Genital tract and plasma human immunodeficiency virus viral load throughout the menstrual cycle in women who are infected with ovulatory human immunodeficiency virus

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OBJECTIVE: The purpose of this study was to determine the vaginal, cervical, and plasma viral load through the menstrual cycle in women who are positive for human immunodeficiency virus.

STUDY DESIGN: A prospective cohort study was performed on 14 women with ovulatory menstrual cycles who have human immunodeficiency virus. Duplicate cervical and vaginal viral load samples (n = 301) were taken at four stages (menstrual, follicular, periovulatory, and luteal) of two consecutive cycles.

RESULTS: Participant characteristics were mean age of 32.7 years, median human immunodeficiency virus helper cell count value of 355, and median plasma viral load of 24,000 copies/mL. Through the menstrual cycle, there was no statistically significant difference in plasma viral load, but there was a significant decrease in genital tract viral load at the periovulatory phase (vagina, $P = .018$; cervix, $P = .007$). Vaginal and cervical viral load were correlated ($r = 0.582$, $P < .001$).

CONCLUSION: Although the plasma viral load remained constant throughout the menstrual cycle, the genital viral load decreased at the periovulatory phase. These results suggest that local factors may affect the genital viral load compartment independent of plasma viral load. (Am J Obstet Gynecol 2003;188:122-8.)

Key words: Human immunodeficiency virus, women, plasma, genital, viral load

Human immunodeficiency virus–1 (HIV-1) RNA is present in the genital tract secretions of most women who are infected with HIV, but there is currently little known regarding viral dynamics within the female genital tract. Few studies have attempted to address the issue of potential variation of genital tract viral load through the menstrual cycle. Several studies have shown an association between plasma viral load and levels of HIV in the female genital tract[1-5]; however, other studies have shown that genital tract levels vary distinctly from the plasma levels and are not well correlated.[6-8] Reichelderfer et al[8] have shown that genital tract HIV RNA levels significantly increase relative to plasma levels in association with cervical inflammation and ulceration. Of note, some studies have shown changes in the genital tract in the presence of infection, so this becomes an important factor to control for in the evaluation of genital tract HIV levels.[9-13]

Several lines of evidence suggest that hormonal variations through the menstrual cycle could have an impact on viral dynamics in the female genital tract. Potential sex differences in plasma viral loads have been reported,[14,15] and a hypothesis is that cyclic estrogen and progesterone levels may have an impact on immunologic control of HIV replication. Greenblatt et al[16] have reported changes in the plasma viral load through the menstrual cycle (a decrease in viral load in the luteal phase) that is related to hormonal variation. Reichelderfer et al[8] have also reported variations in the menstrual cycle and changes in results that are dependent on sampling method.

Given the dramatic changes that occur in the genital tract through the menstrual cycle, it is postulated that there may be changes in genital tract viral load in response to the changing hormonal milieu. In the number of published studies in which cervical and vaginal secretions have

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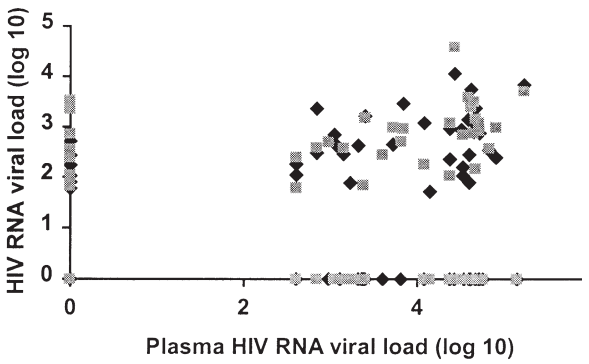

Fig 1. Correlation of plasma and cervical *(closed diamonds)*/vaginal *(hatched squares)* viral loads. Spearman correlation: cervix, $r = 0.396$, $P < .001$; vaginal, $r = 0.275$, $P < .001$.

