Pergamon

Psychoneuroendocrinology, Vol. 20, No. 3, pp. 259–267, 1995

0306-4530/95 $9.50 + .00

0306-4530(94)00057-3

DAILY PLASMA ESTRADIOL AND PROGESTERONE LEVELS OVER THE MENSTRUAL CYCLE AND THEIR RELATION TO PREMENSTRUAL SYMPTOMS

EVA REDEI and ELLEN W. FREEMAN

Departments of Pharmacology, Psychiatry, and Obstetrics and Gynecology, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA

(Received 28 October 1993; in final form 16 May 1994)

SUMMARY

The present study extends a previous report of lower plasma ACTH levels in women with premenstrual syndrome (PMS) compared with asymptomatic controls. Plasma levels of estradiol and progesterone were measured daily in 10 women with confirmed PMS and 8 asymptomatic women. Daily symptom reports were maintained during the same menstrual cycle. Both estradiol and progesterone levels were consistently, but not significantly, higher throughout the cycle in PMS subjects compared with controls. From the follicular to the early luteal phase, estradiol levels were significantly higher in a previously defined PMS subgroup 2 with more severe symptoms throughout the cycle compared with both the less severe PMS subgroup 1 and controls. Progesterone levels were significantly and positively correlated with PMS symptoms along the entire menstrual cycle, preceding the symptoms by 5–7 days. These preliminary results provide support for the hypothesis that the presence of progesterone at early luteal phase levels is required for PMS symptoms to occur.

Keywords—Premenstrual syndrome (PMS); Estradiol; Progesterone; Menstrual cycle.

INTRODUCTION

THERE IS INCREASING evidence that absence of ovulation is involved in the remission of premenstrual syndrome (PMS) (Casson et al., 1990; Freeman et al., 1993; Hammarback & Backstrom, 1988; Mortola et al., 1991; Muse et al., 1984), but no direct relationship between PMS symptoms and the plasma levels of estrogen and progesterone has been identified (Backstrom et al., 1983; Rubinow et al., 1988; Schmidt et al., 1991). While the cyclic gonadal changes appear to be a necessary condition for PMS, the involvment of other hormonal factors may also be needed for PMS to occur in predisposed women.

We recently reported findings of lower plasma ACTH levels in PMS compared to control subjects (Redei & Freeman, 1993). This result was most pronounced in a subgroup of PMS subjects which appeared to be a "pure PMS" group, that is, those with the lowest follicular phase symptoms, large symptom increases premenstrually, and no other

Address correspondence and reprint requests to: Eva Redei, PhD, Departments of Pharmacology and Psychiatry, University of Pennsylvania, Clinical Research Building, 111B, Philadelphia, PA 19104-6141. E-mail: Redei@MSCF.Upenn.edu

psychiatric disorder. The plasma cortisol levels did not significantly differ between PMS and control subjects, although tended to be higher in the luteal phase in the PMS subjects, with the interaction effect reaching a trend level.

Since levels of both estrogen and progesterone can effect plasma ACTH levels, it is of interest to determine whether the two PMS subgroups (defined statistically on the basis of symptom scores) that clearly differed in ACTH levels would also differ in their progesterone and estrogen profiles as well. Furthermore, since ovulation appears to be a necessary condition for the mid- to late luteal phase PMS symptoms to occur, and late luteal endocrine events are not responsible for the manifestation of PMS symptoms (Schmidt et al., 1991), we hypothesized that progesterone levels at the ovulatory/early luteal phases of the cycle would be related to premenstrual symptom levels in the PMS subjects.

METHOD

Subject Selection

The study protocol was approved by the Institutional Review Board, and consent forms were signed by all subjects. Female volunteers were between 19 and 36 years of age (mean = 24.3 ± SD 4.2), had regular menstrual cycles of 22–35 days, were in general good health and had no current major psychiatric disorder as determined by clinical interview. Exclusion criteria for all subjects included current use of any medication including oral contraceptives, and any major physical or mental health condition as previously detailed (Redei & Freeman, 1993). A prior history of depression was reported by 2 PMS and no control subjects.

