ORIGINAL ARTICLE

Alteration in the Hypothalamic-Pituitary-Ovarian Axis in Depressed Women

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Background: Stress and corticotropin-releasing hormone inhibit the reproductive axis. We hypothesized that reproductive axis hormone secretion, particularly luteinizing hormone secretion, is inhibited in women with depression, similar to what has been observed to be caused by stress in numerous species.

Methods: Blood samples were collected every 10 minutes for 12 hours in 25 untreated premenopausal women with depression and 25 nondepressed women who were matched by age and menstrual cycle day. Samples were assayed for luteinizing hormone, follicle-stimulating hormone, estradiol, and progesterone.

Results: The mean plasma estradiol level was 30% lower in the follicular phase in women with depression than in their matched controls: 191 ± 136 vs 261 ± 169 pmol/L (52 ± 37 vs 71 ± 46 pg/mL). The half-life of luteinizing hormone was significantly shorter in women with depression than in their matched controls during both the follicular (22% shorter) and luteal (15% shorter) phases.

Conclusions: The blood levels of reproductive hormones were mostly normal in women with depression, but the blood level of estradiol was significantly lower. Estradiol is known to affect a number of neurotransmitter systems in the brain.

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IT HAS BEEN repeatedly demonstrated that there is a gender difference in depression, with a 2-fold greater prevalence of depression occurring in women than in men.[1-4] Furthermore, the increase in the incidence of depression in girls following the onset of puberty and the destabilization of mood in women in perimenopause has suggested that reproductive hormones may play a role in modulating depression.[4,5] Despite hypotheses about the role of ovarian steroids in mood, no previous studies have conducted a comprehensive assessment of reproductive hormones in women with depression.[5]

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Stress is known to affect the reproductive axis, leading to amenorrhea in the most severe cases. Studies in primates have demonstrated that intracerebroventricular infusion of corticotropin-releasing hormone (CRH) as well as proinflammatory cytokines such as interleukin 1 can decrease luteinizing hormone (LH) secretion.[6-8] Stress-induced (hypothalamic) amenorrhea, as well as exercise-induced amenorrhea and anorexia nervosa, activate the hypothalamic-pituitary-adrenal (HPA) axis, increasing cortisol secretion and decreasing the corticotropin or cortisol response to exogenous CRH.[9-16] These HPA axis abnormalities are similar to those seen in depression, suggesting that activation of the HPA axis may be linked to inhibition of the hypothalamic-pituitary-ovarian (HPO) axis. In addition, increased CRH in the cerebrospinal fluid, which indicates general activation of the extrahypothalamic CRH systems, has been found in depressed patients, lending further support to a hypothesis of decreased reproductive function in depression.[17]

The HPO axis, and LH secretion in particular, is characterized by episodic hormone secretion or "pulses," which can be described using pulse detection algorithms. Pulses are significant increases in hormone levels that occur with a regular frequency such as the once an hour (circhoral) rhythm present in the HPO axis. Hormone pulsatility conveys physiological information in addition to mean hormone levels. The arcuate nucleus of the hypothalamus houses a "pulse generator," which can be characterized electrophysiologically as multiunit activity discharges synchronous with LH pulses.[18] The pulsatile pattern of LH is also synchronous with the pulsatile release of gonadotropin-releasing hormone (GnRH) from