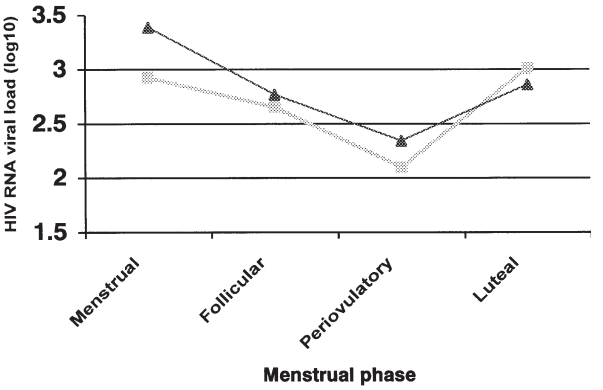

Fig 2. Mean genital tract HIV viral load by menstrual cycle phase; Kruskal-Wallis test was used for comparison between cycle phases. *Gray squares,* Cervical phase ($P < .01$); *closed triangles,* vaginal phase ($P < .05$).

been assessed for HIV-1 in the context of the menstrual cycle, most studies did not document ovulation, nor was the sampling taken at each distinct phase.[17-19]

An improved understanding of genital tract viral dynamics is important because it is becoming clear that the levels of genital tract HIV are likely to be highly relevant to the risk of heterosexual[20] and perinatal transmission.[21,22]

The objectives of this study were to determine whether the plasma HIV-1 viral load changed through the four phases of the menstrual cycle, to measure the level of vaginal and cervical HIV in the four phases of ovulatory menstrual cycles, and to determine the relationship between plasma viral load and genital tract viral load in each phase of the menstrual cycle.

Material and methods

This study was conducted under the approval of the University of British Columbia Clinical Research Ethics Board; a certificate of approval was obtained on April 8, 1997. Women who were infected with HIV and who attended our university-affiliated multidisciplinary HIV clinic (Women and Family HIV Centre, Oak Tree Clinic) in Vancouver, British Columbia, Canada, were asked to participate in this study. Women were eligible if they were regular patients, had regular menstrual cycles for the 6 months before enrollment, had undergone stable antiretroviral therapy regimens for the 3 months before the study, and continued to undergo stable antiretroviral therapy during the 2 months of the study period.

At enrolment, the following information was collected for each of the women from whom we received full consent: estimated time of HIV acquisition, probable mode of acquisition of HIV, history of injection drug use, medical history, obstetric history, menstrual history, history of genital tract infections, Papanicolaou test history, and medications. At the initial visit, which coincided with the beginning of the menstrual cycle, a full examination was conducted. This included a pelvic examination with a Papanicolaou test, cervical samples for chlamydia and gonorrhea, a vaginal Gram stain, and collection of genital tract secretions by SnoStrips (Chavin Pharmaceuticals, Romford, UK). Two samples from the cervix and two samples from the vagina were taken by absorption onto the SnoStrips. The papers were then allowed to air dry and stored at –70°C for batch assays for HIV-1 RNA. At the first study visit, HIV helper cell count (CD4) and HIV plasma viral load were obtained.

Women performed daily basal body temperature charting to further establish the phases of their cycles and subsequently attended the clinic at mid follicular, periovulatory, and midluteal phases of this cycle. At each study visit, plasma viral load samples and duplicate cervical and vaginal viral samples were collected. Serum estradiol level was obtained at the follicular and periovulatory phases and serum progesterone samples were obtained at the luteal-phase time. This protocol was repeated in its entirety over the subsequent menstrual cycle, which resulted in eight sampling times from the plasma and genital tract in each woman.

Laboratory methods. Hormonal assays were conducted at the UBC Reproductive Endocrinology research laboratory. The estradiol level was measured by a Pantex (division of Bio-Analysis, Santa Monica, Calif) radioimmunoassay method, and the progesterone level was measured with a Beckman Access Immunoassay (Beckman Coulter, Mississaugua, Ontario, Canada) system. The Access assay is a paramagnetic, chemiluminescent immunoassay for the quantitative determination of progesterone levels in human serum.

The plasma viral load assays were conducted at the UBC virology laboratory with the use of the Roche Cobas Amplicor HIV-1 Monitor Standard viral load assay (version 1.5; Roche Diagnostic Systems, Branchburg, NJ) with a limit of detection of 400 copies/mL.

