

# Biologic markers of ovarian reserve and reproductive aging: application in a cohort study of HIV infection in women

David B. Seifer, M.D.,<sup>a</sup> Elizabeth T. Golub, Ph.D.,<sup>b</sup> GERALYN Lambert-Messerlian, Ph.D.,<sup>c</sup> Gayle Springer, M.L.A.,<sup>b</sup> Susan Holman, R.N., M.S.,<sup>d</sup> Michael Moxley, M.D.,<sup>e</sup> Helen Cejtin, M.D.,<sup>f</sup> Niyati Nathwani, M.D.,<sup>g</sup> Kathryn Anastos, M.D.,<sup>h</sup> Howard Minkoff, M.D.,<sup>i</sup> and Ruth M. Greenblatt, M.D.<sup>j</sup>

<sup>a</sup> Maimonides Medical Center, Department of Obstetrics & Gynecology, Brooklyn, New York; Mount Sinai School of Medicine, New York, New York; <sup>b</sup> The Johns Hopkins Bloomberg School of Public Health, Department of Epidemiology, Baltimore, Maryland; <sup>c</sup> Department of Pathology and Laboratory Medicine, Brown Medical School and Women and Infants Hospital, Providence, Rhode Island; <sup>d</sup> SUNY Downstate Medical Center, Department of Preventive Medicine and Community Health, Brooklyn, New York; <sup>e</sup> University of Virginia, Charlottesville, Virginia; <sup>f</sup> John H. Stroger Jr. Hospital, Department of Obstetrics and Gynecology, Chicago, Illinois; <sup>g</sup> Los Angeles County Hospital/USC/Norris Medical Center, Los Angeles, California; <sup>h</sup> Montefiore Medical Center, Departments of Medicine and Epidemiology, Bronx, New York; <sup>i</sup> Maimonides Medical Center, Department of Obstetrics & Gynecology, Brooklyn, NY; SUNY Downstate, New York, New York; and <sup>j</sup> University of California, San Francisco School of Medicine, San Francisco, California

**Objective:** To compare Müllerian inhibiting substance (MIS) levels in serum obtained during the early follicular phase to those obtained randomly during the menstrual cycle. To determine whether HIV infection influences early follicular MIS levels, an early marker of ovarian aging.

**Design:** A cross-sectional study.

**Setting:** Women's Interagency HIV Study, a multicenter prospective study.

**Patient(s):** Serum samples obtained from 263 (187 HIV infected and 76 uninfected) participants of the Women's Interagency HIV Study who reported menstrual bleeding during the preceding 6 months and who were not taking exogenous hormones.

**Intervention(s):** Early follicular (cycle days 2–5) MIS samples were compared with serum samples that had been obtained without regard to menstrual cycle phase. Comparison samples were obtained within 6 weeks before or within 3 to 6 months after the early follicular samples. Early follicular FSH, E<sub>2</sub>, inhibin B, and MIS levels were also compared between the HIV infected and uninfected women.

**Main Outcome Measure(s):** Correlation between early follicular MIS and prior and subsequent samples. Comparison of serum markers of ovarian reserve between HIV positive and negative women.

**Result(s):** The MIS values from early follicular and other random cycle phases were highly correlated with each other ( $r > 0.93$ ). In multivariate analysis, increased age and FSH level and lower inhibin B levels were associated with lower MIS level; MIS values did not vary by HIV serostatus.

**Conclusion(s):** Without regard to cycle phase, MIS was similar during early follicular phase and highly correlated with early follicular FSH and inhibin B in women with and without HIV. Measurement of serum MIS offers a simplified method of determining ovarian reserve using specimens obtained without menstrual phase timing. Furthermore, using biologic measures of reproductive aging, we found no evidence that HIV infection influences ovarian aging. (Fertil Steril® 2007;88:1645–52. ©2007 by American Society for Reproductive Medicine.)