SUBJECTS AND METHODS

SUBJECTS

All subjects were premenopausal women who ranged in age from 20 to 49 years. A total of 25 depressed patients and matched nondepressed controls were studied. Depressed women were recruited from patients at the University of Michigan (Ann Arbor) Mood Disorders program who were seeking treatment for new episodes of depression. All studies were approved by the University of Michigan institutional review board. All subjects were medically healthy and had not been treated for the current episode of depression. None were taking psychotropic medications, oral contraceptives, or any other medications, with the exception of aspirin or acetaminophen, for more than 3 months prior to the study. No subject engaged in shift work for more than 3 months prior to the study. No subject was breastfeeding, pregnant, or within 1 year of childbirth. Subjects were studied in the general medical Clinical Research Center, where they were admitted for the duration of the study. All subjects signed an informed consent and received a Structured Clinical Interview for *Diagnostic and Statistical Manual of Mental Disorders* (SCID), *Fourth Edition* (patients) or SCID Non-Patient edition (controls) and structured 17-item Hamilton Rating Scale for Depression interview[26] performed by a trained psychiatric research nurse. A screening physical examination, blood analysis, and urine drug screening test were performed on all subjects and controls. All depressed subjects met *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV)* criteria for nonpsychotic major depression. No other current Axis I diagnoses were permitted except under the category of anxiety disrders other than obsessive-compulsive disorder. No subject had a history of alcohol or substance abuse within the past 3 years. The ethnic composition of the depressed sample was composed of 21 white subjects, 1 Asian subject, 1 African American subject, and 1 Hispanic subject. All controls met SCID criteria for never having a mental illness and had no first degree relatives with an Axis I or II psychiatric disorder. The ethnic composition of the control sample was composed of 23 white subjects and 2 African American subjects. The results of the urine drug screens were negative for all subjects at the time of the study.

STUDY DESIGN

To minimize delay in treatment, each woman with depression was studied in the clinic within 1 week of identification, and thus was studied on a random day of her menstrual cycle. All women were able to identify the date of onset of their last menstrual period and the day of the menstrual cycle on which the study was conducted was calculated from that information. Each control woman was age-matched to each depressed patient and then scheduled for admission to the clinical research center on the same day of her cycle as that of the corresponding depressed patient. To match the subjects by onset of menstrual cycle, we found it necessary to match the control to the depressed patient by menstrual cycle length. If the onset of menses was delayed in a control subject, the study was rescheduled for the appropriate day. Subjects came to the clinical research center at 8 AM, at which time an intravenous catheter was inserted into the antecubital vein. After a 60-minute recovery period, blood samples were drawn every 10 minutes for 12 hours (9 AM to 9 PM). All blood samples were drawn with a plastic syringe and mixed with EDTA in a polypropylene tube. Samples were immediately placed on ice and spun every 1 to 2 hours. Isotonic sodium chloride solution was infused throughout the study. While subjects were able to get up to go to the bathroom, they remained in bed for all 12 hours of the study. Meals were standardized in time and content. Eating between meals was not permitted, but decaffeinated coffee or diet soft drinks were provided at set times.

HORMONAL ASSAYS

Luteinizing hormone, FSH, estradiol, and progesterone were assayed by the automated ACS-180 (Ciba-Corning Diagnostics Corp, East Walpole, Mass) using a chemiluminescence-based assay. Obtained sensitivities for FSH ranged from 0.2 to 200 IU/L and for LH they ranged from 0.075 to 200 IU/L. Within-run coefficient of variation averaged between 2% to 3%; between-run coefficient of variation averaged 4% to 5%. For progesterone the sensitivity is 0.0159 nmol/L and the interassay variability is 4% to 5%. For estradiol, the assay sensitivity is 3.67 pmol/L (1 pg/mL) and the interassay variability is 9% to 11%. Due to changes in availability of reagents from Ciba-Corning, 2 different antibodies were used in the LH and estradiol assays. However, each subject pair always had the same LH or estradiol assay. Luteinizing hormone was assayed in every 10-minute sample and FSH, estradiol, and progesterone were assayed in every third sample (every 30 minutes) for a 12-hour period. All subjects were assayed as matched pairs.