PMS Criteria

All subjects maintained daily symptom reports (DSR) to evaluate PMS symptoms. The DSR listed 17 common PMS symptoms, which the subjects rated daily on a 5-point scale (0, not at all, to 4, overwhelming). Control subjects showed low symptom ratings across the cycle. PMS subjects met the following criteria: 1) a total premenstrual DSR score (days 23–28) of at least 75, with 5 or more symptoms including one mood symptom rated on the same premenstrual day at 3 (distressing and interferes with daily activity) or 4 (overwhelming); 2) clear decrease or relief of symptoms following onset of menses; 3) at least 75% increase in the total premenstrual DSR scores (days 23–28) compared to total postmenstrual DSR scores (days 5–10) and for each of the 5 qualifying symptoms; 4) no current major psychiatric diagnosis; 5) occurrence of PMS for the past year according to subject report; and 6) symptom status confirmed by daily symptom reports during the studied cycle. Nine of the 10 PMS subjects had at least five of the symptoms required for diagnosis of Late Luteal Phase Dysphoric Disorder (LLPDD) (American Psychiatric Association, 1987).

Evidence of Ovulation

All cycles in the analysis were ovulatory as indicated by luteal phase plasma progesterone levels >15 nmol/l.

Subject Exclusions

The final sample for analysis included 10 PMS and 8 control subjects who met all criteria and completed the study procedures. Subjects were excluded if the studied cycle was nonovulatory or abnormal based on daily plasma progesterone and estrogen levels

(5 PMS and 2 control subjects) or if symptom status was not confirmed by the daily symptom reports in the studied cycle (3 PMS and 3 control subjects).

Procedure

Each day for one menstrual cycle, nonfasting blood samples (3 ml) were drawn from the arm between 0800 and 1000h and collected into chilled tubes containing EDTA (1.25 mg/ml blood and Aprotinin (500 KIU/ml). Blood samples were centrifuged at 4°C and plasma was stored at −70°C. Additional testing was conducted every fourth day using an intravenous catheter with heparin lock and drawing three blood samples at 10-min intervals. Analysis of the multiple samples showed no significant changes in hormone secretion during the 30-min intervals, and the mean values on the multiple sample days were used in the analysis.

Symptom Assessments

Subjects maintained daily symptom reports (described above) throughout the study cycle.

Endocrine Methods

Plasma steroid levels were measured in multiple assays, where samples were evenly distributed across groups.

Progesterone concentrations were measured in unextracted plasma using antibody raised against 11α-hydroxyprogesterone-hemisuccinate-BSA (ICN Biomedicals Inc., Carson, CA) and ^{125}I-progesterone tracer. The antibody had less than 5% cross reactivity with any other steroid. The assay sensitivity was 0.6 nmol/l, the intra- and interassay coefficients of variation were 8.7 and 12.3%, respectively. Total plasma estradiol-17β was measured in unextracted samples using antisera (ICN Biomedicals Inc., Carson, CA) raised against 6-keto-estradiol-17β-6-oxime-BSA and ^{125}I-tracer. The antibody cross reacts with estrone (15%). The assay sensitivity was 35 pmol/l, the intra and interassay coefficients of variation were 6.6 and 13.5%, respectively.

Definition of Cycle Phases

The cycle was divided into seven phases, using the phase definitions of Gallant et al. (1992), which kept constant the number of days in the periovulatory and luteal phases and anchored day 28 to the day before bleeding: days 25–28 (late luteal), days 21–24 (mid-luteal), days 17–20 (early luteal), days 14–16 (periovulatory), menses (menstrual), 4 days postmenses (early follicular), and the days remaining between early follicular and periovulatory (late follicular).

Statistical Analysis

Means of the daily measures were calculated for each subject in each cycle phase for each of the hormone and symptom scores. Hormone levels were compared between groups using repeated measures analysis of variance with Tukey studentized range tests in post hoc analyses. Correlations of hormones and symptom scores used the Spearman statistic; single sample *t*-tests were used for the mean correlation with the Bonferroni correction for multiple tests.

The two PMS subgroups were previously derived by a cluster analysis applied to the DSR scores (Redei & Freeman, 1993). The clustering procedure produced two clusters of PMS patients with nonoverlapping symptom scores in the ovulatory and luteal phases.