The laboratory method used to assay HIV-1 in the genital tract specimens was based on the Roche Cobas Am-

Table I. Characteristics of study population (n = 14)

Characteristic	*Absolute*	*Percentage*
Demographics		
Age (y)		
Mean	32.7	
Median	34	
Range	25-44	
Ethnicity (No.)		
White	11	78.6
East Asian	1	7.1
Canadian aboriginal	1	7.1
Hispanic	1	7.1
History of injection drug use (No.)	3	21.4
Obstetrics-gynecology characteristics		
Obstetric history (No.)		
Previous pregnancy	8	57.1
Live birth	5/8	62.5
Therapeutic abortion	4/8	50.0
Spontaneous abortion	1/8	12.5
Sexually transmitted disease history (No.)		
Sexually transmitted disease/vaginitis/vaginosis ever	10	71.4
Sexually transmitted disease/vaginitis/vaginosis current	0	0.0
HIV characteristics (No.)		
Mode of HIV acquisition		
Heterosexual contact	9	64.3
Injection drug use	1	7.1
Combined risk	3	21.4
Unknown	1	7.1
CD4 at visit 1 ($\times 10^6$/L)		
Mean and median	355	—
Range	100-720	—
Plasma viral load at visit 1		
Women with detectable viral load (No.)	10	71.4
Mean ($\log^{10}$)*	4.98	—
Median ($\log^{10}$)	4.24	—
Range ($\log^{10}$)	2.6-6.88	—
Current antiretroviral therapy use (No.)	10	71.4
Dual combination	3/10	30.0
Triple combination	7/10	70.0

*For women with detectable virus in plasma.

plicor HIV-1 Monitor Ultrasensitive viral load assay (version 1.5; Roche Diagnostic Systems, Branchburg, NJ); the lower limit of detection of this assay is 50 copies/mL. The tabs of filter paper from the Sno Strips (Chauvin Pharmaceuticals, Romford, UK) on which the genital secretions were collected were removed from the entire strip and cut into pieces in a sterile fashion. The fragments were then placed into individual 2-mL polypropylene microtubes. One hundred microliters of Roche Monitor QS (Roche Diagnostic Systems, Branchburg, NJ) reagent was added to one vial (900 μL) of lysis buffer, also from the Roche Monitor kit. Six hundred microliters of this mixture was added to each sample tube. The tubes were then placed on a shaker for 45 minutes at 800 revolutions/min. After incubation, the lysis buffer mixture was removed from the sample tube and placed into another corresponding 2-mL microtube. Controls were also prepared according to Roche package insert instructions. Each sample tube had 525 mL of isopropanol added to each tube, which were then vortexed. From this point on, all samples were processed according to Roche Cobas Amplicor HIV-1 Monitor package insert instructions. To validate the Sno strip technique, duplicate specimens, which were then run in duplicate in the laboratory, were obtained at the time of sampling. Validation of this method resulted in a small substudy.[23]

Statistical methods. Measurements (copy numbers) of cervical and vaginal viral loads were log-transformed (base 10). Undetectable viral loads were assigned a copy number of 1 and a corresponding log-transformed value of 0. Duplicated measurements of cervical and vaginal viral loads were averaged. A scatter diagram and Spearman correlation coefficient were used to assess the relationship between cervical and vaginal HIV RNA measurements with the use of these averaged values. All measurements that were obtained over the two menstrual cycles were included in the comparative analyses of interest. Because the distributions of log-transformed cervical and vaginal viral load measurements were not normally distributed, we used nonparametric statistical methods to compare the viral load distributions between phases of the menstrual cycle. We used the Kruskal-Wallis test to

compare HIV RNA measurements among the four phases of the menstrual cycle. Means and SDs of cervical and vaginal viral load measurements were calculated for each phase of the menstrual cycle. The proportions of undetectable viral load measurements by phase of cycle were also calculated. Pairwise comparisons of these measurements between phases (eg, menstrual vs periovulatory) were conducted with the Mann-Whitney test. Mean differences of viral load measurements among phases were also calculated.

Results

Fifteen women were enrolled initially from September 1997 through June 1998. One woman did not complete the study protocol, which left data from 28 cycles in 14 women for analyses. Six of the women did not complete measurements at every cycle point. The demographic characteristics of the women can be seen in Table I. Of note, none of the women had evidence of a current sexually transmitted infection at the time of the study. Most of the women had only moderate immune compromise, as measured by absolute CD4 count (mean CD4 count, 355 $\times 10^9$/L; range, 100-750 $\times 10^9$/L). The plasma viral load that was measured at the first visit was detectable in 10 of the 14 women (median, 24,000 copies/mL; range, <400-750,000+ copies/mL). More than 70% of the women were undergoing antiretroviral therapy, most of whom were undergoing dual or triple combination therapy. Six women were receiving triple combinations that contained protease inhibitors, and 3 women were receiving dual nucleosides that were consistent with patterns of care during the study period. There were no women undergoing monotherapy.

