CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, May 1997, p. 367–374 Vol. 4, No. 3
1071-412X/97/$04.00+0

In Vivo Semen-Associated pH Neutralization of Cervicovaginal Secretions

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Received 30 September 1996/Accepted 3 February 1997

Physiological cervicovaginal acidity can partly inactivate human immunodeficiency virus (HIV). Basic semen components should be able to partially neutralize in vivo cervicovaginal pH. The goals of the study were to evaluate the relationship between cervicovaginal pH and presence of semen components in sexually active African women and to assess whether vaginal douching with water performed just after sexual intercourse could significantly reduce semen components and restore physiological cervicovaginal pH. Cervicovaginal secretion (CVS) from 56 heterosexual African women (19 to 45 years old), living in Bangui, Central African Republic, were evaluated for pH, semen components (prostatic acid phosphatase [PAP] and prostatic specific antigen [PSA]), cellularity, and hemoglobin at inclusion and after vaginal douching with 100 ml of water by using a bock. Before douching, semen components were found in 46 of 56 CVS (82%). The mean vaginal pH was 5.2 (range, 3.6 to 7.7), and concentrations of both PAP and PSA correlated positively and strongly with cervicovaginal pH ($P < 0.001$). After douching, semen components were found in 35 of 56 CVS (62%) ($P = 0.03$). Cervicovaginal PAP and PSA levels were significantly decreased (respectively, $P < 0.0001$ and $P < 0.01$; PAP, −72%; PSA, −87%), as was the total cell count (−60%; $P < 0.0001$). Furthermore, in CVS previously positive for both PAP and PSA, the mean vaginal pH was significantly decreased (6.5 versus 5.3, $P < 0.01$); no genital bleeding was observed. Frequent persistence of semen in CVS from heterosexually active African women leads to a shift from acidity to neutrality that could favor male to female HIV transmission. Vaginal douching provides significant elimination of semen after sexual intercourse; it should be considered for study as a supplementary means for the prevention of heterosexual HIV transmission.

Infection by human immunodeficiency virus type 1 (HIV-1) has been spreading at a high rate among heterosexual populations in the developing world, especially in sub-Saharan Africa, where about 90% of HIV infections have been transmitted by heterosexual intercourse. Despite the identification of numerous cofactors for heterosexual HIV transmission, including multiple sexual partners and sexually transmitted diseases, this striking predominance of heterosexual transmission in sub-Saharan Africa remains incompletely understood.

Among factors that could modulate heterosexual HIV transmission, little attention has been given to the physicochemical interactions between male and female genital secretions. The cervicovaginal pH is physiologically acidic, because the Döderlein flora produces lactic acid from glycogen through fermentation (11). Vaginal acidity accounts for a great part of the nonspecific defenses of the vagina. Both cell-free and cell-associated HIV have been shown in vitro to be either completely (14) or partially (13) inactivated by the pH values found at the entry site of the female genital tract. The semen contains both azoted bases such as spermine from prostate and phosphoryl choline from seminal vesicles, which is dephosphorylized to choline by prostatic acid phosphatase (PAP). In vivo semen-associated neutralization of cervicovaginal pH could be favorable to the virus by hampering the nonspecific antiviral defenses and, therefore, could increase male to female HIV transmission. In an animal model, the presence of seminal plasma in the vaginal tract of nonhuman primates dramatically favored intravaginal inoculation with the simian immunodeficiency virus, likely because of neutralization of vaginal acidity (9, 10).

Previously, we reported that African women sexually exposed to HIV may have semen-borne antibodies to HIV in their cervicovaginal fluids, indicating the persistence of semen components in the vaginal tract after sexual intercourse (1, 2, 4). This finding led us to investigate the persistence of semen as a possible cofactor of male to female transmission of HIV, via an increase of the local pH, in women living in the Central African Republic. In this country, high-risk sexual behavior is very frequent among adults, and HIV infection is highly prevalent (19). We further investigated if vaginal douching with water, performed within hours following sexual intercourse, could significantly eliminate the postintercourse traces of semen and thereby restore the cervicovaginal fluids to a more acidic pH, which would be deleterious to HIV.

MATERIALS AND METHODS

Study population. Fifty-six African women, attending the National Center for Sexually Transmitted Diseases and AIDS in Bangui, Central African Republic, were prospectively included in this study during March and April 1995. Thirty of them were being consulted for genital symptoms, 10 for contraception, 5 for sterility check-up, and 11 for voluntary HIV testing. Their mean age was 26 years (range, 19 to 45 years). All were exclusively heterosexual. Pregnant women, menstruating women, and those with genital bleeding were excluded. None of the included women had used condoms and spermicide in the seven previous

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days nor had any of them practiced vaginal douching. All of them had one or more sexual partners.

Women underwent a standardized face-to-face interview conducted by one female medical practitioner who spoke the national language. The women were asked questions about their demographic characteristics, number of regular sexual partners in the previous year, and prior history of genital ulcer. During the interview, they were also fully briefed on the aims of the proposed study and on the practice of vaginal douching, in order to obtain informed consent as well as good compliance with the protocol.

Serum and cervicovaginal samplings. For each consenting woman, a blood sample was obtained, and the serum was separated. A vaginal smear for Gram staining was obtained by rolling a swab across the vaginal lateral wall, and the swab was then rolled onto a glass slide. Specimens from the posterior fornix and from the endocervix were obtained for wet mount microscopy and microbiological study. Cervicovaginal secretions were collected by a nontraumatic, standardized 1-min vaginal washing with 1 ml of physiologic saline (NaCl ‰), corresponding approximately to a 10-fold dilution of the genital secretions (3). Physiologic saline was chosen for this vaginal washing, to avoid influencing the vaginal pH estimation. Afterwards, a vaginal douching was carried out by a physician (C. T.-B.) using a bock (Marvel; Santé et Beauté, St Jean de Braye, France) filled with 100 ml of water. Water was selected because it is generally available and can be assumed to be innocuous. A second collection of cervicovaginal secretions obtained by vaginal washing with 1 ml of saline was undertaken, on average 90 min after vaginal douching. The cervicovaginal secretions were centrifuged within 1 h at laboratory temperature, at 1,500 × *g* for 10 min, to remove cells and mucus, and the supernatants were aliquoted. Paired sera and cervicovaginal fluids were kept frozen at −30°C until processing.