**Key Words:** Müllerian inhibiting substance, ovarian reserve, HIV

Depletion of ovarian follicles defines the process of women's reproductive aging and is responsible for aging-associated decreases in fertility and gonadal steroids. Recent studies indicate that Müllerian inhibiting substance (MIS) (also known as anti-Müllerian hormone, AMH), may offer the most accurate, simple, and noninvasive method for determining ovarian follicle reserve and reproductive aging (1–11). At present, a variety of methods are used to assess ovarian reserve de-

pending largely on the clinical or research setting. Vaginal ultrasound with antral follicle counts is accurate, but expensive and cumbersome. Follicle-stimulating hormone is correlated with age-related declining fertility. It is a commonly used measure of ovarian aging, but is subject to variance with cycle phase, exogenous hormone use, drinking alcohol, and tobacco smoking (12–14). Early follicular serum inhibin B has been demonstrated to be a more sensitive indicator of ovarian aging (15–17), but is also influenced by cycle phase, as well as body mass and perhaps ethnicity (18).

Both FSH and inhibin B reflect activity of gonadotropin-dependent dominant follicles and are insensitive to the remaining pool of gonadotropin-independent quiescent

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Reprint requests: David B. Seifer, M.D., Maimonides Medical Center, Brooklyn, NY; Mount Sinai School of Medicine, New York, NY (FAX: 718-283-6580; E-mail: drseifer@genesinfertility.com).

follicles. However, it is the size and function of this remaining pool that are the direct determinants of future ovarian function. The MIS is produced by the gonadotropin-independent pool of preantral and small antral follicles and is thus not dependent on cyclic development of individual follicles. Furthermore, MIS is not altered when follicular development is suppressed by exogenous sex steroids. Despite the potential improved utility of MIS as a marker of ovarian reserve over FSH and inhibin B, its behavior in the context of HIV infection is unclear.

The HIV infection, which is associated with neurologic, immunologic, inflammatory, and metabolic perturbations, could influence menstrual pattern and ovarian reserve. Several research groups have reported the occurrence of, or the lack of, altered patterns of reproductive aging in HIV infected women (19–25). These studies have used self-reported menopause and menopausal symptoms, menstrual calendars, or randomly timed FSH levels as measures of reproductive aging, which are recognized to be subject to bias and imprecision. We used the Women's Interagency HIV Study (WIHS) cohort as a platform to test the hypothesis of whether MIS measured in variable phase specimens correlates with early follicular MIS levels. In addition, the WIHS cohort allowed us to investigate whether any correlation between un-timed and early follicular MIS levels remains in the context of HIV infection.

## MATERIALS AND METHODS

### Study Population

This study was nested in the WIHS, the largest ongoing multicenter prospective cohort study of HIV infection and related health conditions among HIV seropositive women and at-risk seronegative comparison women in the United States. The protocols, procedures, and baseline results of the WIHS have been previously described (26). The WIHS enrolled HIV participants between October 1994 and November 1995, and between October 2001 and September 2002, at six study sites (Bronx, NY; Brooklyn, NY; Chicago, IL; Los Angeles, CA; the San Francisco Bay area; and the Washington, D.C. area). The HIV-infected group was similar in terms of race/ethnicity, HIV exposure factors, and age to AIDS cases among US women reported in 1995 and the seronegative group was comparable to the seropositive group in terms of age, race/ethnicity, and a number of sociodemographic characteristics (26, 27). Semiannual WIHS study visits include extensive interview, clinical examination, and collection of biological specimens at any time during the menstrual cycle when bleeding is not present. In addition, during 2003–2005, women who reported having experienced menstrual bleeding during the preceding 6 months and who were not taking exogenous hormones, were asked to notify study staff the day their next cycle began, and then return for study phlebotomy on days 2–5 of that cycle; and hence all laboratory values obtained on day 2–5 visit are referred to as early follicular visits. Early follicular (cycle days 2–5) samples were compared with serum samples that had been

obtained without regard to menstrual cycle phase. Comparison samples were obtained within 6 weeks before and within 3–6 months after the early follicular samples. Written informed consent was obtained from all subjects after approval by the human subjects protection committees at participating institutions. Included in this study are all women for whom specimens were available from both an early follicular visit and a core WIHS visit.

### Assay Methods

The HIV infection was detected by enzyme-linked immunosorbent assay (EIA) with Western blot confirmation (26). Estradiol levels were measured on serum samples by the Coat-A-Count solid phase radioimmunoassay (Siemens Medical Solutions, Malvern, PA) with six-dilution calibration standards and a zero control. FSH levels were measured on serum samples by the ADVIA Centaur FSH two-site immunoassay (Bayer Diagnostics, Tarrytown, NY) with two-point calibration. Both assays were performed by QUEST Diagnostics Laboratories.