Cycles with an abnormal basal body temperature graph or a luteal progesterone level of <16 nmol/L were excluded as anovulatory. The menstrual cycle hormonal data seen in Table II reveal that despite a history of regular cycles, 6 of the 28 complete cycles (21%) were anovulatory and 22 of the 28 complete cycles (79%) were ovulatory.

Mean plasma viral loads that were expressed as $\log^{10}$ values were compared phase by phase, with no differences found in any of the phases. The following data were the median $\log^{10}$ plasma viral load for the four phases: menstrual, 2.6; follicular, 3.3; periovulatory, 3.3; luteal, 3.1 ($P = .942$). Individual patient values for phases were evaluated and also found to be constant throughout the four phases. These findings persist when data are stratified by CD4 count (data not shown).

The data were also analyzed to determine whether there was a relationship between hormone level and plasma viral load. There were no significant correlations between the level of midfollicular–phase estradiol level and plasma viral load ($r = 0.327$, $P = .299$) nor between mid luteal progesterone level and plasma viral load ($r = 0.277$, $P = .317$).

Table II. Study hormonal assessments (n = 14)

Characteristic	*Value*
Total cycles (No.)	28
Annovulatory	6 (21.4%)
Ovulatory	22 (78.6%)
Follicular estradiol level (pmol/L)	
Mean	295
Median	228
Range	61-700
Periovulatory estradiol level (pmol/L)	
Mean	465.9
Median	373.5
Range	177-1287
Luteal progesterone level (nmol/L)	
Mean	23.4
Median	27.3
Range	1.1-48.7

To determine whether the sampling in the genital tract was reproducible, a substudy was performed that compared results on duplicate specimens. Eighty-eight vaginal viral load pairs were compared; the Pearson correlation coefficient was 0.72, and the coefficient of determination was 51.2%. The Pearson correlation coefficient for 92 cervical viral load pairs was 0.72; the coefficient of determination was 51.9%. In an alternate analysis, the Pearson correlation coefficient for 86 paired vaginal viral samples and cervical viral samples (set 1) was 0.58, and the coefficient of determination was 33.7%; for set 2 (n = 86 samples), the Pearson correlation coefficient was 0.56, and the coefficient of determination was 31.3%. Twenty-two samples (25.6%) of set 1 and 23 samples (26.7%) of set 2 had at least one negative viral load result in the pair. The Bland Altman plot for vaginal viral pairs showed that only 11 of 88 samples (12.5%) fell outside the range (the mean of both values ±2 SD); the corresponding Bland Altman plot for cervical samples showed that 10 of 92 samples (10.9%) were out of range. These results show very acceptable reproducibility for this experimental measurement.

Measurable HIV-1 virus was detected in 120 of 301 genital tract samples (40%). The vaginal viral load ranged from undetectable to 36,308 copies, and the cervical viral load ranged from undetectable to 18,000 copies. There was a significant correlation between the cervical and vaginal specimens ($r = 0.582$, $P < .001$). Plasma/vaginal and plasma/cervical viral loads were correlated moderately ($r = 0.2675$, $P < .01$; and $r = 0.396$, $P < .001$, respectively) (Fig 1).

When the genital tract viral load was compared through the four phases of the menstrual cycle, there was a statistically significant decrease in the periovulatory samples (Fig 2). The difference in the cervical viral load was significant at a probability value of <.01; the difference in the vaginal viral load was significant at a probability value of <.05.

Table III. Comparison of cervical viral load measurements (log^{10} copies per milliliter) during four phases of the menstrual cycle, based on measurements from 13 women who were positive for HIV infection

Descriptive measure	*Menstrual*	*Follicular*	*Periovulatory*	*Luteal*
Measurements (No.)	23	24	23	22
Mean	1.68	1.38	0.66	1.34
SD	1.54	1.48	1.37	1.52
Proportion undetectable	0.49	0.51	0.45	0.50

$P = .067$, ANOVA; based on the Kruskal-Wallis test. $P = .009$, significant difference between menstrual and periovulatory phase (mean difference, 1.02 logs); $P = .051$, marginal difference between periovulatory and luteal phase (mean difference, –0.68 logs); based on the Mann-Whitney test.