STATISTICAL ANALYSES

Pulse frequency and amplitude were determined with Pulsefit.[27] **Figure 1** and **Figure 2** show the LH pulse profiles of 2 subjects with graphs of the fitted algorithm. Patients and controls were paired for statistical comparisons of pulse frequencies and amplitudes. For analyses of all hormones, menstrual cycle phase is critical. Thus, following assay of all hormones, the data were reviewed for possible problems in classification of the subjects. For the depressed patients, the estradiol and progesterone data were consistent with the calculated cycle day. However, 1 control was inadvertently studied during the LH surge so was deleted from the study and an additional control was recruited. A second control was reassigned from follicular to luteal phase based on her estradiol and progesterone levels, and another follicular phase control was recruited. Analyses were done by a 2-way repeated-measures analysis of variance with group (patient vs control) as 1 factor and menstrual cycle phase (follicular vs luteal) as the other factor. In these data, missing values were infrequent (1%). For the hormone concentration analysis, each outcome was the mean of the hormone concentration values (missing values were excluded from the mean calculation). For the LH pulsatile analysis, equally spaced observations were required, and missing values were interpolated in a fashion that did not induce pulses. Mean LH, mean amplitude, half-life, baseline, and total input (the summary measures that characterize pulsatile behavior) were log (base e) transformed before analyses. The number of pulses was analyzed after a square root transformation. Mean levels of FSH, mean estradiol, and progesterone were similarly analyzed by a 2-way repeated-measures analysis of variance after log transformation. Since the estradiol data suggested a decrease in the follicular phase only in the depressed patients, the follicular phase data were also analyzed by a signed rank test.