Serodiagnosis of HIV infection. Serodiagnosis of HIV infection was assessed by enzyme-linked immunosorbent assay (ELISA) according to a strategy recommended by the World Health Organization for use in developing countries (24). Antibodies were detected by a second-generation ELISA (Enzygnost Anti-HIV 1/2 Plus; Behring, Marburg, Germany); all positive sera were then confirmed with another second-generation ELISA (Genelavia Mixt; Sanofi-Diagnostics Pasteur, Marnes-La-Coquette, France). A serum positive by both tests was considered HIV positive.

Microbiological study. *Trichomonas vaginalis* was identified by wet mount microscopy. Bacterial vaginosis was diagnosed by microscopic examination of Gram-stained vaginal smears and was defined by a score above 7 according to the scoring method of Nugent (12). *Neisseria gonorrhoeae* was isolated on modified Thayer-Martin medium and identified on the basis of typical colony morphology. Vaginal candidiasis was diagnosed by culture of vaginal secretions on Sabouraud medium. *Chlamydia trachomatis* antigen was detected by indirect microimmunofluorescence. Vaginal discharge without evidence of microorganisms at microbiological evaluation was defined as amicrobial vaginosis.

Laboratory evaluation of cervicovaginal secretions. The presence of semen components, the level of pH, the cellularity, and the detection of hemoglobin traces were estimated in the cervicovaginal secretions collected at inclusion and after vaginal douching. Two semen markers, the prostatic specific antigen (PSA) and the PAP, were quantified in the cervicovaginal secretions (supernatant) by using commercially available immunoenzymatic tests (Tumors Markers, IMX System; Abbott, Chicago, Ill.). According to the prescriptions of the manufacturer, the threshold of positivity for both markers was set at 100 ng/ml, and the reference curves were drawn from dilutions of positive controls measured simultaneously. The pH of each cervicovaginal secretion (supernatant) was measured at room temperature (22°C) with a pH meter (MicropH 2001; Crison, Barcelona, Spain) calibrated with pH 4 and pH 8 reference solutions (Heito, Paris, France). Dead cells were stained by trypan blue, and the remaining epithelial cells and inflammatory cells were counted in each cervicovaginal secretion (pellet) with a Kova slide. Traces of hemoglobin in cervicovaginal secretions (supernatant) were detected by a spectrophotometric technique previously validated for saliva (16); the sensitivity of detection was 0.5 μg of hemoglobin per 100 μl.

In vitro capacity of seminal plasma to neutralize cervicovaginal pH. The capacity of seminal plasma to neutralize cervicovaginal pH was investigated by addition of increasing volumes of a pool of five seminal plasma samples, 100-fold diluted in distilled water, to a constant volume (1,000 μl) of a pool of 15 semen-free cervicovaginal fluids. Measurements of pH were carried out immediately after mixing of genital secretions.

Ex vivo evaluation of sensitivity of HIV-1 to cervicovaginal secretions. One clinical isolate from an HIV-1-infected patient was obtained by a standard technique of cocultivation of peripheral blood mononuclear cells (PBMC) with HIV-negative donor PBMC, which had been previously activated for 48 h with phytohemagglutinin (PHA). Cell-free virus infectivity was quantified by end-point dilution in cell culture and expressed in 50% tissue culture infective doses ($TCID_{50}$) per ml. This HIV-1 isolate was previously found to be non-syncytium inducing on MT2 cells. Two pools of cervicovaginal secretions (supernatants) were constituted: (i) five normal cervicovaginal secretions without detectable seminal plasma (pH of the pool, 3.5) and (ii) five cervicovaginal secretions from women of group I (see below) collected 90 min after vaginal douching (pH 5.3). Pools were passed through a 20-μm-pore-size filter to eliminate bacteria. One-hundred microliters of cell-free viral stock at 100 $TCID_{50}$ per ml was incubated with 200 μl of each pool for 2 h at 37°C, in quadruplicate, on a 24-well plate. A mixture of 100 μl of cell-free viral stock incubated (in triplicate) for 2 h at 37°C with 200 μl of RPMI 1640 at pH 3.5, or with 200 μl of RPMI 1640 at pH 7.0, served as a low pH control and as a culture control, respectively; 300 μl of RPMI 1640 served as negative control (in simplicate). Then, 3×10^6 PBMC from normal donors (previously activated with PHA), in RPMI 1640 supplemented with 2 mM L-glutamine, 15% decomplemented fetal bovine serum, 100 IU of penicillin/ml, 50 μg of streptomycin/ml, and 10% interleukin-2, were added to each well at a final volume of 1.5 ml. The mixture was incubated at 37°C under 5% of CO_2. A 100-μl culture supernatant aliquot was sampled every day for 4 days (D0, D1, D2, and D3) and stored at −30°C. Detection of p24 antigen was performed in these supernatants (diluted 1:25 in phosphate-buffered saline) by using the same kit of immunocapture ELISA (HIVAG-1 Monoclonal, Abbott); the results were expressed in optical density (OD) units. For each day of culture, an inhibition index (I_d) for HIV replication was calculated according to the following formula: $I_d = (OD_{D0} - OD_{Di})/OD_{Di}$, with Di = D1, D2, or D3.