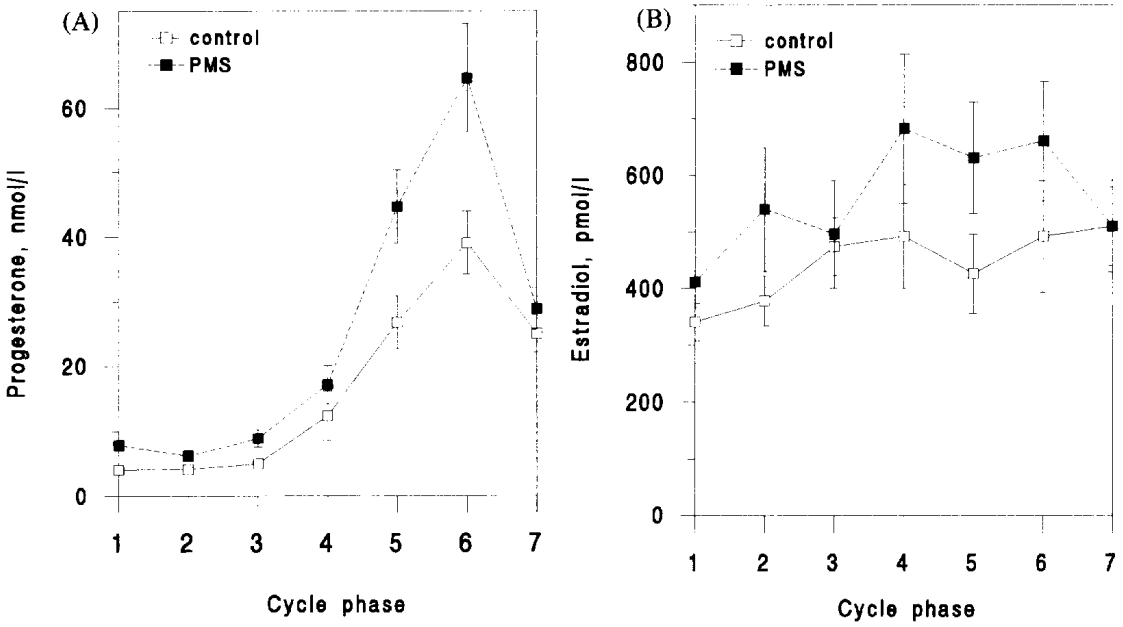

FIG. 1: Plasma levels throughout the menstrual cycle of progesterone (A) and estradiol (B) in subjects with premenstrual syndrome (n = 10) and asymptomatic controls (n = 8). Mean plasma hormone levels for each subject and each cycle phase were calculated from daily hormone measures, where cycle phases were determined as described in the Method Section. Mean and SEM values in the figure were calculated from these individual means for each cycle phase.

The symptom scores overlapped in the follicular phase, indicating that "low" follicular phase symptom levels characterized both PMS cluster groups. PMS subgroup 1 had lower symptom scores in the ovulatory and luteal phases, and was characterized by significantly lower levels of ACTH over the menstrual cycle. PMS subgroup 2 had higher symptom scores and ACTH levels similar to asymptomatic controls.

Probability testing was based on significance levels set at $p < .05$, 2-tailed. The software package was SAS (SAS, 1989).

RESULTS

Plasma progesterone levels were within the normal range and were consistently but not significantly higher over the menstrual cycle ($F[1, 16] = 2.14$, $p = .16$) in the PMS subjects compared to normal controls, as depicted in Fig. 1A. The late follicular phase (phase 3) had the greatest statistical difference between the PMS and control groups (8.87 ± 1.27 nmol/l for PMS and 4.49 ± 0.54 nmol/l for controls, $p < .08$).

Plasma estradiol levels were also within the normal range and were consistently but not significantly higher in the PMS subjects compared to controls ($F[1, 15] = 0.99$), as depicted in Fig. 1B.

