

## Effects of Vaginal Intercourse with and without a Condom on Vaginal Flora and Vaginal Epithelium

David A. Eschenbach,<sup>1</sup> Dorothy L. Patton,<sup>1</sup>  
Thomas M. Hooton,<sup>3</sup> Amalia S. Meier,<sup>1</sup> Ann Stapleton,<sup>2</sup>  
Jan Aura,<sup>1</sup> and Kathy Agnew<sup>1</sup>

Departments of <sup>1</sup>Obstetrics and Gynecology and of <sup>2</sup>Medicine  
and <sup>3</sup>Division of Infectious Diseases, University of Washington, Seattle

Effects of a single episode of intercourse on vaginal flora and epithelium were examined in subjects randomly assigned to groups that used no condom or lubricated nonspermicide condoms. Subjects were evaluated at visits before (1 month and 1–2 days) and after (8–12 h, 2–3 days, and 6–8 days) an index episode of sexual intercourse. The 22 subjects who used no condoms had significantly more *Escherichia coli* and a high concentration ( $\geq 10^5$  cfu/mL) of *E. coli* in the vagina (both,  $P < .001$ ) and urine (all  $< 10^5$  cfu/mL;  $P = .004$ ) at visit 3 than at visits 1 and 2. The 20 subjects who used condoms had a trend toward more vaginal *E. coli* ( $P = .06$ ) and a significant increase in other enteric gram-negative rods ( $P = .001$ ) after intercourse. Intercourse was not associated with gross, colposcopic, or histologic vaginal epithelial abnormalities.

Unprotected sexual intercourse facilitates the transmission of sexually transmitted diseases (STDs), including human immunodeficiency virus (HIV). About 80% of HIV transmission now occurs via vaginal intercourse [1]. Transmission of STDs and HIV is more efficient from men to women than vice versa [2, 3]. This has drawn attention to infections, flora, and epithelial factors in the female genital tract that could enhance or decrease heterosexual HIV transmission [4–6]. Increased acquisition of HIV appears to occur with a wide variety of sexually transmitted cervical infections (*Neisseria gonorrhoeae* [6, 7] and *Chlamydia trachomatis* [6]) and vaginal infections not solely sexually transmitted (e.g., candidiasis [8] and bacterial vaginosis [7, 8]). The absence of *Lactobacillus* organisms also appears to be a factor in the acquisition of HIV [9]. In addition, *Trichomonas vaginalis* is associated with an increased hazard ratio for the acquisition of HIV, although the findings often do not reach statistical significance [6–8].

Because use of a condom could reduce the transmission of all of these microbes, condom use has been advanced as an effective method to prevent HIV transmission [4, 5]. Previous studies showed that intercourse with condoms lubricated with nonoxynol-9 and nonlubricated condoms increases the risk of

urinary tract infection (UTI) [10–12]. Intercourse without condoms is also associated with increased *Escherichia coli* colonization of the vagina and bacteriuria [13]. However, studies have not compared the effect of no condom use with condom use on vaginal epithelium. Here we report the effects of a single index episode of vaginal intercourse in 2 randomly selected study groups: one without a condom and one with a silicone-lubricated condom lacking nonoxynol-9. We examined vaginal flora and vaginal epithelium by gross, colposcopic, and histologic means in women without a bacterial or fungal genital infection, except for bacterial vaginosis. Condoms with nonoxynol-9, the most commonly used spermicide, were not used, because of the variable effects reported of nonoxynol-9 use on genital epithelium and flora [14–16].

### Subjects and Methods

From March 1997 to May 1998, female subjects were enrolled from the University of Washington student and staff population. Subjects were recruited through newspaper ads, flyers, and word-of-mouth referrals. Women were eligible for study if they were 18–40 years old, had regular monthly menses, 1 sex partner, used combination oral contraceptive pills or permanent contraception, and agreed to refrain from vaginal medication. Exclusion criteria were the following groups of factors: (1) complaints of vaginitis, abnormal vaginal discharge, vulvar pruritus, or irritation; (2) chronic illness such as hypertension or diabetes; (3) current use of spermicides or an intrauterine device; (4) antibiotic use in the past month; (5) vaginal suppository or douching in the past week; and (6) allergy to latex. We also excluded subjects who, at baseline, had *N. gonorrhoeae*, *C. trachomatis*, or *T. vaginalis* organisms or symptomatic candidiasis. Asymptomatic bacterial vaginosis was not an exclusion criterion, and subjects with asymptomatic bacterial vaginitis during the study were not treated.

Demographic, sexual, contraceptive, and gynecologic history, including current genital symptoms, were collected on standardized forms at enrollment. Women were asked to refrain from intercourse

Received 8 August 2000; revised 27 November 2000; electronically published 21 February 2001.

Presented in part: Infectious Diseases Society of Obstetrics and Gynecology meeting, Toronto, August 1999.

This study was approved by the University of Washington Human Subjects Review Committee. Written informed consent was obtained from each participating subject, and human experimentation guidelines of the US Department of Health and Human Services were followed in the conduct of the clinical research.