By using a system of cocultivation with donor PBMC, we were not able to evaluate the replication of cell-free HIV-1 after exposure to semen-positive cervicovaginal secretions from women of group I. Indeed, human spermine coagulates with fetal bovine serum (13); furthermore, semen provides per se a powerful inhibitory effect on HIV coculture because spermine suppresses the PHA-induced proliferation of T lymphocytes (17).

Statistical analysis. The Mann and Whitney U test, the rank order Wilcoxon test, the Fisher exact test, and the Spearman's correlation test were used for statistical analysis. Quantitative results are expressed as means ± standard errors.

TABLE 1. Demographic characteristics, sexual behavior, and history of genital ulcer disease in 56 African women attending the National Center for Sexually Transmitted Diseases and AIDS (Bangui), according to their serological status for HIV infection

Characteristic	No. of individuals		Significance for HIV status (*P*)
	HIV positive ($n = 13$)	HIV negative ($n = 43$)	
Educational level			NS[a]
Primary	5	15	
College	8	28	
Marital status			NS
Never married	12	40	
Married	1	3	
Occupation			NS
Housewife	5	19	
Student or worker	8	20	
Self-employed	0	4	
No. of sexual partners in the past year			NS
1	9	31	
>1	4	12	
History of genital ulcer disease	7	10	0.04
Age (yr)[b]	28 ± 1.5	27 ± 1.0	NS

[a] NS, not significant.
[b] Data are means ± standard error of the mean.

RESULTS

Study population. Among the 56 included women, 13 (23%) were seropositive for HIV-1 infection. Demographic characteristics, sexual behavior, and medical history of HIV-seropositive and HIV-seronegative women are compared in Table 1. Only a previous history of genital ulcer was significantly associated with HIV positivity ($P < 0.05$). The majority of included women, 41 of 56 (74%), had some type of genital infection (Table 2).

Cervicovaginal PAP and PSA and vaginal pH at inclusion. The stabilities of PSA and PAP were first evaluated at different pH levels in order to test the validity of their estimations in cervicovaginal fluids. The measurements of both PSA and PAP were not significantly affected by their exposure for 24 h to pH levels between 4.5 and 7.2, i.e., corresponding to the values usually observed in the cervicovaginal secretions (Fig. 1). At inclusion, the PAP or PSA could be detected in 48 of 56 (86%)

TABLE 2. Microbiological evaluation of cervicovaginal samples

Cervicovaginal sample	No. of samples (%)
Normal	15 (27)
Abnormal	41 (73)
Bacterial vaginosis	21 (37)
Sexually transmitted diseases	10 (18)
N. gonorrhoeae	3
T. vaginalis	1
C. trachomatis	6
Vaginal candidiasis	4 (7)
Amicrobial vaginosis	6 (11)

cervicovaginal fluids, the cervicovaginal concentrations of PAP being in general higher than those of PSA (Table 3). The mean cervicovaginal pH was only slightly acidic (Table 3). A positive correlation was found between cervicovaginal pH and the cervicovaginal concentrations of PAP ($P < 0.001$) and PSA ($P < 0.001$) (Fig. 2). The mean cervicovaginal pH in women with genital infection was significantly higher (5.40 ± 0.13; range, 4.06 to 7.73) than that in women with normal vaginal flora (4.72 ± 0.16; range, 3.60 to 5.94) ($P < 0.01$) (Fig. 3). After stratification according to the presence or absence of genital infection, positive correlations between the values of the cervicovaginal pH and PAP as well as PSA concentrations were still observed in women without genital infection ($P < 0.007$ for PAP and $P < 0.0001$ for PSA) and in those with genital infection ($P = 0.01$ for PAP and $P = 0.015$ for PSA). Since the cervicovaginal pH also varies physiologically according to the menstrual cycle, we verified that the correlations between the concentrations of semen markers and the cervicovaginal pH were independent of the date of the menstrual cycle (data not shown). These features strongly suggested a direct causality between the levels of the cervicovaginal pH and the concentrations of semen components, independent of other confounding factors such as genital infection or the date of the menstrual cycle.

Three different groups could easily be delineated according to the cervicovaginal levels of the semen components. In group I ($n = 10$), the cervicovaginal concentrations of both semen markers were above 100 ng/ml, whereas these levels were below 100 ng/ml in group II ($n = 8$), in which seminal plasma was not detected in cervicovaginal secretions. In group III ($n = 38$), the levels of PAP and PSA were discrepant, with PSA being undetectable. In group I, levels of PAP and PSA were both high, with those of PAP (382 ± 10 ng/ml; range, 327 to 426 ng/ml) being higher than those of PSA (275 ± 24 ng/ml; range, 152 to 383 ng/ml) ($P < 0.01$). In group III, the mean cervicovaginal concentration of PAP (244 ± 10 ng/ml; range, 148 to 376 ng/ml) was much lower than that of group I ($P < 0.0001$). Measurement of the cervicovaginal pH showed clear-cut differences between group I (6.47 ± 0.24; range, 5.30 to 7.73) and group II (4.25 ± 0.14; range, 3.60 to 4.89) ($P < 0.005$), between group I and group III (5.14 ± 0.10; range, 4.15 to 6.30) ($P < 0.0001$), and between group II and group III ($P < 0.01$) (Fig. 4). Nevertheless, the pH range was dispersed in all groups, particularly in the semen-positive group I, which included some acid values, and in group III, in which the pH range likely corresponded to a lower or former semen contamination.