The MIS and inhibin B levels were measured at Women and Infants Hospital of Rhode Island. Serum samples were assayed in duplicate by ELISA for MIS/AMH (Diagnostic Systems Laboratories, Inc., Webster, TX). Briefly, samples were incubated in microtiter wells with anti-MIS/AMH antibody. After incubation and washing, the wells were treated with a biotin-labeled antibody followed by streptavidin-horseradish peroxidase. Tetramethylbenzidine was used as substrate and a dual wavelength absorbance was measured at 450 and 620 nm. Samples from each patient were run on the same assay. Intra-assay and interassay coefficients of variation (CV) were <15% and the limit of detection was 0.10 ng/mL.

For inhibin B measurement, a subset of serum samples (days 2–5 only) were also assayed in duplicate using an ELISA method (Oxford Bio-Innovations-DSL, Oxford, United Kingdom). Briefly, samples were pretreated with sodium dodecyl sulfate (SDS), boiled for 3 minutes and incubated with hydrogen peroxide before addition to microtiter wells. After overnight incubation with anti-inhibin B<sub>B</sub> antibody, the wells were washed and incubated with an alkaline phosphatase-labeled, inhibin  $\alpha$  subunit antibody. Substrate was added and absorbance was measured at 405 and 620 nm. The assay is highly specific for dimeric inhibin B, with negligible cross-reactivity reported for pro- $\alpha$  C subunit or activins, and approximately 1% cross-reactivity with inhibin A. The intra-assay and interassay CVs were <15% and the limit of detection was 16 pg/mL. The HIV status of the patients was unknown to the technician performing the assays.

### Statistical Analysis

Early follicular phase characteristics were compared between women with MIS values above and below the median, as well as between those with and without HIV infection, by  $\chi^2$  analysis for categorical variables and *t*-tests for continuous variables. All characteristics were measured at the early

follicular phase study visit. Menopausal stage was defined by an algorithm combining self-reported menstrual history and pregnancy. Early transition signaled decreased cycle predictability, and was defined as women who either: [1] reported at two consecutive visits that they had skipped a period in the past 6 months, [2] reported at two consecutive visits that they had periods at least 3 days early or 3 days late in the past 6 months, or [3] reported at one visit both having skipped a period and having had an early or late period in the past 6 months. Late transition signaled 6–11 months of amenorrhea and was defined as women who reported no period in the past 6 months and were not currently pregnant and had no pregnancies since their last visit (because participants were allowed one skipped semiannual visit, we could assess amenorrhea during the past 11 months). Menopause was defined as  $\geq 12$  months of amenorrhea and was defined as two consecutive visits at which a woman reported no period in the past 6 months, and no pregnancies.

Mean MIS values across visits and between HIV strata were also compared by *t*-tests. Mean early follicular phase FSH, E<sub>2</sub>, and inhibin B values were compared between HIV-infected and HIV-negative women using *t*-tests. Pearson's correlation coefficients, along with their corresponding *P* values, were obtained in examining correlations of log-transformed MIS values between visits. Finally, univariate and multivariate linear regression models were constructed to investigate independent predictors of MIS, adjusting for potential confounders. We examined potential interactions between current smoking and HIV infection, and also between smoking and age, in the multivariate models. In addition, separate group analyses were conducted, restricting the sample to HIV-infected women only, to assess any associations between CD4+ lymphocyte count, HIV viral load, a prior clinical AIDS diagnosis (CDC criteria excluding CD4 cell count), and use of antiretroviral therapies reported by the participant at the core WIHS visit with MIS values. All analyses were conducted using SAS version 9.1 (SAS Institute Inc., Cary, NC).

## RESULTS

Complete early follicular visit data were available at the time of analysis for 263 women, 187 (71.1%) of whom were HIV-infected. Characteristics of those women from the time of the days 2–5 sample draw can be seen in Tables 1 (stratified by MIS above/below the median value) and 2 (stratified by HIV status). Participants ranged in age from 20–54 years (mean 37.1 years), were 68.4% black and 23.6% Latino. Current cigarette smoking was reported by 55.5% of participants, and cohort participants tended to be more than the ideal weight, with the mean body mass index (BMI) (29.1) being just under the lower limit for obesity. Less than 10% (8.8%) had never been pregnant, and almost 40% had at least three children. Very few participants (6.8%) reported having had ovarian surgery. The majority of participants (92.8%) were either premenopausal or were in early transition. Compared to women with MIS values greater than or equal to the

median (1.03 ng/mL), women with values below the median were significantly ( $P < .05$ ) older (mean age 41.7 vs. 32.6 years), more likely to smoke, had higher FSH and lower inhibin B, and reported higher gravidity and parity. The HIV-infected participants differed from the seronegatives only in that they had a significantly higher frequency of reported night sweats. Although mean values of inhibin B, FSH, and E<sub>2</sub> were lower among HIV-infected women, none of these differences was statistically significant.