Financial support: National Institutes of Health (grant HD-33203).

Correspondence (no reprints available): Dr. David Eschenbach, Dept. of Obstetrics and Gynecology, University of Washington, Box 356460, Seattle, WA 98195-6460 (eschen@u.washington.edu).

The Journal of Infectious Diseases 2001;183:913–8

© 2001 by the Infectious Diseases Society of America. All rights reserved.  
0022-1899/2001/18306-0011\$02.00

for 2–5 days before examination at visits 1 and 2. Visit 1 occurred 19–24 days after the last menstrual period, and visit 2 occurred 1 month later (15–22 days from the last menstrual period), so that the vaginal biopsy performed after intercourse was done at a similar time in the menstrual cycle (19–24 days). At visit 1, subjects were randomized to the condom or no condom group by computer-generated random numbers table. After visit 2, subjects were asked to have 1 episode of vaginal intercourse in the next 1–2 days and to return for examination at visit 3 (8–12 h after intercourse), visit 4 (3–4 days after intercourse), and visit 5 (6–8 days after intercourse) after the index episode of intercourse. Subjects refrained from intercourse between visits 3 and 5. At the follow-up visits, we obtained an interval sexual and gynecologic history and performed vaginal and cervical examinations. A midstream clean catch urine was collected for culture before each examination. The urine culture method detected bacteria at  $10^2$  cfu/mL of urine. Recovery of a single uropathogen at  $10^2$ – $10^4$  cfu/mL was distinguished from  $\geq 10^5$  cfu/mL. A second vaginal biopsy was done at visit 3 (8–12 h after intercourse).

At each visit, external genitalia were visually examined. A non-lubricated speculum was inserted for visual and colposcopic examination of the vaginal walls. The amount of vaginal discharge was qualitatively assessed and was recorded. An indicator strip (Color pHast; EM Science) was directly applied to the vaginal sidewall to determine pH. We used a spatula to scrape the right vaginal wall for Gram's stain [17] and for a separate cytologic specimen for Papanicolaou staining.

The left vaginal wall was sampled for Gram's stain and wet mount analysis. The vaginal posterior fornix was swabbed, and the swabs were placed in transport media (Port-A-Cul; Becton Dickinson) and were cultured for aerobic and anaerobic bacteria within 12 h, as reported elsewhere [18]. Hydrogen peroxide ( $H_2O_2$ )-producing lactobacilli were identified by the blue pigment formed when  $H_2O_2$  oxidizes tetramethylbenzidine present in brucella agar base [19]. Cervical samples for cytology and for *C. trachomatis* culture [20] were obtained at enrollment; an endocervical Gram's-stained specimen was obtained at all visits.

A full-thickness biopsy was obtained of the vaginal epithelium in the upper one-third of the vagina at visits 1 and 3, as described elsewhere [21]. We removed a 2- by 4-mm full-thickness sample of vaginal epithelium by cervical biopsy forceps (Mini-Townsend; Cooper Surgical). Biopsy tissue was pinned flat on styrofoam to minimize distortion during formalin fixation. One of us (D.L.P.) counted the number of epithelial layers in hematoxylin-eosin-stained samples in 2 separate fields ( $\times 40$ ) by using an ocular micrometer grid and with no knowledge of other patient data or visit number. Data are shown as a mean of the 2 fields. Epithelial thickness was measured at  $\times 40$  with the micrometer grid: each grid was equivalent to 0.1 mm at  $\times 40$  magnification [22]. Thus, a 0.05-mm difference could be measured accurately.

Table 1 shows categorical baseline characteristics, as compared by  $\chi^2$  or Fisher's exact tests (for cells  $\leq 3$ ). We used a Student's *t* test to compare continuous variables and logistic regression to compare differences in vaginal *E. coli* between the no condom and condom groups. Included in this model were *E. coli* by visit number and study group (no condom or condom) and also  $H_2O_2$ -producing lactobacilli. For tables 2–5, statistical analyses were based on trend over time in the same subject. For data on only 2 points, we used

**Table 1.** Subject demographic and reproductive history characteristics at baseline in no condom and condom group.

Characteristics	No condom group ( <i>n</i> = 22)	Condom group ( <i>n</i> = 20)	<i>P</i>
Mean age $\pm$ SE, years	22.7 $\pm$ 0.9	23.0 $\pm$ 0.9	.8
Not married	18 (82)	16 (80)	.9
Student	15 (68)	15 (75)	.8
Ethnicity			
White	13 (59)	17 (85)	.09
Black	0	0	
Other	9 (41)	3 (15)	
Smoking history			
Yes, not now	6 (27)	5 (25)	.9
Yes, still smoke	3 (14)	4 (20)	
No. of alcoholic drinks per week			
1–2	6 (27)	11 (55)	.2
$\geq 3$	13 (59)	7 (35)	
No. of lifetime sex partners			
1–2	6 (27)	6 (30)	.8
3–4	5 (23)	6 (30)	
$>4$	11 (50)	8 (40)	
>1 Sex partner in last			
3 months	1 (5)	1/19 (5) <sup>a</sup>	.5
New sex partner since visit 1	2 (9)	3/19 (16) <sup>a</sup>	.5
Frequency of intercourse/week			
0–2	8 (36)	11/19 (58) <sup>a</sup>	.2
$\geq 3$	14 (64)	8/19 (42) <sup>a</sup>	
Condom use			
Never	22 (100)	18/19 (95) <sup>a</sup>	.9
Sometimes	0	1/19 (5) <sup>a</sup>	
Combination oral contraceptives	21	20	.9

NOTE. Data are no. (%) of subjects unless otherwise noted.