Table IV. Comparison of vaginal viral load measurements (log^{10} copies per milliliter) during four phases of menstrual cycle, based on measurements from 13 women who were positive for HIV infection

Descriptive measure	*Menstrual*	*Follicular*	*Periovulatory*	*Luteal*
Measurements (No.)	23	23	23	19
Mean	2.17	1.12	1.04	1.19
SD	1.53	1.54	1.51	1.33
Proportion undetectable	0.22	0.56	0.56	0.47

$P = .047$, ANOVA; based on Kruskal-Wallis test. $P = .028$, significant difference between menstrual and follicular phase (mean difference, 1.05 logs); $P = .017$, significant difference between menstrual and periovulatory phase (mean difference, 1.13 logs); based on the Mann-Whitney test.

Of note, six of seven women with a viral load of <400 copies/mL in plasma had detectable virus in the genital tract at levels that ranged from 120 to 1023 copies in cervical specimens and from 132 to 6310 copies in vaginal specimens. Of note, viral load values quoted at <400 copies are less accurate than higher values because of the nature of the Roche assay.

Table III shows a phase comparison of the cervical viral load measurements, with the menstrual phase as the reference level. As presented, there is a significant difference between the menstrual level and the periovulatory phase ($P = .009$), with a marginal difference between periovulatory and luteal phases ($P = .051$). Table IV shows the same results from the vaginal viral load measurements with a significant difference between menstrual and periovulatory ($P = .017$) and with a difference seen between menstrual and follicular. Of note, the menstrual phase is the phase with the highest genital tract viral load, but in particular at the level of the cervix, the periovulatory phase is significantly lower in viral load.

Comment

In this study of HIV-1 levels through ovulatory menstrual cycles, we have shown significant changes in the genital tract compartment through the cycle in the absence of a corresponding change in plasma viral load. There was a consistently observed, statistically significant decline in both cervical and vaginal HIV-1 viral load in the periovulatory phase. Although our study involves relatively few women, this finding was reproducible and was seen in both of the ovulatory menstrual cycles that were studied in most of the women. There was no change in plasma viral load throughout the cycle, and we observed no correlation between the level of midfollicular-phase estradiol or midluteal-phase progesterone level and plasma viral load. At all other phases of the cycle, there was strong correlation between plasma and genital tract virus levels.

It appears that different body compartments and reservoirs exist and that low levels of HIV RNA can be detected in a variety of cells and tissues, even when the plasma viral load is undetectable.[24] We found detectable virus in the genital tract of some women who had undetectable plasma viral loads. It is thought that genital tract virus comes from both distant transport (ie, through blood/plasma) and local production.

We hypothesize that the decrease in genital tract viral load at the periovulatory phase of the cycle is related most likely to a decrease in local HIV replication/production. This may be related to the impact of hormones on the local cervical microenvironment. There is enhanced production of cervical mucous and local immunoglobulin A at midcycle in ovulatory women, in preparation for conception. These local changes may well also result in the inhibition of HIV replication or expression. In addition, the enhanced production of cervical mucus may result in a washout effect of virus, particularly at the level of the cervix. The highest genital tract viral levels were seen in the menstrual phase; this may illustrate an increase in the risk for sexual transmission at this stage of the menstrual cycle.

Multiple factors that may have an impact on the level of HIV-1 in the genital area have been evaluated and include the relationship of local HIV levels to β-chemokine secretion. Kutteh et al have reported an interesting cytokine and antibody pattern in the menstrual cycle, with a peak of interleukin just before ovulation and with a increase in immunoglobulin G and A at this time also. One can speculate that this may, in part, be the cause of the decrease in HIV-1 that we observed in the genital tract during the periovulatory phase of the initial cycle.[24] The complexity of this problem is reflected in varying reports in the literature regarding the patterns of cytokines through the menstrual cycle. Al-Harthi et al[25] have suggested that an increase in vaginal cytokine levels occurs during menses as opposed to during the periovulatory period. The common thread with all of these studies, including ours, is that local factors appear to be affecting local viral load specifically, independent of plasma viral load.

Most published data regarding HIV-1 in the female genital tract has used a cervical-vaginal lavage for sampling. We used a Sno strip absorption technique, in the hope of producing better quantification, because the strips absorb a specific volume of fluid directly from the vaginal mucosa or the cervical os. By performing duplicate sampling at each study visit from both the vaginal mucosa and cervical os and measuring the two sets on separate assay runs, we were able to validate this technique.[23] It is also noteworthy that other investigators recently have used the same technique to sample rectal mucosa for local production of HIV-1.[26] More recently, there has been a comparison of various sampling techniques that has suggested that the use of "wicks" as a similar system to this study was the most sensitive technique for sampling.[27]

It is important to note that the women in our study were asymptomatic and that none of them had any active inflammatory disorders of the female genital tract. All of the women had apparently normal and intact vaginal mucosa, and none of the women had significant cervical cytologic abnormalities. Other studies have shown changes in genital tract viral load in the presence of inflammation, but our study does not address these issues or the potential impact of the menstrual cycle on the genital tract viral levels with coexistent inflammatory disorders.