The MIS values from randomly drawn samples (the before and after visits are within 6 months of the index visit) were highly correlated with those from the early follicular phase samples (index visit), as seen in Figure 1. Comparing the before visit samples with the index visit samples, MIS values were very highly correlated ( $r = 0.948$ ,  $P < .0001$ ). Early follicular MIS values were also highly correlated with values from randomly drawn samples taken 3–6 months later ( $r = 0.944$ ,  $P < .0001$ ). Stratified by HIV status, both pairwise visit comparisons were still significantly highly correlated ( $r > 0.93$ ,  $P < .0001$ ).

Table 3 shows the mean ( $\pm$  standard error of the mean) MIS values by HIV status, at each of the three study visits (before, index, and after). The HIV-infected women had significantly lower ( $P < .05$ ) MIS values than uninfected women at both the before and index visits, and marginally lower ( $.05 < P < .10$ ) values at the after visit. In addition, although obesity was common in both cohort groups, BMI was significantly lower among the HIV-infected women (29 vs. 31,  $P = .027$ ; data not shown).

Next, we investigated factors that were independently associated with MIS levels among our participants. Table 4 shows the results of univariate and multivariate linear regression analyses, with log-transformed MIS value as the outcome. In univariate analysis, the following factors were significantly associated with lower MIS values: older age, greater number of pregnancies/children, ovarian surgery, being in late transition or menopausal, smoking, and higher FSH levels. In addition, higher inhibin B was associated with higher MIS. In multivariate analysis, older age and higher FSH remained associated with lower MIS values, whereas higher inhibin B remained associated with higher MIS. Adjusting for age, FSH, and inhibin B, HIV infection was not associated with MIS. All potential interactions that we examined were found not to be statistically significant (data not shown). Finally, group analyses were conducted among HIV-infected women only to investigate potential associations of virologic and immunologic factors with MIS levels. We examined CD4+ lymphocyte count, plasma HIV RNA quantity, the occurrence of an AIDS-defining condition, and receipt of antiretroviral therapy; none was found to be significantly associated with MIS (data not shown).

## DISCUSSION

It has been previously shown that MIS measured in the follicular phase may be an earlier and more sensitive serum marker

**TABLE 1**

**Early follicular phase characteristics of 263 participants in the Women's Interagency HIV Study, April 2003–February 2005.**

Characteristic	Total (N = 263)	MIS < 1.03 ng/mL <sup>a</sup> (n = 131)	MIS ≥ 1.03 ng/mL (n = 132)	P value <sup>b</sup>
HIV Serostatus <sup>c</sup>				
Seronegative	76 (28.9)	36 (27.5)	40 (30.3)	.614
Seropositive	187 (71.1)	95 (72.5)	92 (69.7)	
Age <sup>d</sup>	37.1 ± 0.43	41.7 ± 0.44	32.6 ± 0.50	<.0001
Race <sup>c</sup>				
White	13 (4.9)	5 (3.8)	8 (6.1)	.756
Black	180 (68.4)	90 (68.7)	90 (68.2)	
Latina	62 (23.6)	31 (23.7)	31 (23.5)	
Other	8 (3.0)	5 (3.8)	3 (2.3)	
BMI <sup>d</sup>	29.1 ± 0.49	29.2 ± 0.72	29.1 ± 0.67	.886
Smoking <sup>c</sup>				
Current	146 (55.5)	77 (58.8)	69 (52.3)	.035
Former	35 (13.3)	22 (16.8)	13 (9.9)	
Never	82 (31.2)	32 (24.4)	50 (37.9)	
Gravidity (# pregnancies) <sup>c</sup>				
None	23 (8.8)	9 (6.9)	14 (10.6)	.013
1–2	55 (20.9)	19 (14.5)	36 (27.3)	
≥ 3	185 (70.3)	103 (78.6)	82 (62.1)	
Parity (# children) <sup>c</sup>				
None	49 (18.6)	16 (12.2)	33 (25.0)	.009
1–2	109 (41.4)	53 (40.5)	56 (42.4)	
≥ 3	105 (39.9)	62 (47.3)	43 (32.6)	
Ovarian surgery <sup>c</sup>	18 (6.8)	12 (9.2)	6 (4.6)	.138
Night sweats <sup>c</sup>	31 (11.8)	19 (14.5)	12 (9.1)	.174
Stage of menopause <sup>c</sup>				
Premenopause/early transition	244 (92.8)	119 (90.8)	125 (94.7)	.227
Late transition/menopausal	19 (7.2)	12 (9.2)	7 (5.3)	
Inhibin B <sup>d</sup>	74.6 ± 4.74	63.4 ± 8.29	85.7 ± 4.51	.018
FSH <sup>d</sup>	8.0 ± 0.58	10.9 ± 1.10	5.2 ± 0.14	<.0001
E <sub>2</sub> <sup>d</sup>	44.6 ± 1.63	47.0 ± 2.87	42.1 ± 1.55	.134