Figure 2 depicts the progesterone and estradiol levels for the two previously defined PMS subgroups (Redei & Freeman, 1993) compared to the normal controls. PMS subgroup 1 (n = 6), which was characterized by significantly lower plasma levels of ACTH over the menstrual cycle, had gonadal hormone levels similar to the normal controls (Fig. 2A). In contrast, PMS subgroup 2 (n = 4), which was previously characterized by

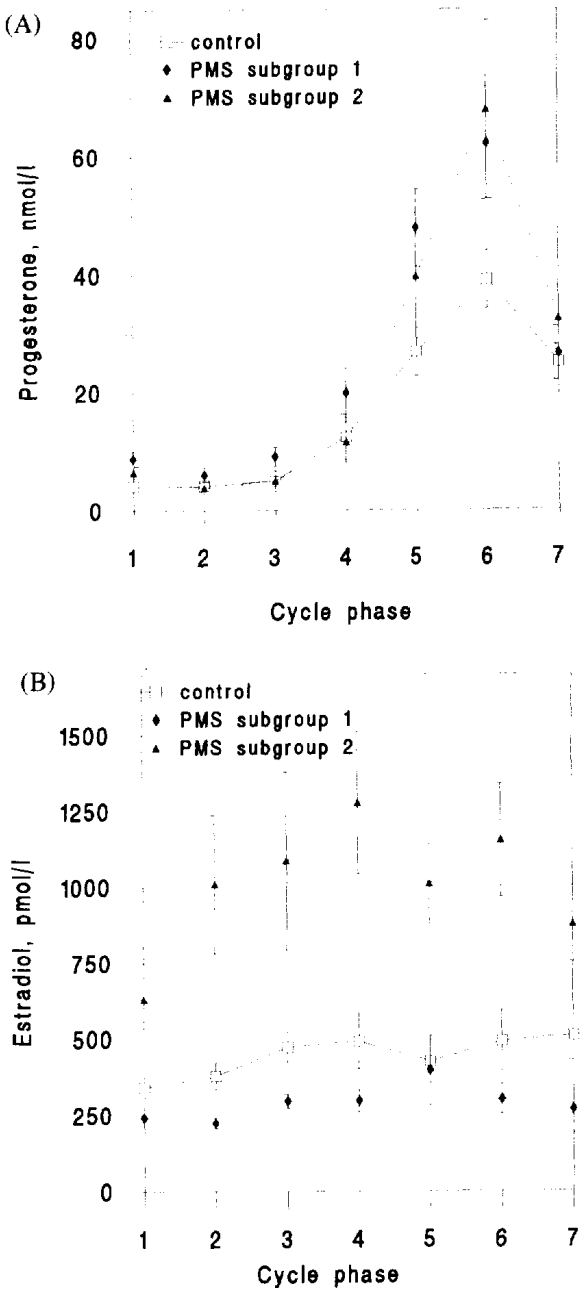

FIG. 2: Plasma levels of progesterone (A) and estradiol (B) in the previously defined PMS subgroup 1 (n = 6) and PMS subgroup 2 (n = 4). Asymptomatic controls are the same as in Fig. 1. Values are means with SEM, calculated as described in the Method Section and Fig. 1.

normal plasma levels of ACTH over the menstrual cycle, had similar progesterone levels but significantly higher estradiol levels compared to the normal controls and to PMS subgroup 1 (F[2, 15] = 8.05, $p < .004$) (Fig. 2B). Comparisons of estadiol levels in each phase between subgroup 2 and controls showed significant differences from the follicular to the early luteal phases (phases 2–5), with the greatest difference around ovulation

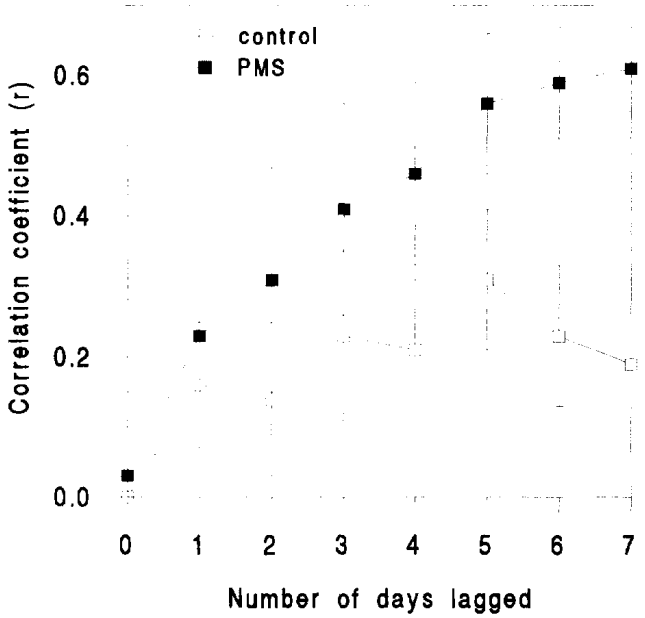

FIG. 3: Within subject correlations between plasma levels of progesterone and daily symptom scores along the entire menstrual cycle on the same day and 1–8 days later. Values represent means ± SEM of individual correlations.