Because of the era in which this study was conducted, many of the women in our clinic were undergoing antiretroviral therapy, which limited our ability to enroll women who were undergoing no antiretroviral therapy. Overall, the study numbers are small, and we cannot make meaningful conclusions regarding the impact of antiretroviral therapy on genital tract viral secretion. This is clearly an area of importance in terms of both heterosexual and perinatal transmission, and further investigation is needed.

We believe that this study clearly demonstrates the need for more investigation regarding the dynamics of HIV-1 in the female genital tract. More women must be studied over time to gain a fuller understanding of the effects of ovulatory and anovulatory menstrual cycles and menopause on the level of HIV-1 in the genital tract. Studies that evaluate the effects of other factors such as genital tract infections and the influence of antiretroviral therapies must take into consideration the hormonal milieu in which any samples are being taken (ie, which phase of the menstrual cycle). Full evaluation of this will enhance our understanding of the risks of heterosexual and perinatal transmission of HIV.

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REFERENCES

1. Hart CE, Lennox JL, Pratt-Palmore M, Wright TC, Schinazi RF, Evans-Strickfaden T, et al. Correlation of human immunodeficiency virus type 1 RNA levels in blood and the female genital tract. J Infect Dis 1999;179:871-82.
2. Cu Uvin S, Caliendo AM. Cervicovaginal human immunodeficiency virus secretion and plasma viral load in human immunodeficiency virus-seropositive women. Obstet Gynecol 1997;90: 739-43.
3. Iverson AKN, Larsen AR, Jensen T, Fugger L, Balslev U, Wahl S, et al. Distinct determinants of human immunodeficiency virus type 1 RNA and DNA loads in vaginal and cervical secretions. J Infect Dis 1998;177:1214-20.
4. Goulston C, McFarland W, Katzenstein D. Human immunodeficiency virus type 1 RNA shedding in the female genital tract. J Infect Dis 1998;177:1100-3.
5. Shaheen F, Sison AV, McIntosh L, Mukhtar M, Pomerantz RJ. Analysis of HIV-1 in the cervicovaginal secretions and blood of pregnant and nonpregnant women. J Human Virol 1999;2: 154-66.
6. Rasheed S, Li Z, Zu D, Kovacs A. Presence of cell-free human immunodeficiency virus in cervcovaginal secretions is independent of viral load in the blood of human immunodeficiency virus-infected women. Am J Obstet Gynecol 1996;175:122-30.
7. John GC, Nduati RW, Mbori-Ngacha D Overbaugh J, Welch M, Richardson BA, et al. Genital shedding of human immunodeficiency virus type 1 DNA during pregnancy: association with immunosuppression, abnormal cervical or vaginal discharge and severe vitamin A deficiency. J Infect Dis 1997;175:57-62.
8. Reichelderfer PS, Coombs RW, Wright DJ, Cohn J, Burns DN, Cu-Uvin S, et al. Effect of menstrual cycle on HIV-1 levels in the peripheral blood and genital tract: WHS 001 study team. AIDS 2000;14:2101-7.
9. Wright TC, Subbareo S, Ellerbrock TV, Lennox JL, Evans-Strickfaden T, Smith DG, et al. Human immunodeficiency virus 1 expression in the female genital tract in association with cervical inflammation and ulceration. Am J Obstet Gynecol 2001; 184:279-85.
10. Ghys PD, Fransen K, Diallo MO, Ettiegne-Traore V, Coulibaly I-M, Yeboue KM, et al. The associations between cervicovaginal HIV shedding, sexually transmitted diseases and immunosuppression in female sex workers in Abidjan, Cote d'Ivoire. AIDS 1997;11:F85-93.
11. Cu-Uvin S, Hogan JW, Caliendo AM, Harwell J, Mayer KH, Carpenter CCJ, for the HIV Epidemiology Research Study. Association between bacterial vaginosis and expression of human immunodeficiency virus type 1 RNA in the female genital tract. Clin Infect Dis 2001;33:894-6.