Note: MIS = Müllerian inhibiting substance; BMI = body mass index.

<sup>a</sup> Median split.

<sup>b</sup> Obtained by *t*-test or  $\chi^2$  analysis.

<sup>c</sup> N (%).

<sup>d</sup> Mean ± standard error of the mean.

Seifer. Random MIS in ovarian reserve. *Fertil Steril* 2007.

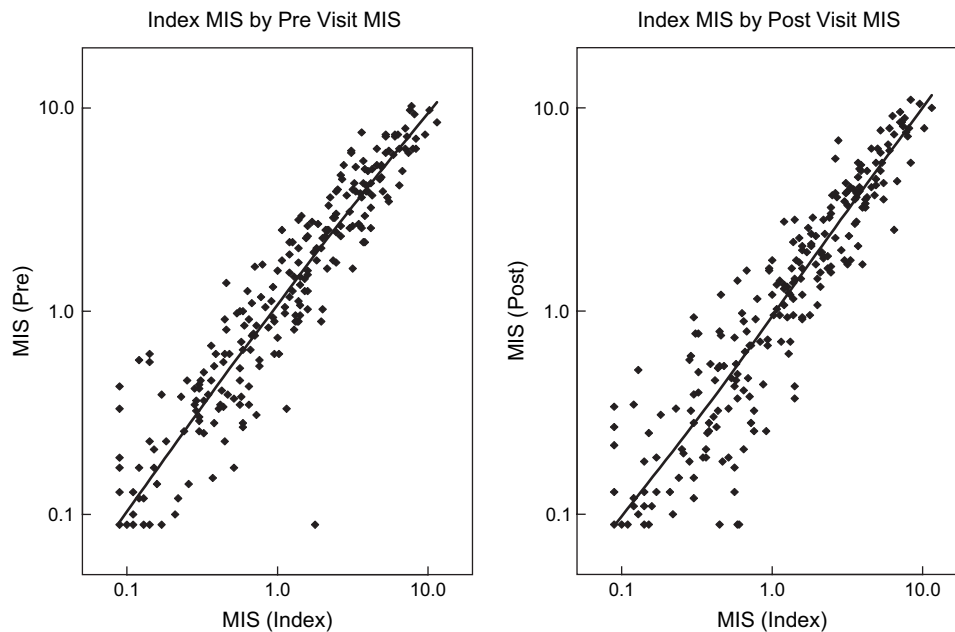
of changes in ovarian reserve than conventional early follicular phase markers (FSH, E<sub>2</sub>, or inhibin B) (4–11). Recently a prospective study of 81 Dutch volunteers who were followed for 4 years (mean age 39.6 and 43.6 years at the beginning and at the end of the study, respectively) found that MIS levels were more accurate indicators of reproductive capability, determined by ultrasound measurement of antral follicle counts, than were FSH, E<sub>2</sub>, and inhibin B (4). The MIS values have been shown to have negligible variation through the menstrual cycle (intracycle) (28, 29), between menstrual cycles (intercycle) (30), in response to pituitary desensitization by exogenous GnRH agonist (3, 31), or during pregnancy

(32). These findings are consistent with the concept that MIS serum concentrations are independent of gonadotropin secretion.