Cervicovaginal PAP and PSA and vaginal pH after vaginal douching. After douching with water, semen components were less frequently detected, being detected in 35 of 56 (62%) cervicovaginal fluids ($P = 0.03$), and cervicovaginal PAP and PSA concentrations were significantly decreased (respectively, $P < 0.0001$ and $P < 0.01$) (Table 3). The mean cervicovaginal pH also showed a trend to be decreased (5.02 ± 0.07), but the difference from the mean value before douching did not reach statistical significance (Table 3). In fact, the influence of vaginal douching on vaginal retention of semen and on pH variation was more obvious in groups I and III. Thus, in group I, cervicovaginal secretions became more acidic after douching (pH 5.36 ± 0.17; $P < 0.01$) (Fig. 5), and the mean concentrations of PAP and PSA decreased significantly (PAP, 229 ± 57 ng/ml; $P < 0.02$; PSA, 161 ± 47 ng/ml; $P < 0.004$). In group III, the mean concentration of PAP also decreased significantly (PAP, 138 ± 14 ng/ml; $P < 0.0001$), and the mean cervicovaginal pH was slightly decreased (4.91 ± 0.07) but not significantly. By contrast, the mean cervicovaginal pH in group II increased significantly after douching (4.64 ± 0.43; $P < 0.05$) (Fig. 5).

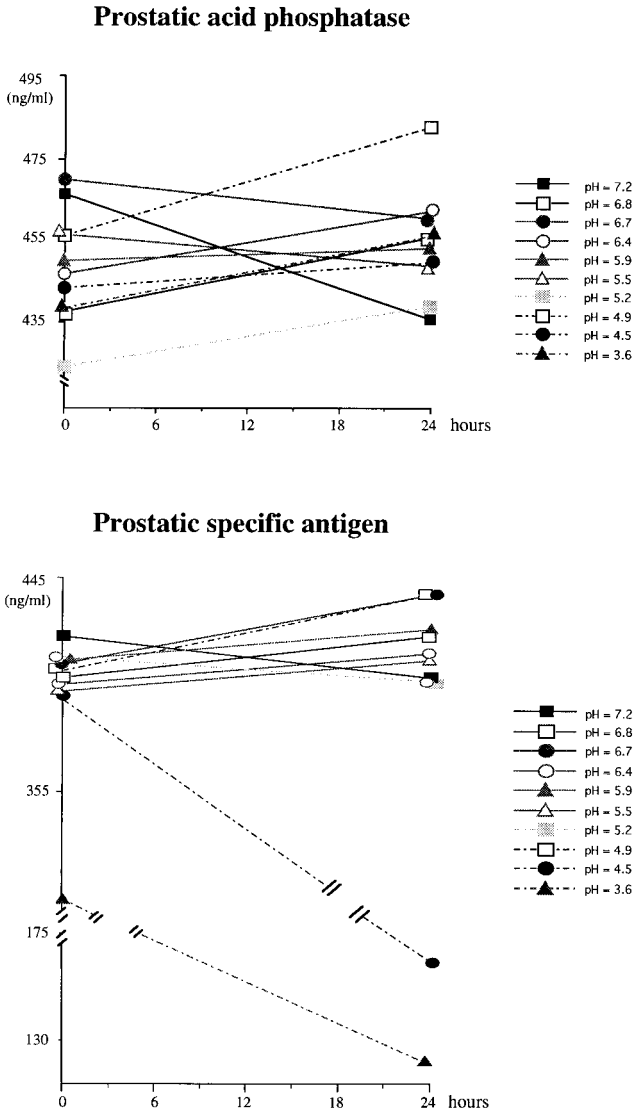

FIG. 1. Immuno-enzymatic detection of PAP (upper) and PSA (lower) in a pool of five seminal plasmas, diluted 1:100 in distilled water, in which increasing volumes of a 1 N H_2SO_4 solution were added to obtain indicated final pH values immediately and after a 24-h incubation.

Cellularity and hemoglobin traces at inclusion and after douching. Dead, epithelial, and inflammatory cell counts in

TABLE 3. Biological parameters (means ± standard errors) in cervicovaginal washings from 56 African women before and after vaginal douching

Time of sample collection	pH (range)	PAP [ng/ml (range)]	PSA [ng/ml (range)]	Cellularity (10^6/ml)			
				No. of cells (range)	No. of dead cells	No. of epithelial cells	No. of inflammatory cells
Inclusion	5.19 ± 0.11 (3.60–7.73)	235 ± 16 (0–425)	53 ± 15 (0–382)	3.2 ± 0.24 (0.7–8.4)	2.3 ± 0.17	0.3 ± 0.05	0.4 ± 0.07
After douching	5.02 ± 0.07 (4.00–6.62)	135 ± 119 (0–437)	31 ± 12 (0–347)	1.3 ± 0.11 (0.3–5.1)	0.9 ± 0.09	0.2 ± 0.03	0.1 ± 0.02
P^a	NS[b]	<0.0001	0.002	<0.0001	<0.0001	<0.01	0.0001

[a] Wilcoxon rank order test.
[b] NS, not significant.

cervicovaginal secretions decreased significantly after douching ($P < 0.0001$) (Table 3). The prevalence of cervicovaginal secretions with detectable traces of hemoglobin at inclusion was similar to that found after douching (1 of 56 versus 2 of 56). The HIV-infected women did not differ from HIV-negative women, according to the levels of cervicovaginal pH, semen components, and cellularity, at inclusion or after vaginal douching.

Capacity of seminal plasma to neutralize cervicovaginal pH in vitro. Investigation of the buffering strength of seminal plasma showed that a small amount of semen was sufficient to neutralize cervicovaginal acidity. The resulting curve typified the neutralization of a weak acid by a weak base (Fig. 6).

Ex vivo evaluation of sensitivity of HIV-1 to cervicovaginal secretions. The exposure of cell-free virus to semen-free acidic cervicovaginal secretions, to cervicovaginal secretions collected after vaginal douching from women who had had recent sexual intercourse, and to low pH medium resulted in a transitory decrease of HIV replication, in contrast with the level of replication induced by untreated virus (Fig. 7). The degree of inhibition of viral replication appeared to be pH dependent and was more pronounced for the more acidic pool of cervicovaginal secretions. We verified that a 2-h exposure of donor PBMC at pH 3.5 did not significantly affect their viability, which remained over 95%.

