)		16 (50)		16 (43)		16 (44)	
Intermediate (4–6)		10 (29)		8 (24)		9 (28)		10 (27)		10 (28)	
Bacterial vaginosis (7–10)		7 (21)		10 (30)		7 (22)		11 (30)		10 (28)	
Visit 4	22		20		25		23		21		.7
Normal (0–3)		14 (64)		11 (55)		12 (48)		11 (48)		10 (48)	
Intermediate (4–6)		3 (14)		7 (35)		8 (32)		6 (26)		5 (24)	
Bacterial vaginosis (7–10)		5 (23)		2 (10)		5 (20)		6 (26)		6 (29)	

Values are n (%) unless otherwise specified.

* Indicates significant paired change between visits within the given group.

Table 3

Odds Ratios of Microbe Colonization by Nonoxynol-9 Delivery System, Adjusted for Baseline Colonization

Microbe/Nonoxynol-9 Formulation	Odds Ratio (95% Confidence Interval)
H_2O_2+ Lactobacilli	
52.5-mg gel	1.0 (referent)
100-mg gel	0.8 (0.3–2.0)
150-mg gel	0.7 (0.4–1.4)
100-mg film	0.5 (0.2–1.1)
100-mg suppository	0.7 (0.3–1.5)
H_2O_2– Lactobacilli	
52.5-mg gel	1.0 (referent)
100-mg gel	0.9 (0.4–1.9)
150-mg gel	1.2 (0.5–2.9)
100-mg film	0.8 (0.4–1.7)
100-mg suppository	1.3 (0.6–2.9)
Gardnerella vaginalis	
52.5-mg gel	1.0 (referent)
100-mg gel	1.1 (0.5–2.6)
150-mg gel	0.8 (0.4–1.9)
100-mg film	1.4 (0.7–3.0)
100-mg suppository	1.0 (0.4–2.2)
Escherichia coli	
52.5-mg gel	1.0 (referent)
100-mg gel	0.7 (0.4–1.3)
150-mg gel	1.0 (0.6–1.8)
100-mg film	0.7 (0.4–1.4)
100-mg suppository	1.4 (0.8–2.6)
Enterococcus	
52.5-mg gel	1.0 (referent)
100-mg gel	0.5 (0.2–0.9)
150-mg gel	0.6 (0.3–1.2)
100-mg film	0.5 (0.3–1.0)
100-mg suppository	0.9 (0.4–1.9)
Anaerobic gram-negative rods	
52.5-mg gel	1.0 (referent)
100-mg gel	3.6 (1.3–10.4)
150-mg gel	1.7 (0.7–4.0)
100-mg film	1.0 (0.5–2.3)
100-mg suppository	2.3(0.9–5.6)
Bacterial Vaginosis gram stain	
52.5-mg gel	1.0 (referent)
100-mg gel	2.0 (0.8–5.4)
150-mg gel	1.2 (0.5–2.4)
100-mg film	1.4 (0.6–3.5)
100-mg suppository	0.7 (0.4–1.4)
Yeast	
52.5-mg gel	1.0 (referent)
100-mg gel	0.9 (0.4–2.4)
150-mg gel	0.4 (0.1–1.1)
100-mg film	0.1 (0.02–0.5)
100-mg suppository	0.4 (0.1–0.96)

Table 4

Odds Ratios of Microbe Colonization by Nonoxynol-9 Dose, Adjusted for Baseline Colonization

Microbe/Average Nonoxynol-9 Dose per Wk	Odds Ratio (95% Confidence Interval)	*P*
H_2O_2+ Lactobacilli		
< 100 mg	1.0 (referent)	
100–174 mg	1.2 (0.7–2.1)	.6
175–281 mg	0.9 (0.5–1.7)	.8
> 281 mg	0.7 (0.4–1.4)	.3
H_2O_2– Lactobacilli		
< 100 mg	1.0 (referent)	
100–174 mg	1.2 (0.7–2.2)	.7
175–281 mg	0.9 (0.4–1.8)	.7
> 281 mg	2.0 (1.0–4.1)	.04
Gardnerella vaginalis		
< 100 mg	1.0 (referent)	
100–174 mg	1.8 (1.0–3.2)	.06
175–281 mg	1.6 (0.9–2.8)	.1
> 281 mg	1.8 (0.9–3.4)	.08
Escherichia coli		
< 100 mg	1.0 (referent)	
100–174 mg	1.0 (0.6–1.7)	.9
175–281 mg	0.9 (0.5–1.5)	.7
> 281 mg	1.3 (0.8–2.1)	.4
Enterococcus		
< 100 mg	1.0 (referent)	
100–174 mg	1.3 (0.7–2.2)	.4
175–281 mg	0.9 (0.5–1.5)	.6
> 281 mg	1.1 (0.6–2.1)	.7
Anaerobic gram-negative rods		
< 100 mg	1.0 (referent)	
100–174 mg	1.2 (0.6–2.2)	.6
175–281 mg	1.4 (0.7–2.6)	.3
> 281 mg	2.4 (1.1–5.3)	.02
Bacterial vaginosis gram stain		
< 100 mg	1.0 (referent)	
100–174 mg	1.7 (0.9–3.3)	.1
175–281 mg	1.6 (0.8–3.2)	.2
> 281 mg	2.3 (1.1–4.7)	.02
Yeast		
< 100 mg	1.0 (referent)	
100–174 mg	1.7 (0.9–3.3)	.1
175–281 mg	1.6 (0.8–3.2)	.2
> 281 mg	2.3 (1.1–4.7)	.02

Table 5

Average Coital Acts, Product Use, and Nonoxynol-9 Use Per Week

Formulation	n	Coital Acts/Wk	*P*	Product Use/Wk	*P*	Nonoxynol-9 Dose/Wk (mg)	*P*
52.5-mg gel	46	2.1 ± 1.3		1.9 ± 1.2		101.4 ± 63.3	
100-mg gel	43	2.9 ± 2.4		2.8 ± 2.3		280.5 ± 233.6	
150-mg gel	46	2.1 ± 1.2		2.0 ± 1.2		296.2 ± 176.5	
100-mg film	51	2.1 ± 1.6		1.9 ± 1.5		194.6 ± 150.0	
100-mg suppository	49	2.1 ± 1.4		1.9 ± 1.4		190.9 ± 143.8	
			.06		.03		< .001

Values are mean ± standard deviation unless otherwise specified.