The collection of serum samples at precise times in the early follicular phase is not always feasible, both in a research and in a clinical setting. Additional visits for phlebotomy may be required and the timed specimen cannot be obtained unless menstrual bleeding occurs and is identified accurately. A serum measure of ovarian reserve that is independent of cycle phase, such as MIS, would be a great advantage in the investigation of reproductive aging in

**FIGURE 1**

Correlation of Müllerian inhibiting substance (MIS) values at different times in the menstrual cycle among Women's Interagency HIV Study (WIHS) participants.



Seifer. Random MIS in ovarian reserve. *Fertil Steril* 2007.

cohort studies and in clinical care, but most clinical studies to date examining MIS have focused on women with infertility. With increasing interest in the inclusion of women in clinical research and a growing women's health research agenda, MIS measurements could be a highly useful tool for assessing reproductive age and menopausal status.

This large cohort study of women who were not preselected for infertility demonstrates that serum MIS sampled at varying cycle times during a prior or subsequent cycle is highly correlated with early follicular serum MIS and does not vary by HIV status. This has a potentially significant impact on the practice of physicians who evaluate women's ovarian reserve. The current standard for such an evaluation is limited to ultrasound antral follicle counts or early follicular serum samples (i.e., days 2–5 of the menstrual cycle) for various ovarian biomarkers such as FSH, inhibin B, E<sub>2</sub>, or MIS. Because a single serum sample for MIS taken randomly during the menstrual cycle or within up to 6 months of that cycle has a high correlation with a timed sample in the early follicular phase, it may no longer be necessary to restrict serum collection to a narrow window of time to assess ovarian reserve. Most recently La Marca et al. (33) demonstrated that a serum MIS on any day of the menstrual cycle is associated with ovarian response in assisted reproductive technology (ART). Our study suggests that an untimed serum MIS within as well as between cycles may be useful in a clinical setting of women without a history of infertility. These findings have broad clinical utility in the treatment of couples with infertility as

well as in the design of clinical studies examining changes in organ system physiology secondary to ovarian aging.

Furthermore, the data from this study suggest that HIV-infected women have neither earlier nor delayed ovarian aging compared to HIV-uninfected women. Although mean values for early follicular phase serum MIS concentrations were not similar between HIV-infected and HIV-negative women, multivariate analysis, which accounts for potential confounders, demonstrated that HIV status was not an independent predictor of serum MIS concentration. This is consistent with another analysis undertaken among a larger WIHS sample, which showed no difference in ovarian failure (defined as serum FSH >25 mIU/mL and amenorrhea for at least 1 year) by HIV status, adjusting for age, BMI, albumin, and parity (27). Increasing age, early follicular serum inhibin B, and FSH concentrations were independently associated with MIS level in our study, demonstrating that MIS co-varies with ovarian aging and biologic measures of ovarian reserve. Thus, it appears that neither HIV infection nor antiretroviral therapies accelerates ovarian aging with its concomitant morbidities of osteoporosis, cardiovascular disease, and urogenital atrophy.

Measurement of serum MIS offers a simplified method of determining ovarian reserve using specimens obtained without menstrual phase timing. The fact that serum MIS is independent of gonadotropin secretion highlights its clinical relevance and potential benefits of being randomly sampled

**TABLE 2**

**Characteristics by HIV serostatus of 263 participants in the Women's Interagency HIV Study, April 2003–February 2005.**