<sup>a</sup> One person in the condom group did not have that variable checked either positive or negative on the form.

either McNemar sign test for binary data or paired Student's *t* test for continuous data. For  $>2$  time points, we used logistic regression for correlated binary data and linear regression for correlated continuous data [23] (significance was set at  $P < .05$ ). These were fitted with the GEE function in SPlus with an AR-1 correlation structure. Software used included SPSS for Windows 7.5 (SPSS) and SPlus for Unix (Mathsoft).

## Results

*Comparison of demographic, contraceptive, and sex history.* Subjects randomized to no condoms (*n* = 22) or condoms (*n* = 20) are compared in table 1. The groups were similar in all demographic characteristics examined. Most subjects were young, single, white, nonsmoking students, but whites made up a slightly higher proportion of subjects in the condom group. The groups had similar smoking, alcohol, sex, and contraceptive histories. To prevent pregnancy during the study, all but 1 subject in the no condom group used oral contraceptives (that woman had a tubal ligation). There were no differences between the 2 groups in current genital symptoms or menstrual history (data not shown). Cervical ectopy of  $\geq 25\%$  of the cervical surface was present in 9% of the no condom group and in 40% of the condom group ( $P = .03$ ), and minimal cervical mucus was present in 95% of the no condom group and 68% of the condom group ( $P = .04$ ). There were no significant differences

**Table 2.** Clinical examination data before and after an index episode of vaginal intercourse (IC) in groups that did not use or did use condoms.

Group	Time related to index IC <sup>a</sup>				<i>P</i> <sup>b</sup>
	Visit 2	Visit 3	Visit 4	Visit 5	
No condom use					
No. of subjects	22	22	22	19	
Vulvar erythema	4 (18)	6 (27)	4/21 (19)	4 (21)	.2/.5
Vaginal erythema	0	1 (5)	0	0	ND
Abnormal vagina by colposcopy	0	1 (5)	0	0	ND
Mean pH ± SE	4.4 ± 0.1	4.5 ± 0.1	4.5 ± 0.1	4.4 ± 0.1	.1/.9
Mean no. of epithelial layers <sup>c</sup>	28 ± 0.9	26 ± 0.9	—	—	.1
Mean no. of PMNL/5 HPF <sup>c</sup>	3.3 ± 0.4	4.1 ± 0.6	—	—	.1
Condom users					
No. of subjects	20	20	19	18	
Vulvar erythema	4 (20)	7 (35)	2 (11)	2 (11)	.007/.2
Mean pH ± SE	4.3 ± 0.1	4.4 ± 0.1	4.4 ± 0.1	4.4 ± 0.1	.6/.6
Mean no. of epithelial layers <sup>c</sup>	27 ± 0.8	28 ± 1.1	—	—	.9
Mean no. of PMNL/5 HPF <sup>c</sup>	4.3 ± 0.6	4.3 ± 0.8	—	—	.4

NOTE. Data are no. (%) of subjects unless otherwise indicated. No subjects in condom group had vaginal erythema or abnormal vagina by colposcopy. HPF, high-powered field; ND, not done; PMNL, polymorphonuclear leukocytes; —, vaginal biopsy was not done.

<sup>a</sup> Visit 2, 1–2 days before index IC; visit 3, 8–12 h after index IC; visit 4, 3–4 days after index IC; visit 5, 6–8 days after index IC.

<sup>b</sup> *P* values for test 1/test 2: test 1, visit 3 differs from visits 1 and 2, as assessed by logistic regression for correlated data; test 2, visits 3–5 show linear trend by logistic regression for correlated data.

<sup>c</sup> Visit 1 biopsy vs. visit 3 biopsy.

in gross or colposcopic abnormalities of the vagina, amount of vaginal discharge, or viscosity of vaginal discharge between groups (data not shown).

*Clinical and vaginal epithelial findings before and after intercourse.* Clinical findings at visit 1 and 1 month later at visit 2 were almost identical (first visit data are not shown). In table 2, selected results of the clinical examination are provided for visit 2 (no intercourse in previous 2 days), visit 3 (8–12 h after index episode of intercourse), visit 4 (3–4 days after intercourse), and visit 5 (6–8 days after intercourse). In both groups, there was an increase in vulvar erythema at visit 3, compared with that at visits 1 and 2, but the difference was statistically significant only in the condom group (*P* = .007, logistic regression for correlated data). The presence of erythema decreased by visit 4 in both groups. The linear decrease in erythema among visits 3, 4, and 5 was not statistically significant in the condom group (*P* = .2). The visual and colposcopy appearance of the vagina and the mean vaginal pH did not change after intercourse in either group. The mean number of epithelial cell layers of the vaginal mucosa and the mean number of neutrophils per 5 high-powered fields were similar before and after intercourse in both groups. The presence of lymphocytes and plasma cells also did not change in the vaginal submucosa between visits 1 and 3 in either group (data not shown).