Mathematical approach. Assuming that the quantity of semen eliminated from the vagina each time is proportional to the cervicovaginal concentration of semen and that the vaginal production of lactic acid after sexual intercourse does not

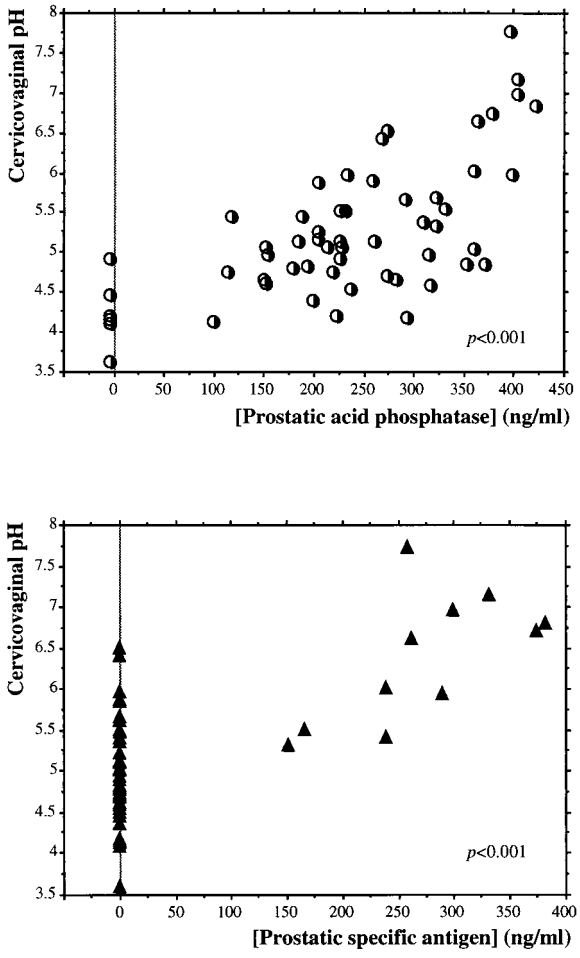

FIG. 2. Distribution of cervicovaginal pH according to cervicovaginal concentrations of PAP (upper) and PSA (lower) in 56 African women at risk for heterosexual HIV transmission.

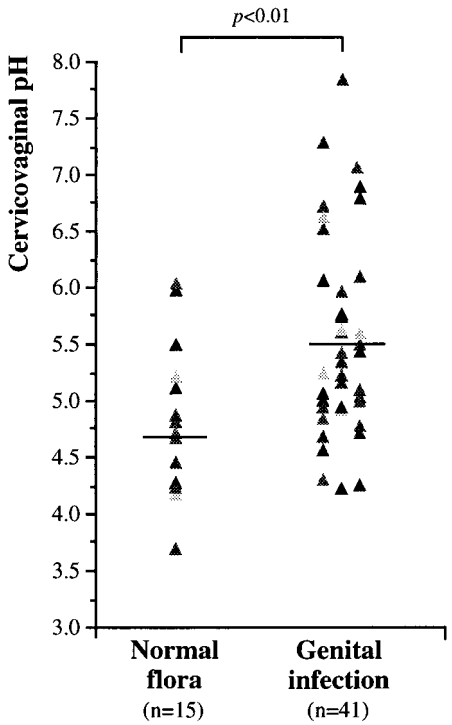

FIG. 3. Distribution of cervicovaginal pH in 56 African women according to the absence or presence of genital infection.

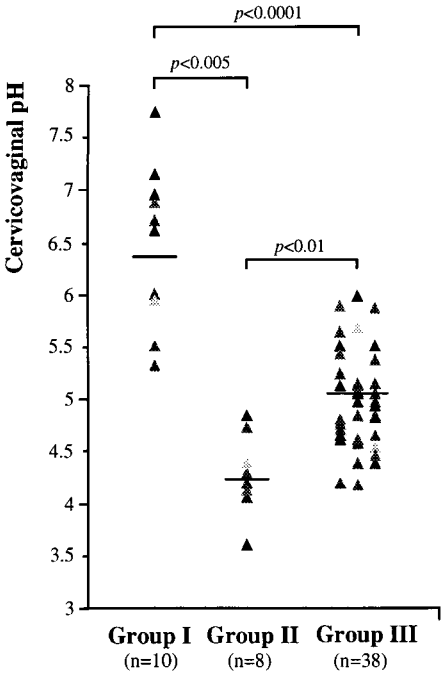

FIG. 4. Cervicovaginal pH at inclusion among women from semen-positive groups I (PAP and PSA concentrations of >100 ng/ml) and III (PAP concentration of >100 ng/ml and PSA concentration undetectable) and from semen-free group II. The horizontal lines represent the means of the distributions.