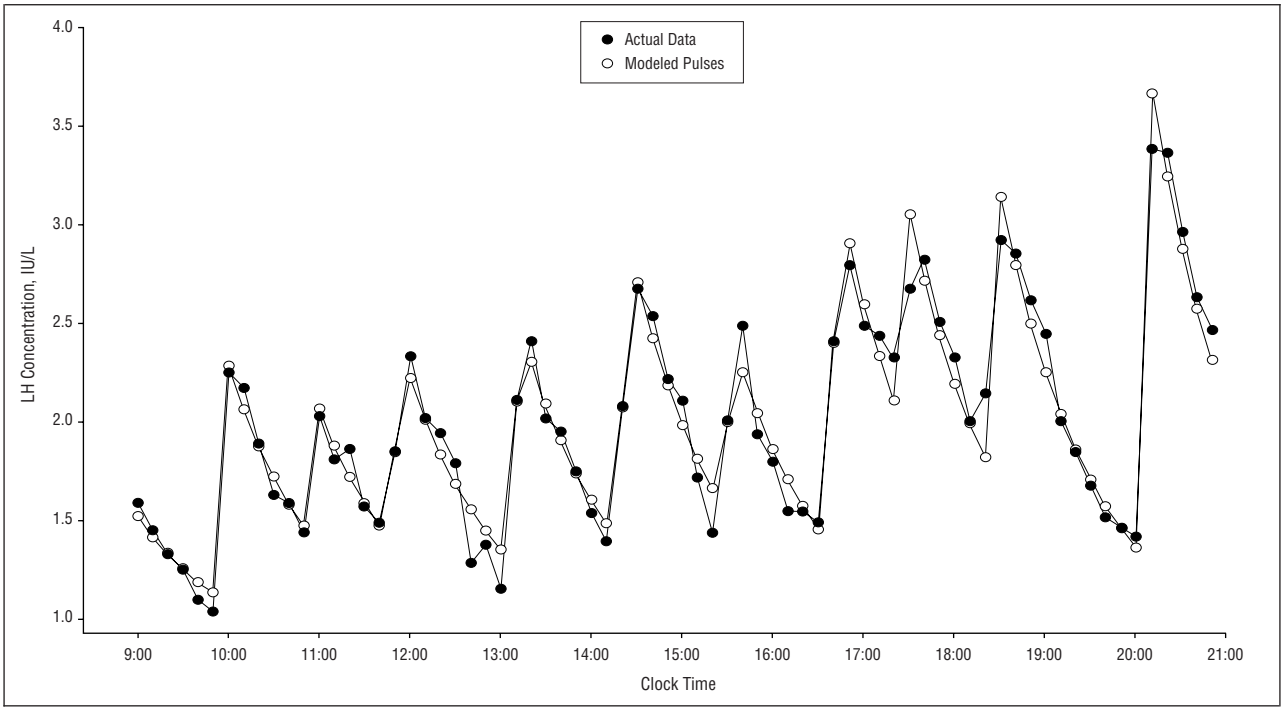

Figure 1. *Profile of luteinizing hormone (LH) secretion produced by actual data and modeled pulses produced by Pulsefit[27] in one subject in the follicular phase. Note the close agreement between actual data and fitted data.*

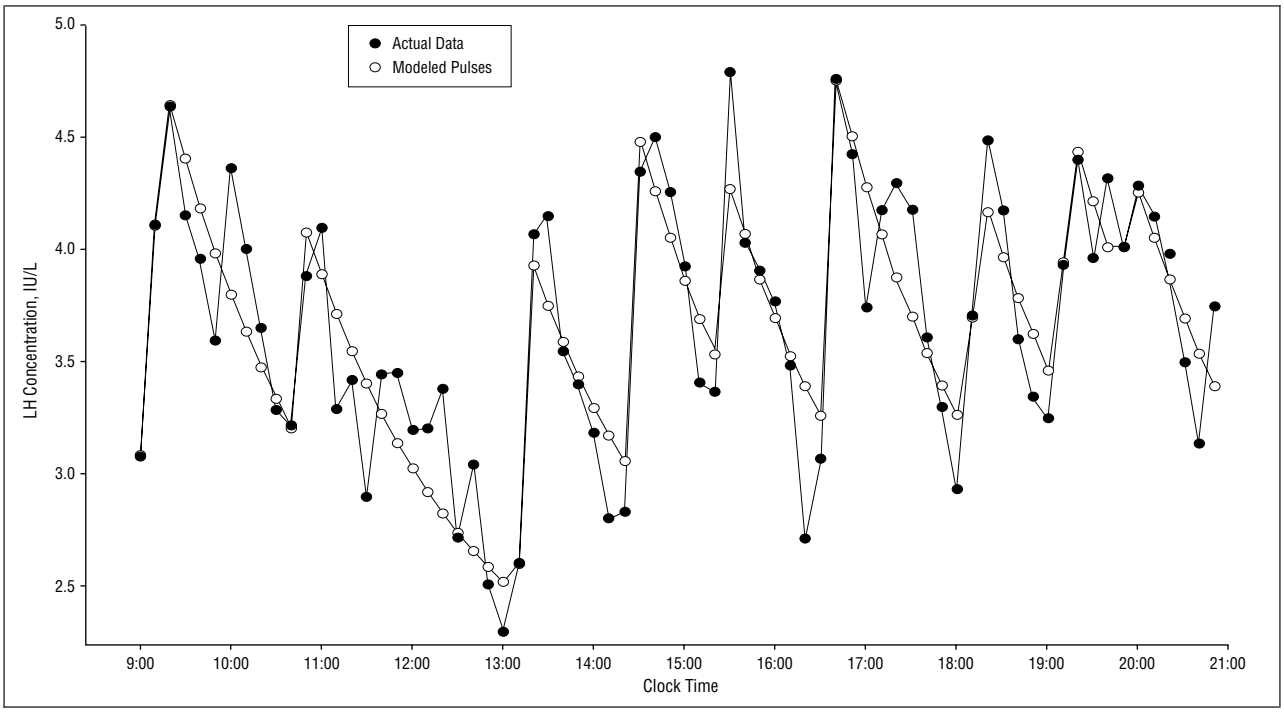

Figure 2. *Profile of luteinizing hormone (LH) secretion produced by actual data and modeled pulses produced by Pulsefit[27] in a different subject in the follicular phase. While the agreement between actual data and fitted data is still quite good, note the irregular pattern of pulses occurring at time 11:00, which was modeled as one pulse.*