(subgroup 2 mean estradiol in phase 4 = 1277.51 ± 234.94 pmol/l and 491.91 ± 91.78 pmol/l for controls, $p < .02$).

When the progesterone levels for each subject were correlated over the entire menstrual cycle with lagged DSR scores, the mean correlations were not significant in controls, but were highly significant in the PMS group when DSR scores were lagged 5–7 days (Fig. 3, means = 0.56, 0.59, 0.61, respectively, $p < .001$ with Bonferroni correction). The correlation of progesterone and lagged symptom scores was observed in both PMS subgroups. The relationship between progesterone levels and symptom scores lagged 5–7 days was found only for progesterone and not for estrogen, ACTH or cortisol.

To determine which symptoms contributed to the observed relationship between progesterone levels and lagged symptom scores, the same correlation procedures were repeated for each of the DSR symptoms. Significant differences compared to controls were found for the correlations of progesterone with symptoms of swelling ($r = 0.54$), cramps ($r = 0.54$), irritability ($r = 0.53$), nervous tension ($r = 0.48$), food cravings ($r = 0.48$), fatigue ($r = 0.45$), and anxiety ($r = 0.43$).

DISCUSSION

In this study, estradiol and progesterone levels, measured daily throughout the menstrual cycle, were consistently but not significantly higher in PMS subjects compared to asymptomatic controls. There were differences in plasma estrogen levels between the two previously defined subgroups with levels being significantly higher in the subgroup of patients with higher symptom scores. Furthermore, when hormone levels were correlated with DSR scores lagged 5–7 days along the entire menstrual cycle, there was a significant correlation between the DSR scores and progesterone but no other studied

hormone. This latter relationship suggests that progesterone plays a role in PMS symptoms, but the mechanisms are undefined.

The present data do not indicate the cause of elevated estrogen levels in PMS subgroup 2. There is a possible connection between this increased estrogen and the unaltered levels of ACTH and cortisol found in PMS subgroup 2 with higher symptom scores (Redei & Freeman, 1993). Estrogen has been shown to directly increase gene expression of corticotropin releasing factor in the hypothalamus (Vamvakopoulos & Chrousos, 1993) which can lead to elevation of ACTH levels into the control range in PMS subgroup 2. Alternatively, this association may lie in the effect of estrogen in increasing the production of corticosteroid binding globulin (CBG) (Sandberg et al., 1964). Although estrogen-induced increases in CBG usually coincide with increases in total cortisol levels and no changes in free cortisol, increased levels of CBG have been seen in women taking oral contraceptives without significant increase in total cortisol levels (Scott et al., 1990). Since we have similarly not found increased total cortisol levels in the PMS subgroup 2, we hypothesize that elevated estrogen levels may lead to decreased levels of free cortisol and reduced negative feedback inhibition of ACTH secretion in this PMS subgroup. This hypothesis could be tested by measuring levels of CBG and free cortisol in these two PMS subgroups and in controls.

It is noteworthy that the significant findings in this study occurred primarily around ovulation, the late follicular or early luteal phases. Previously, Schmidt et al. (1991) clearly showed that endocrine events in the late luteal phase did not directly generate PMS symptoms, and that there was no correlation between gonadal hormone levels and same day symptom scores. However, Halbreich et al. (1986) found significant correlations between plasma progesterone levels and symptom scores lagged 5–7 days, results that were replicated in the present study and suggest that hormonal events prior to the luteal phase provoke PMS symptoms.