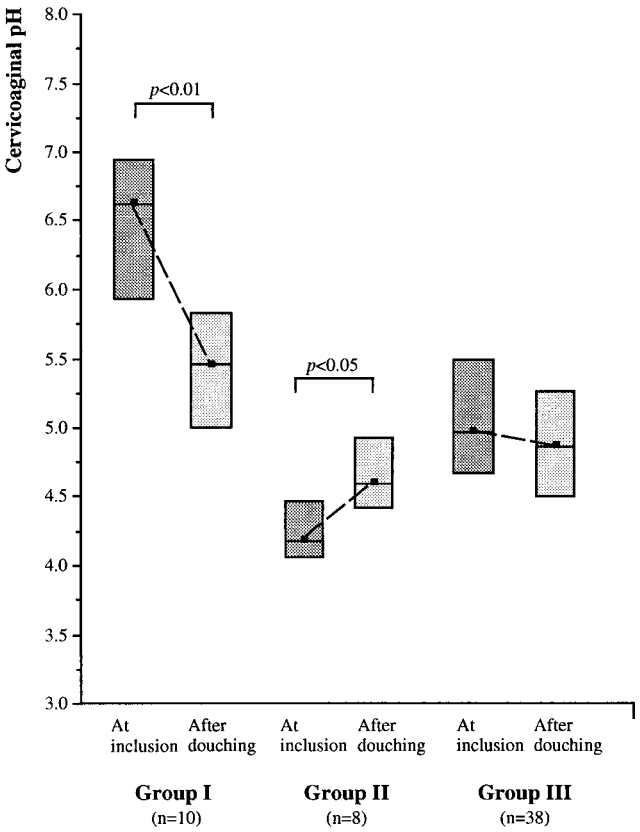

FIG. 5. Variations of cervicovaginal pH, at inclusion and 90 min after vaginal douching with 100 ml of water, among women from semen-positive groups I and III and from semen-free group II. Note the dramatic decrease of cervicovaginal pH after douching in women from group I, in whom high cervicovaginal concentrations of semen components at inclusion demonstrate recent sexual intercourse. Results are shown as boxes including interquartiles, and the horizontal lines represent the medians of the distributions.

significantly alter the neutralizing capacity of semen azoted bases, the residual quantity, $q(t)$, of semen in cervicovaginal secretions follows a decreasing exponential function (Fig. 8), $q(t) = V \cdot c(0) \cdot e^{-kt}$, where V is the volume of cervicovaginal secretions and $c(0)$ is the mean cervicovaginal concentration of semen, just after an ejaculation.

The cervicovaginal pH appears directly related to $q(t)$:

$$\mathrm{pH}(t) = \alpha \cdot q(t) = \alpha \cdot V \cdot c(0) \cdot e^{-kt} \qquad (1)$$

where α and k are constants.

After sexual intercourse, the cervicovaginal pH shifts from acidity ($\mathrm{pH_{min}}$) to neutrality ($\mathrm{pH_{max}}$): $\mathrm{pH_{max}} = \alpha \cdot V \cdot c(0)$ and $\mathrm{pH_{min}} = \alpha \cdot V \cdot c(0) \cdot e^{-kt_m} = \mathrm{pH_{max}}\, e^{-kt_m}$, where t_m is the time at which the semen traces are no longer detectable. Thus, $k = (1/t_m) \cdot \ln(\mathrm{pH_{max}}/\mathrm{pH_{min}})$.

At a time T, the cervicovaginal pH_T reaches the threshold under which HIV is assumed to be inactivated:

$$\mathrm{pH}_T = \alpha \cdot V \cdot c(0) \cdot e^{-kT},$$

$$\text{and } T = (1/k) \cdot \ln[\alpha \cdot V \cdot c(0)/\mathrm{pH}_T],$$

$$T = (1/k) \cdot \ln(\mathrm{pH_{max}}/\mathrm{pH}_T),$$

$$T = t_m \cdot \ln(\mathrm{pH_{max}}/\mathrm{pH}_T)/\ln(\mathrm{pH_{max}}/\mathrm{pH_{min}}) \qquad (2)$$

We can further apply this mathematical model according to our results and those reported in the literature to evaluate the time T during which the cervicovaginal secretions after sexual intercourse are not able to inactivate HIV because of their high pH. t_m is approximated at about 48 h by Haimovici and Anderson (7). The value of pH_T has been variably estimated in vitro at 4.0 (13) or 5.7 (14); we can approximate pH_T by taking the mean of both reported values, e.g., at about 4.8. The value of $\mathrm{pH_{max}}$ can be estimated by the mean of the distribution of the cervicovaginal pH in group I (6.5). Finally, the value of $\mathrm{pH_{min}}$ can be estimated at 3.5 as reported by Fox and colleagues (5). Using these values, T can be approximated by equation 2 as about 23.5 h. After a postcoital vaginal douching, $\mathrm{pH_{max}}$ can be estimated by using the mean of the distribution of the cervicovaginal pH in group I after douching (5.3); T' decreases then to 11.5 h. Finally, according to this mathematical approach, the time during which the pH of cervicovaginal secretions is favorable to viral replication is shortened by a factor of 2 by the use of a simple vaginal douching after sexual intercourse.

DISCUSSION

In investigating the influence of vaginal retention of semen on cervicovaginal pH in African women at high risk for heterosexual HIV transmission, a frequent persistence of semen was observed, leading the cervicovaginal fluid pH to rise from acidity to neutrality. After recent sexual intercourse, the cervicovaginal pH correlated positively with the cervicovaginal concentrations of semen components, independently of the presence of genital infection or dysmicrobism and of the date of the menstrual cycle.

The increase in cervicovaginal pH involves both seminal and vaginal parameters, which can vary according to the respective volumes of each fluid and to the duration of the intravaginal

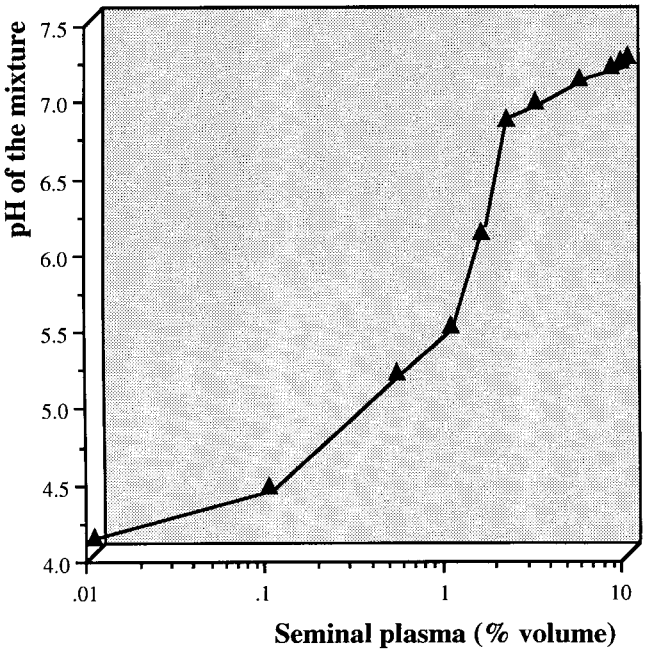

FIG. 6. In vitro neutralization curve of a pool of semen-free cervicovaginal fluids by a pool of seminal plasmas (diluted 1:100 in distilled water). The pH of the mixture rapidly shifted to neutrality when the volume of seminal plasma exceeded 0.5%.