*Comparison of vaginal flora between visits 1 and 2.* The vaginal flora and Gram's-stain specimen were first compared at a similar time (19–24 days) in 2 consecutive menstrual cycles separately for subjects randomized to no condoms and condoms. Only microbes present in ≥5 subjects or at a concentration ≥log 4.0 were considered. The vaginal flora was stable over the 2 months before the scheduled index episode of intercourse among subjects in both

groups for all isolates, with the exception of *Enterococcus* organisms, which increased significantly in the no condom group between visits 1 (*n* = 5) and 2 (*n* = 10; *P* = .04).

Lactobacilli were the dominant organism in both groups, present in 68%–77% of the no condom group and 85%–95% of the condom group at visits 1 and 2, and were the dominant bacteria, comprising 67%–99% of all microbes in the vagina, as determined by concentrations of microbes recovered. Lactobacilli and other microbes occurred at baseline in a similar concentration in the no condom and the condom groups. The number of subjects with intermediate scores or bacterial vaginosis by Gram's stain at the first and second months were 4 and 5 in the no condom group and 3 and 5 in the condom group, respectively.

*Vaginal microflora before and after index intercourse.* Table 3 lists all vaginal microflora before and after the index episode of intercourse in the no condom and condom groups, except for facultative gram-negative rods (GNRs). As mentioned above, only microbes present in ≥5 subjects or at a ≥log 4.0 concentration were considered. No significant change in these vaginal microorganisms occurred between visit 3 (8–12 h after intercourse) and visits 1 (not shown) and 2 in the groups without or with condoms. When present, lactobacilli were recovered at ≥10<sup>5</sup> cfu/mL at all visits in the condom group (data not shown). In the no condom group, *Lactobacillus* organisms were recovered at ≥10<sup>5</sup> cfu/mL in all but 2 patients at visits 1–3 and in all but 1 patient at visit 4. We used logistic regression analysis to examine the presence of lactobacilli by visit number and by no condom and condom groups but found no difference in the prevalence of lactobacilli by visit between groups.

Table 4 shows comparisons of the presence (and, in parentheses, for elevated concentrations ≥10<sup>5</sup> cfu/mL of vaginal fluid)

**Table 3.** Vaginal microflora except for facultative gram-negative bacteria before and after an index episode of intercourse (IC) by groups that did not or did use condoms.

Group	Time related to IC <sup>a</sup>			
	Visit 2	Visit 3	Visit 4	Visit 5
<b>No condom use</b>				
No. of subjects	22	22	22	19
Total lactobacilli $\geq 10^5$	14 (65)	13 (59)	14 (64)	13 (68)
H <sub>2</sub> O <sub>2</sub> positive	6 (27)	7 (32)	8 (36)	7 (37)
H <sub>2</sub> O <sub>2</sub> negative	4 (18)	3 (14)	4 (18)	5 (26)
Non- <i>Lactobacillus</i> species $\geq 10^5$	15 (68)	17 (77)	14 (64)	13 (68)
<i>Candida albicans</i>	5 (23)	4 (18)	3 (14)	5 (26)
<i>Prevotella</i> species	7 (32)	8 (36)	11 (50)	9 (47)
Anaerobic gram-positive cocci	8 (36)	11 (50)	9 (41)	7 (37)
Gram's stain				
Bacterial vaginosis	4 (18)	5 (23)	3 (14)	2 (11)
Intermediate	1 (5)	1 (5)	4 (18)	1 (5)
<b>Condom users</b>				
No. of subjects	19	19	19	18
Total lactobacilli $\geq 10^5$	14 (74)	14 (74)	13 (68)	11 (61)
H <sub>2</sub> O <sub>2</sub> positive	11 (58)	10 (53)	10 (53)	10 (56)
H <sub>2</sub> O <sub>2</sub> negative	5 (26)	5 (26)	5 (26)	5 (28)
Non- <i>Lactobacillus</i> species $\geq 10^5$	8 (40)	11 (55)	13 (68)	12 (67)
<i>Candida albicans</i>	3 (15)	4 (20)	3 (16)	3 (17)
<i>Prevotella</i> species	6 (30)	4 (20)	3 (16)	9 (50)
Anaerobic gram-positive cocci	4 (20)	7 (35)	8 (42)	9 (50)
Gram's stain				
Bacterial vaginosis	2 (10)	2 (10)	1 (5)	4 (22)
Intermediate	3 (15)	2 (10)	5 (26)	2 (11)

NOTE. Data are no. (%) of subjects unless otherwise noted.