Previous data on plasma progesterone and estrogen levels in PMS studies are characterized by inconsistent results and lack of agreement (Adamopoulos et al., 1972; Backstrom & Carstensen, 1974; Eriksson et al., 1992; Halbreich et al., 1986; Hammerback et al., 1989; Munday et al., 1981; O'Brien et al., 1980; Rubinow et al., 1988; Trunnell et al., 1988; Watts et al., 1985). Cycles with higher plasma estradiol and progesterone levels in the periovulatory and luteal phases were reported to correlate with increased severity of premenstrual symptoms (Hammerback et al., 1989), observations similar to the results in the present study. Interestingly, Dennerstein et al. (1984) reported a bimodal distribution in urinary estrogen values of PMS women in the luteal phase, a finding that parallels the subgroup differences in the present study. Similarly, Taylor (1979) found that premenstrual estradiol levels were lower in women with low affective symptoms compared with the levels of women with more severe affective symptoms. Both reports are consistent with our findings of differing estrogen secretion in the PMS subgroups, and both utilized daily hormonal measurements and daily symptom assessments, as was done in the present study.

Several lines of previous evidence suggest gonadal hormone involvement in PMS, but the mechanisms remain speculative. Gonadal steroids most likely act in the central nervous system where time-dependent genomic events in specific areas of the brain may affect mood and behavior (McEwen, 1991). While there are no data regarding direct effects of changing levels of progesterone on the brain, a large body of literature describes neural progestin receptors that are not different from peripheral progesterone receptors (MacLusky & McEwen, 1978, 1980). A finding that some progesterone metabolites are

barbiturate-like ligands for the GABA receptor-chloride ion channel complex (Majewska et al., 1986) suggests an alternate type of membrane-related mechanism through which progesterone may have another direct action in the brain. Estrogen may also affect the brain directly or indirectly and can influence central availability of glucocorticoids, which affect central nervous system activity and influence mood and behavior (McEwen, 1987).

In summary, it appears from this small pilot study that, regardless of PMS group heterogeneity, there is an indirect involvment of gonadal hormones prior to the luteal phase symptoms, and that progesterone levels are associated with PMS symptoms directly in a time-lagged manner. Thus, we hypothesize that ovulation-dependent increases in progesterone levels are necessary but not sufficient to cause PMS symptoms. Further study in larger samples of other hormonal and behavioral characteristics, superimposed on the permissive role of progesterone in the PMS population, could aid in identifying the etiology of PMS.

Acknowledgments: This study was supported by grants from the Eberhard Foundation, grants MH 45862 and HD 18633 from the National Institute of Health and by the Clinical Research Center grant 5 MO1 PR00040 from the National Institute of Health. Both authors contributed equally to this study.

The authors express appreciation for the expert technical assistance provided by Barbara Altimari, Beatriz Garcia-Espana and Li-Fang Li.

REFERENCES

Adamopoulos DA, Loraine JA, Lunn SF, Coppen AJ, Daly RJ (1972) Endocrine profiles in premenstrual tension. Clin Endocrinol 1:283–292.

American Psychiatric Association (1987) Diagnostic and Statistical Manual for Mental Disorders: DSM-III-R, Third Edition Revised. American Psychiatric Association, Washington DC.

Backstrom T, Carstensen H (1974) Estrogen and progesterone in plasma in relation to prementrual tension. J Steriod Biochem 5:257–260.

Backstrom T, Sanders D, Leask R, Davidson D, Warner P, Bancroft J (1983) Mood, sexuality, hormones and the menstrual cycle. II. Hormone levels and their relationship to the premenstrual syndrome. Psychosom Med 45:503–507.

Casson P, Hahn PM, Van Vugt DA, Reid RL (1990) Lasting response to ovariectomy in severe intractable premenstrual syndrome. Am J Obstet Gynecol 162:99–105.

Dennerstein L, Spencer-Gardner C, Brown JB, Smith MA, Burrows GD (1984) Premenstrual tension: Hormonal profiles. J Psychosom Obstet Gynecol 3:37–51.

Eriksson E, Sundblad C, Lisjo P, Modigh K, Anderch B (1992) Serum levels of androgens are higher in women with premenstrual irritability and dysphoria than in controls. Psychneuroendocrinology 17:195–204.

Freeman EW, Sondheimer SJ, Rickels K, Albert J (1993) Gonadotropin-Releasing Hormone agonist in treatment of premenstrual symptoms with and without comorbidity of depression: A pilot study. J Clin Psychiatry 54:192–195.

Gallant SJ, Popiel DA, Hoffman DM, Chakraborty PK, Hamilton JA (1992) Using daily ratings to confirm premenstrual syndrome/late luteal phase dysphoric disorder. Part II. What makes a "real" difference. Psychosom Med 54:167–181.