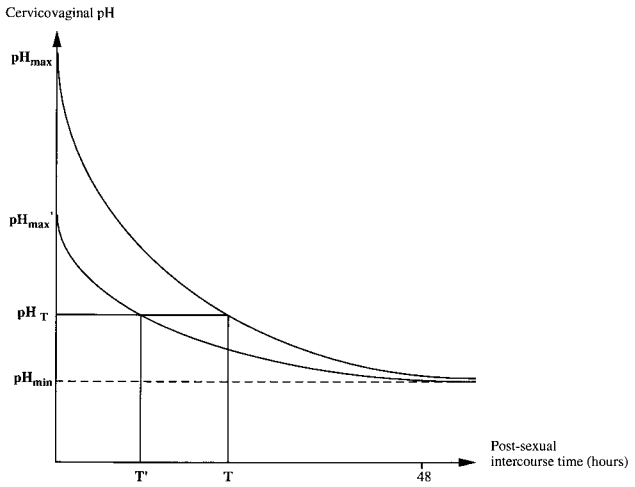

FIG. 8. Theoretical curves representing variation of the cervicovaginal pH after sexual intercourse (upper curve) and after a postcoital vaginal douching with water (bottom curve), as expressed by equation 1 (see text). A vaginal douching performed just after sexual intercourse reduces the postcoital cervicovaginal pH_{max} to pH_{max}'; the time T' during which the cervicovaginal pH is favorable to virus replication ($\geq pH_T$) is significantly reduced. The pH_{min} represents the normal acid pH of semen-free cervicovaginal secretions.

semen storage. The cationic charge of semen is primarily carried by spermine, a polyamine having immunosuppressive activities (17), whereas the anionic charge comes from citric acid. Both of these components are synthesized by the prostate. The buffering capacity of semen must be relative to independent variations of the concentration of these two components. In contrast, the anionic charge of the vaginal fluid is carried by lactic acid, which maintains low pH values (11). Our results demonstrate a postintercourse rise of the vaginal pH, as shown by the differences between group I and group II and by the in vitro neutralization curve of the cervicovaginal fluid by semen, which possibly protects semen HIV against acidity. During the following hours, the vaginal pH progressively comes back to its normal acid value (5); as observed in group III, the duration of this period likely depends on the initial amount of spermine. Prostatic proteins PAP and PSA are progressively eliminated with the mucus stream (20), and in addition to variations of their initial levels, different losses of antigenic determinants during degradation by vaginal proteases could explain the discrepant levels of these markers in group III.

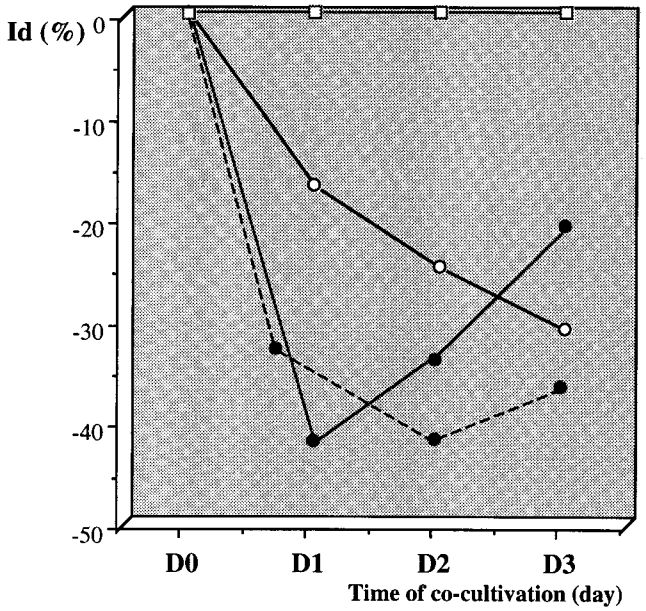

FIG. 7. Ex vivo evaluation of sensitivity of cell-free HIV-1 to semen-free, acid cervicovaginal secretions and to cervicovaginal secretions collected after vaginal douching soon after sexual intercourse. — —○—, postdouching cervicovaginal fluid (pH 5.3); —●—, semen-free cervicovaginal fluid (pH 3.5); —□—, culture control; - - ● - -, low pH medium control (pH 3.5).

The loss of normal vaginal acidity, secondary to neutralization of genital secretions through semen azoted bases, may favor heterosexual HIV transmission. Indeed, a complete and irreversible inactivation of free HIV virions has been demonstrated in vitro with pH values below 5.4 after a 20-min incubation and below 5.7 after a 2-h incubation at 37°C (14). Inactivation is similar for cell-associated virus, but it is partially reversible (14), unless the pH values can degrade the cells themselves, a phenomenon usually occurring below a pH of 6 (23). O'Connor and colleagues reported similar observations, but a substantial reduction in HIV infectivity was not observed in vitro until pH levels were reduced below 4.5 (13). In vitro, we have verified that normally acidic, semen-free cervicovaginal secretions strongly depress HIV cocultivation with HIV-negative donor PBMC for a period of about 24 h.