<sup>a</sup> Visit 2, 1–2 days before index IC; visit 3, 8–12 h after index IC; visit 4, 3–4 days after index IC; visit 5, 6–8 days after index IC.

of *E. coli* and of other enteric GNRs in vaginal fluid. In the no condom group, a significant increase occurred in the proportion of subjects with *E. coli* ( $P < .001$ ) and any GNRs ( $P < .001$ ) between visit 3 and visits 1 and 2. A significant linear decrease also occurred in the proportion of subjects with *E. coli* ( $P = .03$ ) and any GNRs ( $P = .01$ ) at visits 4 and 5, compared with that at visit 3. In the no condom group, more subjects also had a high concentration of *E. coli* ( $\geq 10^5$  cfu/mL) in vaginal fluid at visit 3 ( $n = 5$ ) than at visits 1 ( $n = 4$ ) and 2 ( $n = 1$ ;  $P = .001$ ).

In the condom group, a slight increase occurred in the proportion of subjects with vaginal *E. coli* at visit 3, compared with that at visits 1 and 2 ( $P = .06$ ), and there was a significant increase in the proportion of subjects with other vaginal enteric GNRs ( $P = .001$ ) and any GNRs (*E. coli* and other enteric GNRs) at visit 3, compared with that at visits 1 and 2 ( $P = .008$ ). In the condom group, no significant change occurred in the number of subjects with a high concentration of either *E. coli* or GNRs after intercourse.

Subjects were randomly assigned to the groups, and it was possible to address the question of whether vaginal *E. coli* after intercourse was significantly increased in the no condom group versus the condom group. We developed a logistic regression model to compare the presence of *E. coli* by visit number, study group (no condom or condom), and presence or absence of H<sub>2</sub>O<sub>2</sub>-producing lactobacilli. In these analyses, we confirmed that the prevalence of *E. coli* in the vagina was significantly

increased at visit 3, compared with that at visits 1 and 2 ( $P < .001$ ) and was significantly decreased at visits 4 and 5, compared with that at visit 3 ( $P = .03$ ). However, there was no significant difference in the prevalence of vaginal *E. coli* between the no condom and condom groups after intercourse ( $P = .5$ ).

These analyses also allowed us to examine whether lactobacilli in the vagina protected against *E. coli* colonization. High ( $\geq 10^5$  cfu/mL) concentration of H<sub>2</sub>O<sub>2</sub>-producing lactobacilli was not correlated with the presence of *E. coli* in the overall model ( $P = .7$ ), but there was an association between condom use, vaginal *E. coli*, and  $>10^5$  cfu/mL of H<sub>2</sub>O<sub>2</sub>-producing lactobacilli. In the no condom group, H<sub>2</sub>O<sub>2</sub>-producing lactobacilli were not associated with vaginal *E. coli* ( $P = .7$ ), whereas, in the condom group, H<sub>2</sub>O<sub>2</sub>-producing lactobacilli were associated with a decreased prevalence of vaginal *E. coli* ( $P = .01$ ).

Urine culture results are shown in table 5. In the no condom group, a statistically significant increase occurred in the proportion of subjects with *E. coli* at visit 3, compared with that at visits 1 and 2 ( $P = .004$ ). *E. coli* was the only uropathogen isolated from urine in the no condom group. Only 1 subject (in the no condom group at visit 1) had  $\geq 10^5$  cfu/mL. In the condom group, there was a slight, but not statistically significant, increase in the proportion of subjects with *E. coli* and any GNR in the urine at visit 3 compared with visits 1 and 2. All but 1 subject had *E. coli* in urine, and she had an *Enterobacter* organism in urine at visits 2 and 3.

## Discussion

The potential effects of vaginal intercourse on vaginal physiology are important to examine since STD and UTI are associated with

**Table 4.** Comparison of selected vaginal gram-negative rods (GNRs) before and after index episode of intercourse (IC) with and without a condom.

Group	Time related to index IC <sup>a</sup>					<i>P</i> <sup>b</sup>
	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	
<b>No condom use</b>						
No. of subjects	22	22	22	22	19	
<i>Escherichia coli</i>	6 (4)	3 (1)	10 (5) <sup>c</sup>	2 (1)	2 (0)	<.001/.03
Other GNRs	0	2 (1)	1 (1)	0	0	.5/.5
Any GNRs	6 (4)	5 (2)	10 (6) <sup>c</sup>	2 (1)	2 (0)	<.001/.01
<b>Condom users</b>						
No. of subjects	20	20	20	19	18	
<i>E. coli</i>	2 (1)	1 (1)	3 (1)	1 (1)	1 (1)	.06/.4
Other GNRs	0	0	3 (0)	2 (1)	1 (1)	.001/.4
Any GNRs	2 (1)	1 (1)	5 (1)	3 (2)	2 (2)	.008/.7

NOTE. Nos. in parenthesis are no. of subjects with  $\geq 10^5$  microorganisms/mL of vaginal fluid. Any GNRs, all gram-negative rods, including *E. coli*; other GNRs, all gram-negative rods, excluding *E. coli*.