Characteristic	Total (N = 263)	HIV seronegative (n = 76)	HIV seropositive (n = 187)	P value <sup>a</sup>
Age <sup>b</sup>	37.1 ± 0.43	36.1 ± 0.96	37.5 ± 0.47	.142
Race <sup>c</sup>				
White	13 (4.9)	3 (4.0)	10 (5.4)	.371
Black	180 (68.4)	58 (76.3)	122 (65.2)	
Latina	62 (23.6)	13 (17.1)	49 (26.2)	
Other	8 (3.0)	2 (2.6)	6 (3.2)	
BMI <sup>b</sup>	29.1 ± 0.49	29.4 ± 0.85	29.0 ± 0.60	.705
Smoking <sup>c</sup>				
Current	146 (55.5)	50 (65.8)	96 (51.3)	.101
Former	35 (13.3)	8 (10.5)	27 (14.4)	
Never	82 (31.2)	18 (23.7)	64 (34.2)	
Gravidity (# pregnancies) <sup>c</sup>				
None	23 (8.8)	9 (11.8)	14 (7.5)	.245
1–2	55 (20.9)	19 (25.0)	36 (19.2)	
≥3	185 (70.3)	48 (63.2)	137 (73.3)	
Parity (# children) <sup>c</sup>				
None	49 (18.6)	18 (23.7)	31 (16.6)	.365
1–2	109 (41.4)	28 (36.8)	81 (43.3)	
≥3	105 (39.9)	30 (39.5)	75 (40.1)	
Ovarian surgery <sup>c</sup>	18 (6.8)	3 (4.0)	15 (8.0)	.236
Night sweats <sup>c</sup>	31 (11.8)	4 (5.3)	27 (14.4)	.037
Stage of menopause <sup>c</sup>				
Premenopause/early transition	244 (92.8)	72 (94.7)	172 (92.0)	.434
Late transition/menopausal	19 (7.2)	4 (5.3)	15 (8.0)	
Inhibin B <sup>b</sup>	74.6 ± 4.74	75.7 ± 5.82	74.2 ± 6.25	.881
FSH <sup>b</sup>	8.0 ± 0.58	8.5 ± 1.50	7.9 ± 0.54	.610
E <sub>2</sub> <sup>b</sup>	44.6 ± 1.63	48.2 ± 3.00	43.1 ± 1.94	.157

Note: BMI = body mass index.

<sup>a</sup> Obtained by *t*-test or  $\chi^2$  analysis.

<sup>b</sup> Mean ± standard error of the mean.

<sup>c</sup> N (%).

Seifer. Random MIS in ovarian reserve. Fertil Steril 2007.

**TABLE 3**

**Mean (± standard error of the mean) Müllerian inhibiting substance values by HIV status at three time points.**

Visit	Total	HIV+	HIV–	P value <sup>a</sup>
Pre (random)	2.00 ± 0.14	1.82 ± 0.15	2.45 ± 0.31	.043
Index (days 2–5)	1.89 ± 0.13	1.69 ± 0.14	2.36 ± 0.30	.024
Post (random)	1.92 ± 0.14	1.77 ± 0.16	2.31 ± 0.31	.086
P value (pre vs. index)	.021	.030	.374	
P value (index vs. post)	.465	.185	.655	

<sup>a</sup> Comparing HIV+ and HIV- values; P values obtained by *t*-tests.

Seifer. Random MIS in ovarian reserve. Fertil Steril 2007.

**TABLE 4**

**Independent correlates of early follicular phase (log) Müllerian inhibiting substance values among Women's Interagency HIV Study participants.**

Covariate	Univariate analysis		Multivariate analysis	
	Unadjusted parameter estimate ( $\beta$ )	P value	Adjusted parameter estimate ( $\beta$ )	P value
Age (1-y increments)	-0.063	<.0001	-0.058	<.0001
# pregnancies	-0.074	<.0001		
# children	-0.155	<.0001		
Night sweats	-0.207	.076		
Ovarian surgery	-0.484	.001		
BMI	-0.001	.817		
Stage of menopause <sup>a</sup>				
Early transition	-0.148	.092		
Late transition/menopausal	-0.382	.016		
Race <sup>b</sup>				
Black	-0.305	.082		
Latina	-0.318	.088		
Other	-0.083	.761		
Smoking <sup>c</sup>				
Current	-0.270	.001		
Former	-0.395	.001		
Inhibin B	0.002	<.0001	0.001	<.0001
FSH	-0.024	<.0001	-0.008	.004
E <sub>2</sub>	-0.001	.411		
HIV+	-0.096	.251		

Note: BMI = body mass index.

<sup>a</sup> Reference category = premenopause.

<sup>b</sup> Reference category = white.

<sup>c</sup> Reference category = never smokers.

Seifer. *Random MIS in ovarian reserve. Fertil Steril* 2007.

during the menstrual cycle in contrast to traditional gonadotropin dependent markers (i.e., FSH, E<sub>2</sub>, and inhibin B), which require early follicular phase sampling to determine ovarian reserve. These findings should be of substantial interest to those practitioners and investigators who believe ovarian reserve to be relevant to the care of their patients or the outcomes of their clinical research studies.

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