In the Central African Republic, as in sub-Saharan Africa, multiple sexual partnership is frequent; sexual exposure to HIV is also common, since the prevalence of HIV in the general adult population is between 10 and 15% (19). In this context, we found that 90% of women consulting the National Center for Sexually Transmitted Diseases and AIDS in Bangui had semen traces in their genital secretions, indicating recent sexual intercourse (20). We can hypothesize that a significant proportion of the female adult population of Bangui presents

a similarly high frequency of sexual intercourse and identical modification of the cervicovaginal pH due to in vivo semen neutralization. The rise of cervicovaginal pH in women at high risk for HIV could have important implications for heterosexual HIV transmission. First, the semen could be a cofactor increasing heterosexual HIV transmission from male to female. Indeed, the neutral pH observed in most women with high levels of semen markers in the vaginal tract shows how the local acidity is actually neutralized by semen, which may favor the male to female transmission of HIV by vaginal intercourse. According to our mathematical approach, the semen may act as a potential cofactor of sexual HIV transmission during an about 20-h period (*T*) of its vaginal elimination. The more frequently a woman has sexual intercourse, the greater will be her susceptibility to HIV due to in vivo semen neutralization of her cervicovaginal pH. If a woman has sexual intercourse several times per day, which is common among commercial sex workers, the cervicovaginal pH becomes permanently neutral, resulting in a loss of nonspecific, pH-related, antimicrobial defenses of the vagina. Second, when a woman has multiple sexual partners, the intravaginal storage of semen-derived HIV under neutral pH conditions may even allow HIV transmission to another man. This male to male transmission via an unaffected female has been hypothesized for prostitutes in developed countries (15). Third, the cervicovaginal rise of pH can also increase the infectivity of the genital secretions of an HIV-infected woman, favoring a female to male transmission during further sexual contact. Finally, the risk of heterosexual HIV transmission via penio-vaginal intercourse appears likely to depend on the frequency of intercourse of the female sexual partner and on the HIV status of her male partner(s).

In Africa, the prevention of heterosexual HIV transmission relies essentially on sexual behavior changes, condom promotion, and treatment of genital infections, particularly sexually transmitted diseases (8). Programs of condom promotion and regular treatment of sexually transmitted diseases can result in a major decline of HIV-1 incidence among females (8). However, preventive measures remain difficult to apply, in part because of ethnocultural resistance. Indeed, women in sub-Saharan Africa generally have little economic independence and lack control over their sexual activity, as men are generally the sexual decision-makers. For example, men often refuse to use condoms (22). Consequently, in the absence of a prophylactic vaccine, there is an urgent need to develop female-controlled means to prevent sexual transmission of HIV. We have demonstrated that after a postintercourse vaginal douching with water (women from group I), a significant fraction of semen traces can be eliminated from the vagina and that the cervicovaginal pH decreases significantly from neutrality to acidity. The pH decrease in this group (only 90 min after douching) did not drop to a normal level; however, it is likely that after significant elimination of semen residues, the cervicovaginal pH will then rapidly return to physiological acidity. Ex vivo, the exposure of virus to the cervicovaginal fluids collected after vaginal douching resulted in transitory inhibition of HIV replication, as observed with native semen-free cervicovaginal fluids. The cellularity of cervicovaginal secretions decreased significantly after vaginal douching, suggesting that cell-associated viruses, like HIV, can be partially eliminated if deposited in the vagina during sexual intercourse. Vaginal douching appeared safe, without causing any mucosal bleeding. Finally, a postcoital vaginal douching eliminates a significant amount of semen, providing a partial reversion of the in vivo semen-associated pH neutralization of cervicovaginal secretions and permitting a reduction of the contact duration between both cell-free and cell-associated HIV and the cervicovaginal mucosa. Vaginal douching is also well accepted, very simple, cheap, and female controlled. Epidemiological studies performed in the field suggest that vaginal douching would partially prevent heterosexual HIV transmission. In Bangui, we have observed that consistent genital douching with commercially available detergents, such as mercurobutol and triclocarban, reduced the risk of HIV infection by 40% (confidence interval, 30 to 90%); this reduction persisted after adjustment for number of sexual partners and marital status (6). In Chiang Mai, Thailand, Siraprapasiri and colleagues have reported an apparent protective effect of genital cleaning with soap and water, and to a lesser degree with soap and commercial detergent, after sexual intercourse among female sex workers (18). These reports and our observations suggest that vaginal douching following sexual intercourse might constitute a means for partially preventing heterosexual HIV transmission. Vaginal douching with a soft acid detergent could be beneficial, since it should permit acidification of the cervicovaginal secretions in an easier manner. Clearly, our suggestion must be viewed as being only preliminary. For example, vaginal douching could be proposed when other more classical, and efficient, methods of preventing sexual HIV transmission, such as the use of condom, have not been utilized during sexual intercourse. However, a regular practice of postintercourse vaginal douching could probably not be recommended, since by modifying the vaginal flora, it may increase the susceptibility to sexually transmitted diseases in cases of abusive use (21).

In sub-Saharan Africa, it is likely that sexual hygiene rules, simple and respected, could be extremely useful in limiting the risk of heterosexual HIV transmission. Post-sexual intercourse vaginal douching could constitute a supplementary means for the prevention of heterosexually acquired HIV infection which could be associated with the other classical methods already proposed for AIDS prevention in Africa, like the use of a condom. In the absence of an efficient vaccine, these rules de facto constitute the only means of AIDS prevention we currently possess.

ACKNOWLEDGMENTS

We thank Abbott Laboratories, Rungis, France, for providing assays for semen detection and Geffroy from Santé et Beauté, for providing bocks for vaginal douching. We also thank J.-C. Nicolas and D. Meillet for their advice and technical assistance.

Grant support was provided by SIDACTION, and C. Tevi-Bénissan was the recipient of a fellowship from SIDACTION.

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