<sup>a</sup> Visit 1, 30 days before index IC; visit 2, 1–2 days before index IC; visit 3, 8–12 h after index IC; visit 4, 3–4 days after index IC; visit 5, 6–8 days after index IC.

<sup>b</sup> *P* values for test 1/test 2. Test 1 compares whether visit 3 differs from visits 1 and 2. Test 2 assesses whether linear trend occurred among visits 3–5. Both tests use logistic regression for correlated data models.

<sup>c</sup>  $P < .001$ , no. of subjects with  $\geq 10^5$  microorganism/mL of vaginal fluid at visit 3 vs. at visits 1 and 2.

**Table 5.** Comparison of urinary gram-negative rods (GNRs) before and after an index episode of intercourse (IC) with and without a condom.

Group	Time related to index IC <sup>a</sup>					<i>P</i> <sup>b</sup>
	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	
No condom use						
No. of subjects	22	22	22	22	19	
<i>Escherichia coli</i>	3 <sup>c</sup>	1	7	2	2	.004/.3
Condom users						
No. of subjects	20	20	20	19	18	
<i>E. coli</i>	0	1	3	5	2	.08/.2
Any GNRs	0	2	4	5	2	.09/.5

<sup>a</sup> Visit 1, 30 days before index IC; visit 2, 1–2 days before index IC; visit 3, 8–12 h after index IC; visit 4, 3–4 days after index IC; visit 5, 6–8 days after index IC.

<sup>b</sup> *P* values for test 1/test 2. Test 1 compares whether visit 3 differs from visits 1 and 2. Test 2 assesses whether linear trend occurred among visits 3–5. Both tests use logistic regression for correlated data models.

<sup>c</sup> One subject had  $\geq 10^5$  cfu/mL of urine.

intercourse. Intercourse could lead to trauma of vaginal epithelium that, in turn, would increase the ability of microbes to attach, colonize, and cause infection. Furthermore, a comparison was needed of the effect of intercourse without and with a condom on the vaginal epithelium and vaginal flora. Although condoms protect against STD infectious agents, including HIV, an examination was needed for potential adverse effects of condoms, such as trauma, on the vaginal physiology [12].

We observed virtually no vaginal erythema or lesions of the vaginal epithelium after intercourse without or with condoms, although vulvar erythema increased in both groups. The mean number of epithelial cell layers and the number of subepithelial polymorphonuclear leukocytes were not altered by intercourse in either group. It was reassuring that intercourse produced no effect on the vaginal epithelium; however, assessment of trauma by more sensitive methods (e.g., hemoglobin measurement) is needed.

In addition, we observed no colposcopic evidence of intercourse-related trauma to the cervix in either the condom or no condom group. Ectopy consists of only a few cell layers of epithelium between the surface and blood vessels, compared with the 26–28 squamous cells layers in the vagina [22]. Oral contraception is associated with ectopy [24], and the area of cervical ectopy is the most likely area of the cervix to receive trauma during intercourse. However, these subjects had little ectopy (only 24% of the women had  $\geq 25\%$  ectopy), despite all but 1 using oral contraception to prevent pregnancy.

Lactobacilli use glycogen to produce lactic acid, which helps maintain both the low pH and the dominance of lactobacilli and other acidophilic bacteria in the vagina [25, 26]. The low pH of the vagina is also maintained by lactic acid production by vaginal epithelial cells. The pH of the vagina is reported to have a buffer system, so one would predict that semen with a pH of 7–8 in the vagina would be buffered back to the baseline pH, probably the reason that our subjects who did not use condoms had only a small insignificant increase in vaginal pH at 8–12 h after intercourse.

Intercourse with or without a condom had no effect on vaginal lactobacilli, which suggests that semen does not adversely impact vaginal colonization with lactobacilli. This is of interest, since the production of H<sub>2</sub>O<sub>2</sub> by lactobacilli appears to represent an important mechanism by which lactobacilli maintain their dominance over other vaginal flora [25]. The H<sub>2</sub>O<sub>2</sub> produced by lactobacilli may inhibit or kill other vaginal flora, particularly flora that lack or have low levels of H<sub>2</sub>O<sub>2</sub>-scavenging enzymes, such as catalase [26]. It is possible that H<sub>2</sub>O<sub>2</sub>-producing lactobacilli decrease the risk of HIV acquisition by directly killing free virus present in the vagina at intercourse, preventing bacterial vaginosis [25], which is associated with an increased risk of HIV acquisition [6, 8], and by helping to maintain a low pH in the vagina, which may inhibit HIV [27].

One of the most interesting findings in this study was a significant increase 8–12 h after intercourse in the proportion of subjects in the no condom group with *E. coli* isolated from the vagina and a lesser but significant increase in the proportion of subjects in the condom group with enteric GNRs in the vagina. These findings do not appear to be by chance. First, subjects were analyzed by logistic regression for correlated data in which the subjects were used as their own control subjects. The prevalence of *E. coli* and enteric GNRs at the 2 visits before the indexed episode of intercourse at visit 3 was relatively stable, and the prevalence of *E. coli* decreased significantly at visits 4 and 5, compared with that at visit 3 (8–12 h after intercourse). Second, a parallel increase in *E. coli* and enteric GNRs occurred in the urine of both groups.