Halbreich U, Endicott J, Goldstein S, Nee J (1986) Premenstrual changes and changes in gonadal hormones. Acta Psychiatry Scand 74:576–586.

Hammarback S, Backstrom T (1988) Induced anovulation as a treatment of premenstrual tension syndrome: A double-blind crossover study with GnRH-agonist vs. placebo. Acta Obstet Gynecol Scand 67:159–166.

Hammarback S, Damber JE, Backstrom T (1989) Relationship between symptom severity and hormone changes in women with premenstrual syndrome. J Clin Endocrinol Metab 68:125–130.

MacLusky NJ, McEwen BS (1978) Oestrogen modulates progestin receptor concentrations in some rat brain regions but not in others. Nature 274:276–278.

MacLusky NJ, McEwen BS (1980) Progestin receptors in rat brain: Distribution and properties of cytoplasmic progestin-binding sites. Endocrinology 106:192–202.

Majewska MD, Harrison NC, Schwartz RD, Barker JL, Paul SM (1986) Steroid hormone metabolites are barbiturate-like modulators of the gaba receptor. Science 232:1004–1007.
McEwen BS (1987) Glucocorticoid-biogenic amine interactions in relation to mood and behavior. Biochem Pharmacol 36:1755–1763.
McEwen BS (1991) Nongenomic and genomic effects of steroids on neural activity. Trends Pharmacol Sci 12:141–147.
Mortola JF, Girton L, Fischer U (1991) Successful treatment of severe premenstrual syndrome by combined use of gonadotropin-releasing hormone agonist and estrogen/progesterone. J Clin Endocrinol Metab 72:252A–F.
Munday MR, Brush MG, Taylor RW (1981) Correlations between progesterone, oestradiol and aldosterone levels in the premenstrual syndrome. Clin Endocrinol 14:1–9.
Muse KN, Cetel NS, Futterman LA, Yen SSC (1984) The premenstrual syndrome: Effects of "medical ovariectomy." N Engl J Med 311:1345–1349.
O'Brien PMS, Selby C, Symonds EM (1980) Progesterone fluids and electrolytes in premenstrual syndrome. Br Med J 1:1161–1163.
Redei E, Freeman EW (1993) Preliminary evidence for plasma adrenocorticotropin levels as biological correlates of premenstrual symptoms. Acta Endocrinol 128:536–542.
Rubinow DR, Hoban MC, Grover GN, Galloway DS, Roy-Byrne P, Andersen R, Merriam GR (1988) Changes in plasma hormones across the menstrual cycle in patients with menstrually related mood disorder and in control subjects. Am J Obstet Gynecol 158:5–11.
Sandberg AA, Woodruff M, Rosenthal HE, Nienhouse A, Slaunwhite WR Jr (1964) Transcortin: A corticosteroid-binding globulin of plasma. VII. Half-life in normal and oestrogen treated subjects. J Clin Invest 43:461–464.
SAS/STAT User's Guide, Version 6, 4th Ed. (1989) SAS Institute Inc., Cary, NC.
Schmidt PJ, Nieman LK, Grover GN, Muller KL, Merriam GR, Rubinow DR (1991) Lack of effect of induced menses on symptoms in women with premenstrual syndrome. N Engl J Med 324:1174–1179.
Scott EM, McGarrigle HG, Lachelin GCL (1990) The increase in plasma and saliva cortisol levels in pregnancy is not due to the increase in corticosteroid binding globulin levels. J Clin Endocrinol Metab 71:639–644.
Taylor JW (1979) Plasma progesterone, oestradiol 17β and premenstrual symptoms. Acta Psychiatry Scand 60:76–86.
Trunnell EP, Turner CW, Key WR (1988) A comparison of the psychological and hormonal factors in women with and without premenstrual syndrome. J Abnorm Psychol 92:429–436.
Vamvakopoulos C, Chrousos GP (1993) Evidence of direct estrogenic regulation of human corticotropin-releasing hormone gene expression. J Clin Invest 92:1896–1902.
Watts JFF, Butt WR, Edwards RL (1985) Hormonal studies in women with premenstrual tension. Br J Obstet Gynecol 92:247–255.