the median eminence into the hypophyseal portal system.[19] Pulse frequency is critical in maintaining ovulatory function. Luteinizing hormone frequencies that are either too fast (polycystic ovary disease) or too slow (hypothalamic amenorrhea) lead to anovulation.[20,21] Furthermore, GnRH pulse frequency can differentially regulate messenger RNA (mRNA) levels for LH and follicle-stimulating hormone (FSH) β subunits.[22,23] Luteinizing hormone pulse frequency is the critical variable shown to be altered in hypothalamic amenorrhea or following stressors such as missed meals or intracerebroventral CRH infusions.[21,7,24,25] Thus, LH pulse frequency seems to be

Table 1. Luteinizing Hormone Pulse Data

	Mean ± SD				Statistics					
	Normal Controls		Depressed Patients		Group by Phase		Group		Menstrual Phase	
	Follicular (n = 12)	Luteal (n = 13)	Follicular (n = 12)	Luteal (n = 13)	$F_{1,23}$	*P*	$F_{1,23}$	*P*	$F_{1,23}$	*P*
Pulse No./12 h	11.7 ± 3.5	5.1 ± 2.4	11.6 ± 3.7	5.1 ± 2.5	<.001	.98	.01	.94	73.46	<.001
Total secretion, IU/L	24.3 ± 19.0	16.6 ± 6.4	15.0 ± 6.0	18.6 ± 11.4	1.01	.33	1.19	.29	0.39	.54
Pulse amplitude IU/L	2.1 ± 1.4	3.8 ± 1.6	1.4 ± 0.54	3.8 ± 1.9	1.77	.20	1.66	.21	26.78	<.001
Baseline, IU/L	3.9 ± 4.6	1.8 ± 1.5	2.5 ± 1.7	1.9 ± 1.7	0.30	.59	0.24	.63	3.57	.07
Half-life, min	43.5 ± 21.1	56.9 ± 13.6	34.1 ± 13.6	47.1 ± 10.0	0.10	.75	5.57	.027	14.21	.001

Table 2. Mean Reproductive Hormone Levels in Depressed Women and Matched Controls*

	Mean ± SD				Statistics					
	Normal Controls		Depressed Patients		Group by Phase		Group		Menstrual Phase	
	Follicular (n = 12)	Luteal (n = 13)	Follicular (n = 12)	Luteal (n = 13)	$F_{1,23}$	*P*	$F_{1,23}$	*P*	$F_{1,23}$	*P*
FSH, IU/L†	4.1 ± 0.58	3.4 ± 1.3	4.1 ± 1.0	3.4 ± 2.3	0.06	.81	0.98	.33	5.81	.02
LH, IU/L	6.0 ± 5.9	3.7 ± 2.0	3.6 ± 1.9	3.9 ± 2.8	0.83	.37	1.28	.27	1.18	.29
Progesterone, nmol/L	0.02 ± 0.007	0.18 ± 0.14	0.015 ± 0.007		0.97	.33	0.53	.47	67.94	<.001
Estradiol, pmol/L (pg/mL)	261 ± 169 (71 ± 46)	352 ± 235 (96 ± 64)	191 ± 136 (52 ± 37)	(92 ± 58)	1.11	.30	3.41	.09	2.37	.14
Estradiol in follicular phase, pmol/L (pg/mL)	261 ± 169 (71 ± 46)	. . .	191 ± 136 (52 ± 37)	. . .	. . .	. . .	. .‡	.03	. . .	. . .

**FSH indicates follicle-stimulating hormone; LH, luteinizing hormone. Ellipses indicate not applicable.*
†*Perimenopausal pair deleted from mean values of follicular phase.*
‡*Rank value = –27.*

the most sensitive marker of subtle reproductive abnormalities. Finally, LH pulse frequency reflects the integrity of the central rather than peripheral components of the axis and thus provides a better "window to the brain" than do mean hormone levels. Our study was undertaken to evaluate the HPO axis and LH pulse profiles in women with depression.