Of interest, in the condom group, H<sub>2</sub>O<sub>2</sub>-producing lactobacilli appeared to suppress *E. coli* colonization, whereas, in the no condom group, H<sub>2</sub>O<sub>2</sub>-producing lactobacilli were not associated with reduced colonization with *E. coli*. These data support the previous report that H<sub>2</sub>O<sub>2</sub>-producing lactobacilli appear to decrease vaginal *E. coli* colonization in women with recurrent UTI [28]. These findings also suggest that intercourse without a condom introduces some male factor not present with condom use that reduces the suppressive effect of H<sub>2</sub>O<sub>2</sub>-producing lactobacilli on *E. coli* in the vagina.

These findings are compatible with previous findings that intercourse is associated with a transient increase of *E. coli* colonization in the vagina and urine [13, 29]. *E. coli* and other GNRs that enter the bladder presumably originate in the rectum and subsequently colonize the periurethral area. The apparently greater effect of intercourse in vaginal *E. coli* in the no condom than condom group was not statistically different by logistic regression analysis but nevertheless could be due to  $\geq 1$  of the following explanations. First, it could be because of the reduced effect of H<sub>2</sub>O<sub>2</sub>-producing lactobacilli on vaginal *E. coli* colonization in the no condom group. Second, it is possible that the male sexual contacts of the subjects in the no condom group carried *E. coli* in the urethra or, if uncircumcised, under the foreskin. *E. coli* in male sex partners has been shown to be identical to that causing urinary infection in their female part-

ners [29], although no causal association has been shown. Third, it is possible that intercourse without a condom (vs. intercourse with a lubricated condom) more readily carries *E. coli* from the periurethral area into the vagina and lower urinary tract. Intercourse with unlubricated condoms may increase the risk of urinary infections, and trauma has been speculated to be the cause [13]. Although trauma may be an explanation, in our study, vulvar erythema was present in both groups, and we found no differences in the no condom and condom groups in vaginal erythema, even as observed by colposcopy. Furthermore, *E. coli* adheres to vaginal epithelial cells within minutes in an in vitro model, and it is possible that the skin of the penis allows for more adherence of bacteria than a condom surface.

In summary, vaginal intercourse both without and with a condom produced no discernable effect on vaginal or cervical epithelium and produced only erythema on vulvar epithelium. It was reassuring that 8 h after vaginal intercourse, no effect was found on vaginal pH, vaginal lactobacilli, or other normal vaginal flora. However, it appears that the mechanical effect of vaginal intercourse acts to introduce *E. coli* and other enteric bacteria into the vagina and lower urinary tract. These data are consistent with previous reports in which vaginal intercourse was associated with UTI in women. It will be important in longer studies to randomize couples to condom and no condom use to determine whether consistent condom use can significantly reduce the entry of *E. coli* and other enteric bacteria into the vagina and urine after intercourse.