12. McClelland RS, Wang CC, Mandaliya K, Overbaugh J, Reiner MT, Panteleef DD, et al. Treatment of cervicitis is associated with decreased cervical shedding of HIV-1. AIDS 2001;15:105-10.
13. Wang CC, McClelland RS, Reilly M, Overbaugh J, Emery SR, Mandaliya K, et al. The effect of treatment of vaginal infections on shedding of human immunodeficiency virus type 1. J Infect Dis 2001;183:1017-22.
14. Farzadegan H, Hoover DR, Astemborski J, Lyles CM, Margolick JB, Margham RB, et al. Sex differences in HIV-1 viral load and progression to AIDS. Lancet 1998;352:1510-4.
15. Sterling TR, Vlahov D, Astemborski J, Hoover DR, Margolick JB, Quinn TC, et al. Initial plasma HIV-1 RNA levels and progression to AIDS in women and men. N Engl J Med 2001;344:720-5.
16. Greenblatt RM, Ameli N, Grant RM, Bacchetti P, Taylor RN. Impact of the ovulatory cycle on virologic and immunologic markers in HIV infected women. J Infect Dis 2000;181:82-90.
17. Clemetson DBA, Moss GB, Willerford DM, Hensel M, Emonyi W, Holmes KK, et al. Detection of HIV DNA in cervical and vaginal secretions: prevalence and correlates among women in Nairobi, Kenya. JAMA 1993;269:2860-4.
18. Vogt MW, Witt DJ, Craven DE, Byington R, Crawford DF, Hutchinson MS, et al. Isolation patterns of the human immunodeficiency virus from cervical secretions during the menstrual cycle of women at risk for the acquired immunodeficiency syndrome. Ann Intern Med 1987;106:380-2.
19. Mostad SB, Jackson S, Overbaugh J, Reilly M, Chohan B, Mandaliya K, et al. Cervical and vaginal shedding of human immunodeficiency virus type 1-infected cells throughout the menstrual cycle. J Infect Dis 1998:178:983-91.
20. Chakraborty H, Sen PK, Helms RW, Vernazza PL, Fiscus SA, Eron JJ, et al. Viral burden in genital secretions determines male-to-female sexual transmission of HIV-1: a probabilistic empiric model. AIDS 2001;15:621-7.
21. Dickover RE, Garratty EM, Herman SA, Sim, M-S, Plaeger S, Boyer PJ, et al. Identification of levels of maternal HIV-1 RNA associated with risk of perinatal transmission: effect of maternal zidovudine treatment on viral load. JAMA 1996; 275:599-605.
22. Mofenson LM, Lambert JS, Stiehm ER, Bethel J, Meyer WA, Whitehouse J, et al. Risk factors for perinatal transmission of human immunodeficiency virus type 1 in women treated with zidovudine. N Engl J Med 1999;341:385-93.
23. Sherlock CH, Money D, Craib KJP, Merrick L, Arikan Y, Remple V, et al. Are measurements of genital HIV-1 viral load in women reproducible? [abstract] Proceedings of the 8th Conference on Retroviruses and Opportunistic Infections; 2001 February 4-8; Chicago, Ill. Chicago: The Conference; 2001.
24. Kutteh WH, Moldoveanu Z, Mestecky J. Mucosal immunity in the female reproductive tract: correlation of immunoglobins, cytokines, and reproductive hormones in human cervical mucus around the time of ovulation. AIDS Res Hum Retroviruses 1998;14(1 Suppl):S51-5.
25. Al-Harthi L, Kovacs A, Coombs RW, Reichelderfer PS, Wright DJ, Cohen MH, et al. A menstrual cycle pattern for cytokine levels exists in HIV-positive women: implication for HIV vaginal and plasma shedding. AIDS 2001;15:1535-43.
26. Collis T, Celum C, Whittington W, Lucchetti A, Sanchez J, Lockhart D, et al. Compartmentalization and variability in HIV-1 shedding in blood, semen, rectum and pharynx [abstract]. Proceedings of the 8th Conference on Retroviruses and Opportunistic Infections; 2001 February 4-8, 2001; Chicago, Ill. Chicago: The Conference; 2001.
27. Coombs RW, Wright DJ, Reichelderfer PS, Burns DN, Cohn J, Cu-Uvin S, et al. Variation of human immunodeficiency virus type 1 viral RNA levels in the female genital tract: implications for applying measurements to individual women. J Infect Dis 2001;184:1187-91.