Protocol Title: An Endocrine Model for Postpartum Mood Disorders
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Total requested accrual: 100
(40) Patients
(40) Volunteers
(20) Past Major Depression

IND/IDE ☐ No ☒ Yes *(attach FDA documentation)*
Drug/Device/#__163205 Lupron_____
Sponsor _NIMH (Maryland Pao, MD is the official representing the Institute)

Durable Power of Attorney ☒ No ☐ Yes
Multi-institutional Project ☐ No ☒ Yes
Institution_____ FWA #_____
Date of IRB approval_____ *(attach IRB documentation)*

Data and Safety Monitoring Board ☒ No ☐ Yes

Technology Transfer Agreement ☐ No ☒ Yes
Agreement type and number MTA 2017-0327_____Expiration Date__None
_____

Confidential Disclosure Agreement ☒ No ☐ Yes

Samples are being stored ☐ No ☒ Yes

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Precis:

The appearance of mood and behavioral symptoms during pregnancy and the postpartum period has been extensively reported. While there has been much speculation about possible biologically based etiologies for postpartum disorders (PPD), none has ever been confirmed. Preliminary results from two related studies (protocols 90-M-0088, 92-M-0174) provide evidence that women with menstrual cycle related mood disorder, but not controls, experience mood disturbances during exogenous replacement of physiologic levels of gonadal steroids. The present protocol is designed to create a "scaled-down" hormonal milieu of pregnancy and the puerperium in order to determine whether women who have had a previous episode of postpartum major affective episode will experience differential mood and behavioral effects compared with controls and to determine whether it is the abrupt withdrawal of gonadal steroids or the prolonged exposure to gonadal steroids that is associated with mood symptoms. Supraphysiologic plasma levels of gonadal steroids will be established, maintained, and then rapidly reduced, simulating the hormonal events that occur during pregnancy and parturition. This will be accomplished by administering estradiol and progesterone to women who are pretreated with a gonadotropin releasing hormone (GnRH) agonist (Lupron). After eight weeks, administration of gonadal steroids will be stopped in one group of patients and controls, and a sudden decline in the plasma hormone levels will be precipitated. Another group will be maintained on supraphysiologic levels of estradiol and progesterone for an additional month. Outcome measures will include mood, behavioral, and hormonal parameters.

1. Introduction:

a. Background and Introduction

Affective syndromes that occur during the postpartum period have traditionally been divided into three categories: 1) postpartum "blues", 2) postpartum depression, 3) puerperal psychosis. The present protocol will only deal with the last two categories, which are associated with more persistent symptoms and with a higher rate of morbidity.

Puerperal psychosis is the most severe form of psychiatric disturbance in the puerperium. This condition is characterized by extreme agitation and confusion, lability of mood, severe sleep disturbances, possible paranoid or grandiose delusions, and in some cases prominent hallucinations. Results of both retrospective case register studies and longitudinal prospective studies have observed that the majority of cases of puerperal psychosis begin during the first few weeks after parturition (1,2). The prevalence of psychosis in the month after childbirth is approximately 22 times that of any month in the preceding two years (3). Women with a history of bipolar or schizoaffective illness are particularly at risk, and about 50% of these women relapse in the first few months after delivery (4-6). In fact most cases of postpartum psychosis are considered to be an extreme manifestation of a bipolar disorder (7), and the overall incidence of this condition is very low, occurring in 0.2% of all deliveries.

Postpartum depression is clinically similar to the DSM-IV defined major depression, but its onset has a temporal relationship to parturition. The main symptoms are appetite and sleep disturbance, excessive fatigue, sadness and anhedonia, excessive guilt, psychomotor and cognitive disturbances and suicidal ideation. The two to three month prevalence rates of postpartum depression in studies using conventional diagnostic criteria (8) (e.g., RDC, DSM-III) have been reported to be in the range of 8.2% to 14.9% (9,10). Some studies (1,10,11), but not

all (12), have reported that the incidence of depression is increased significantly during the first 3 months after birth as compared to during pre-pregnancy, pregnancy or the period after the first postpartum year. Others have disputed this association, arguing that the prevalence of depression during the postpartum period is no greater than that for depression at other periods of a woman's life (12). Factors associated with postpartum depression are a history of psychiatric illness, family history of psychiatric illness, marital disharmony and lack of confiding relationships, maternal age and the number of life events in the previous year (13-15). Additionally, several studies have suggested that a past history of postpartum disorders (PPD) may predispose women to develop an episode of PPD during subsequent pregnancies. In a longitudinal prospective study (16) women with a past history of PPD had a significantly higher rate of PPD during a subsequent pregnancy than women without a history of PPD, specifically, 28-40% compared with 10%. These findings are corroborated by preliminary results from a longitudinal prospective study conducted at the Massachusetts General Hospital suggesting a higher relative risk of PPD during subsequent pregnancies in women with a prior episode of PPD (17,18).

b. Biological Factors in Postpartum Disorders:

Hormonal factors have been implicated as having an important etiological role in the development of PPD. Pregnancy is accompanied by a slow but sustained rise in various steroid and peptide hormones, followed by a sudden drop over the first few days after delivery. During the third trimester of pregnancy, plasma progesterone levels are approximately 150 ng/ml and estradiol reaches plasma levels of approximately 10-15 ng/ml. These levels represent a 10 and 50 fold increase, respectively, of maximum menstrual cycle levels (19). After parturition progesterone and estradiol levels drop to early follicular phase levels within days (20).