## References

- Royce RH, Sena A, Cates W Jr, Cohen MS. Sexual transmission of HIV. *N Engl J Med* **1997**;336:1072–8.
- Downs AM, de Vincenzi I. Probability of heterosexual transmission of HIV: relation to number of unprotected sexual contacts. *J Acquir Immune Defic Syndr Hum Retrovirol* **1996**;11:388–95.
- Nicolosi A, Correa Leite ML, Musicco M, Arici C, Gavazzeni G, Lazzarin A. The efficiency of male-to-female and female-to-male sexual transmission of the human immunodeficiency virus: a study of 730 stable couples. Italian Study Group on HIV Heterosexual Transmission. *Epidemiology* **1994**;5:570–5.
- de Vincenzi I. A longitudinal study of human immunodeficiency virus transmission by heterosexual partners. European Study Group on Heterosexual Transmission of HIV. *N Engl J Med* **1994**;331:341–6.
- Guimaraes MD, Munoz A, Boschi-Pinto C, Castillo EA. HIV infection among female partners of seropositive men in Brazil. Rio de Janeiro Heterosexual Study Group. *Am J Epidemiol* **1995**;142:538–47.
- Laga M, Manokoa A, Kivuvu M, et al. Non-ulcerative sexually transmitted diseases as risk factors for HIV-1 transmission in women: results from a cohort study. *AIDS* **1993**;7:95–102.
- Taha TE, Hoover DR, Dallabetta GA, Kumwenda NI, Mtimalvalye LA, Yang LP. Bacterial vaginosis and disturbances of vaginal flora: association with increased acquisition of HIV. *AIDS* **1998**;12:1699–706.
- Martin HL, Nyange PM, Richardson BA, et al. Hormonal contraception, sexually transmitted diseases, and risk of heterosexual transmission of human immunodeficiency virus type 1. *J Infect Dis* **1998**;178:1053–9.
- Martin HL, Richardson BA, Nyange PM, Lavreys L, Hillier SL, Chohan B. Vaginal lactobacilli, microbial flora and risk of immunodeficiency virus type 1 and sexually transmitted disease acquisition. *J Infect Dis* **1999**;180:1863–8.
- Fihn SD, Boyko EJ, Chen CL, Normand EH, Yarbzo P, Scholes D. Association between use of spermicide-coated condoms and *Escherichia coli* urinary tract infection in young women. *Am J Epidemiol* **1996**;144:512–20.
- Fihn SD, Boyzo EJ, Chen CL, Normand EH, Yarbzo D, Scholes D. Use of spermicide-coated condoms and other risk factors for urinary tract infection caused by *Staphylococcus saprophyticus*. *Arch Intern Med* **1998**;158:281–7.
- Foxman B, Marsh J, Gillespie B, Rubin N, Koopman JS, Spear S. Condom use and first time urinary tract infection. *Epidemiology* **1997**;8:637–41.
- Hooton TM, Hillier S, Johnson C, Roberts PL, Stamm WE. *Escherichia coli* bacteriuria and contraceptive method. *JAMA* **1991**;265:64–9.
- Kreiss J, Ngugi E, Holmes K, Ndinya-Achola J, Waiyaki P, Roberts PL. Efficacy of nonoxynol 9 contraceptive sponge use in preventing heterosexual acquisition of HIV in Nairobi prostitutes. *JAMA* **1992**;268:477–82.
- Zekeng L, Feldblum PJ, Oliver RM, Kaptive L. Barrier contraceptive use and HIV infection among high-risk women in Cameroon. *AIDS* **1993**;7:725–31.
- Roddy RE, Zekeng L, Ryan KA, Tamoufe U, Weir SS, Wong EL. A controlled trial of nonoxynol-9 film to reduce male-to-female transmission of sexually transmitted diseases. *N Engl J Med* **1998**;339:504–10.
- Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *J Clin Microbiol* **1991**;29:297–301.
- Hillier SL, Krohn MA, Rabe LK, Klebanoff SJ, Eschenbach DA. Normal vaginal flora, H<sub>2</sub>O<sub>2</sub>-producing lactobacilli and bacterial vaginosis in pregnant women. *Clin Infect Dis* **1993**;16(Suppl 4):S273–81.
- Eschenbach DA, Davick PR, Williams BL, et al. Prevalence of hydrogen peroxide-producing *Lactobacillus* species in normal women and women with bacterial vaginosis. *J Clin Microbiol* **1989**;27:251–6.
- Stamm WE, Tam MR, Koester M, Cles L. Detection of *Chlamydia trachomatis* inclusions in McCoy cell cultures with fluorescein-conjugated monoclonal antibodies. *J Clin Microbiol* **1983**;17:666–8.
- Miller L, Patton D, Meier A, Thwin SS, Hooton TM, Eschenbach DA. Depomedroxyprogesterone-induced hypoestrogenism and changes in vaginal flora and epithelium. *Obstet Gynecol* **2000**;96:431–9.
- Patton DL, Thwin SS, Hooton TM, Stapleton AE, Eschenbach DA. Cell layers and immune cells in vaginal epithelium at three times in the normal menstrual cycle. *Am J Obstet Gynecol* **2000**;183:967–73.
- Disser PJ, Liang KY, Zeger SL. Analysis of longitudinal data. Oxford: Oxford University Press, **1994**:146–68.
- Critchlow CW, Wølner-Hanssen P, Eschenbach DA, et al. Determinants of cervical ectopy and of cervicitis: age, oral contraception, specific cervical infection, smoking, and douching. *Am J Obstet Gynecol* **1995**;173:534–43.
- Hawes SE, Hillier SL, Benedetti J, Stevens CE, Koutsky LA, Wølner-Hanssen P. Hydrogen peroxide producing lactobacilli and acquisition of vaginal infection. *J Infect Dis* **1996**;174:1058–63.
- Klebanoff SJ, Hillier SL, Eschenbach DA, Waltersdorph AM. Control of the microbial flora of the vagina by H<sub>2</sub>O<sub>2</sub>-generating lactobacilli. *J Infect Dis* **1991**;164:94–100.
- O'Connor TJ, Kinchington D, Kangro HO, Jeffries DJ. The active candidate viricidal agents, low pH and genital secretions against HIV-1 in vitro. *Int J STD AIDS* **1995**;6:267–72.
- Gupta K, Stapleton AE, Hooton TM, Roberts PL, Fennel CL, Stamm WE. Inverse association of H<sub>2</sub>O<sub>2</sub>-producing lactobacilli and vaginal *Escherichia coli* colonization in women with recurrent urinary tract infections. *J Infect Dis* **1998**;178:446–50.
- Foxman B, Zhang L, Tallman P, Andree BC, Geiger AM, Koopman JS. Transmission of uropathogens between sex partners. *J Infect Dis* **1997**;175:989–92.