RESULTS

All patients met *DSM-IV* criteria for major depression. The mean±SD age of the patients was 29±7.8 years. The mean±SD age of the controls was 29±7.8 years. The mean±SD severity of depression by the Hamilton Rating Scale for Depression was 17.4±4.5. Of the 25 patients with major depression, 7 patients were studied in the first episode of major depression, 17 patients met criteria for recurrent unipolar depression, and 1 patient met criteria for bipolar II, depressed. In 7 subjects, dysthymia preceded the onset of the current episode of depression. Six patients met Research Diagnostic Criteria for endogenous depression for the current episode, 12 for probable endogenous depression, and 7 for nonendogenous depression. Nine of 25 subjects met criteria for at least 1 anxiety disorder: 2 with generalized anxiety, 4 with panic disorder, 5 with simple phobias, and 2 with social phobia.

Twelve of the patients and their matched controls were studied in the follicular phase and 13 in the luteal phase. We found no significant difference between patients and controls on any measure of LH except the half-life of LH, which was significantly shorter in patients than in controls (**Table 1**). We found decreased mean estradiol levels in the follicular phase in women with depression (**Table 2**, **Figure 3**). We also found the expected follicular-luteal phase differences in mean FSH levels, mean estradiol levels, LH pulse amplitude, and LH pulse half-life (Tables 1 and 2). There was 1 patient-control pair of regularly cycling women older than 45 years who were studied as part of this protocol. Examination of their hormone data revealed elevated mean LH and FSH levels, suggestive of perimenopause particularly in the control subject of this pair. All statistical analyses were also performed without this pair and there was no change in significant and nonsignificant findings.

COMMENT

Our study represents the first comprehensive assessment of reproductive hormones in women with major depression including detailed analyses of the LH pulse profile. Older studies that examined LH levels in depressed patients generally examined 1 to 4 samples collected before a GnRH challenge.[28-30] These studies included both premenopausal and postmenopausal women and men, and made no distinction between menstrual cycle phases. Since LH is secreted in pulses with ampli-

tude varying across the menstrual cycle (Table 1), and since removal of ovarian feedback following menopause leads to greatly increased LH levels, meaningful data cannot be obtained from examining mean LH levels across these groups. Furthermore, examining mean LH levels ignores pulse frequency, a critical component of the reproductive axis. The LH pulse frequency and amplitude were normal in each woman with depression. However, mean estradiol levels were lower in the follicular phase of the menstrual cycles of women with depression. A recent report by Schweiger et al[31] found decreased mean testosterone levels in men with major depression, leading the authors to conclude that gonadal function may be disturbed in men with major depression. Our data on women complement these findings suggesting that alterations in gonadal function can be associated with major depression in both men and women.

Given the normal LH pulsatility and normal FSH levels in women with depression, low levels of estradiol could occur secondary to an ovarian problem. The shorter half-life of LH in depressed patients could also contribute to lower estradiol levels. However, the lower estradiol levels in women with depression are not low enough to drive rebound LH secretion, since mean LH levels are similar in patients and controls. Furthermore, given the normal progesterone levels during the luteal phase in women with depression, there seems to be no ovulatory defect. This suggests that the critical neuronal circuits controlling both GnRH secretion and GnRH pulsatility are normal in women with depression. A previous study by Meller et al,[32] examining LH pulsatility in 10 depressed women in the follicular phase and 13 control women, also found no difference in the number of LH pulses during an 8-hour period, although significantly increased LH amplitude and LH area under the curve were observed in women with depression. In addition, our data are in agreement with the study of O'Toole and Rubin,[33] which examined mean LH and FSH levels in women with depression and menstrual status but not phase-matched control women during a 16-hour period, finding normal mean levels of LH and FSH in women with depression. Our data complement other data that have found LH response to GnRH administration in depression to be normal for the most part.[28-30,33] However, it should be pointed out that response to GnRH is also normal in women with hypothalamic amenorrhea, despite the profound alteration in reproductive functioning and LH pulsatility.

The finding of lower estradiol levels in women with depression may have implications for our understanding of mood disorders in women. Estradiol has effects on multiple brain systems including memory, synaptic density, and the neurotransmitter systems of serotonin and norepinephrine.[34-37] Estradiol also has behavioral effects, acting as an anxiolytic.[38] However, since the levels of estradiol are normal during the luteal phase in women with depression, the central nervous system effects of lowered estradiol levels are probably transient across the menstrual cycle. Finally, estrogen has been proposed as an adjunct to treatment of major depression, with some studies finding benefit in open-label studies,[39,40] although the results of placebo-controlled trials were mixed.[41,42] These findings of lowered estradiol levels in depressed premenopausal women lend support to further placebo-controlled trials of estrogen augmentation, which should be performed in conjunction with careful measurements of baseline estrogen during a 6- to 12-hour period.

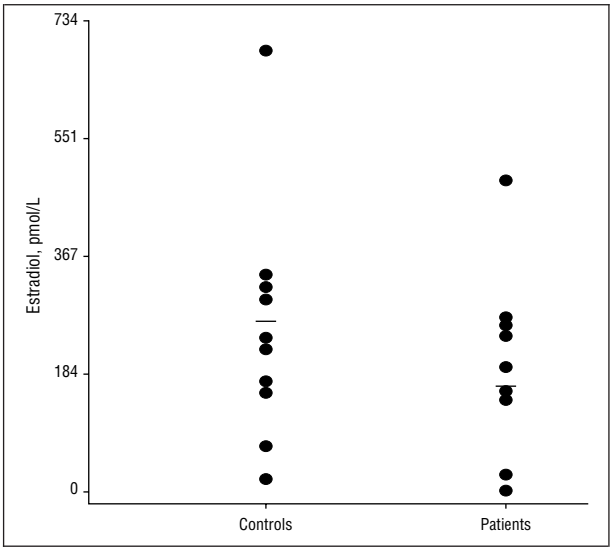

Figure 3. *Scatterplot of estradiol data from the follicular phase in women with depression and matched control subjects. There are 12 subjects in each group. The bar indicates mean values.*

Limitations of this study include the entry of depressed patients at random phases of the menstrual cycle rather than selecting 2 preset times such as early follicular and mid-luteal phases to assess the reproductive hormone profile. In addition, because we were recruiting from a treatment-seeking sample, we had to rely on self-reports for menstrual cycle day, although we did find that mean estradiol and progesterone levels in the patients concurred with their self-reports. However, we did not document the occurrence of ovulatory cycles in the patients and controls. If we had restricted the definition of luteal to ovulatory cycles in controls but not patients, this could have introduced an artificial bias. Similarly, if we had excluded patients with anovulatory cycles, we might have missed one of the abnormalities in reproductive functioning in women with depression. Finally, the sample size in the follicular phase was quite small, and we did not study women in the perimenopause or postpartum stages. Consequently, we do not know if lower levels of estradiol occur during times of major reproductive transitions in women with depression.

In conclusion, our study found generally normal reproductive function in women with depression in both the follicular and luteal phases compared with control women matched by age and menstrual cycle day. However, we did find significantly decreased levels of estradiol in women with depression. The estradiol data collected here are the means of 24 samples assayed every 30 minutes for 12 hours. We observed a 2-fold fluctuation of estradiol levels during the course of 12 hours, and thus a larger number of samples need to be collected to define accurately the mean estradiol level. Further studies are needed to confirm that women with depression have lower mean levels of estradiol. If true, this difference could affect a number of central nervous system systems modulated by estradiol.

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