A role for progesterone and estradiol in postpartum mood disorders is suggested by their manifold neuroregulatory effects (21).

1). Progesterone - In addition to its genomic effect, progesterone has rapid effects on neuronal membranes (22). The rapid membrane action of progesterone metabolites is thought to be responsible for the anaesthetic action of progesterone (23) and occurs through positive modulation of the gamma-aminobutyric acid-A (GABA-A) receptor (24). These progesterone metabolites (e.g., allopregnanolone) may be formed from circulating progesterone or may be synthesized de novo in the brain and are, therefore, known as neurosteroids. Some researchers have postulated that the levels of neurosteroids reached in the brain are physiologically significant and compatible with their playing a neuromodulatory role during the estrous cycle and pregnancy, possibly associated with behavioral changes seen in these conditions (25,26). Studies, using different outcome measures, confirm that in female rats, GABA-A receptor sensitivity to specific neurosteroids such as allopregnanolone (26) is cycle-dependent. Pregnancy and the postpartum period have also been reported to both increase and decrease the affinity of GABA receptors in the brain for a variety of ligands (26,27). These effects of progesterone could provide the theoretical ground for a "progesterone withdrawal" hypothesis for PPD.

Studies have been unable to identify a significant association between progesterone levels and either the "blues" (28,29) or postpartum depression (30). However, one study did report a significant correlation between progesterone levels around parturition and the "blues." Additionally, Dalton treated women at risk for the development of PPD with open label progesterone following parturition and observed no recurrence of PPD (31), suggesting a preventive effect of progesterone on the onset of PPD in this sample. In contrast, there is at least one study that indicates that giving progestins during the postpartum period actually increases

postpartum depressive symptoms (32). It remains unclear whether there is a causal role for changing levels of progesterone in the onset of symptoms of PPD (30).

2). Estradiol - Estradiol has been found to alter brain neurotransmitter activity in several ways: in the hypothalamus, estradiol (E2) is associated with decreased norepinephrine synthesis and increased choline acetyltransferase activity (33), decreased glutamic acid decarboxylase activity (34), and increased muscarinic receptors (35,36); in the cerebral cortex, E2 replacement in ovariectomized rats decreases beta-adrenergic and 5HT-1 receptors but increases 5HT-2 receptors (37); and E2 facilitates excitability of hippocampal neurons in vitro (38,39) and in vivo (40), through both short term and long term mechanisms, and decreases the threshold to electrically-induced seizures in the medial amygdaloid nucleus (41,42). Additionally, E2 increases NMDA binding sites in the CA1 region of the hippocampus (43). An anti-dopaminergic effect of estradiol at both the pituitary and striatum has been described (44). Elevated levels of estradiol have been reported to decrease tyrosine hydroxylase activity and hence dopamine synthesis (45), while even low doses can modulate the striatal agonist affinity states of the D_2 receptor (46). Consistent with these putative antidopaminergic effects is the observation of improvement of L-DOPA-induced tardive dyskinesia with estradiol (47) and the amelioration of psychotic symptoms in schizophrenic women during the high estradiol phase of their menstrual cycle (48). Conversely, it should be noted that enhanced dopamine receptor sensitivity following estradiol administration has been reported (49,50). Regional brain MAO activity has been shown to be increased by progesterone (51) and decreased by estradiol (52).

Most studies have failed to show an association between blues or depression and estradiol plasma levels around parturition (29,30). However, there are preliminary data that indirectly suggest a role for estradiol in postpartum disorders. Women who later have a recurrence of PPD, as opposed to women who remain well, show an increased D_2 sensitivity postpartum as reflected

by exaggerated growth hormone response to apomorphine (6,53). This modification in D_2 sensitivity may result from altered effects of estradiol on D_2 receptors in women with PPD, given the ability under normal conditions of E_2 to inhibit D_2 activity by uncoupling the D_2 receptor from its second messengers and to decrease D_2 receptor sensitivity (46,54). Furthermore, estradiol replacement has been reported to be an effective treatment for postpartum depression under double-blind, placebo-controlled conditions (55).

3) Other hormones - A significant association has been found between postpartum thyroid dysfunction and the development of postpartum depression (56,57). It has also been shown that women with a positive thyroid antibody status, which occurs in approximately 10% of normal women, are prone to episodes of postpartum depression (58), and up to 1% of all postpartum women may develop a depression associated with autoimmune thyroid disease. More recently, Pedersen et al., in press, reported a negative correlation between antenatal thyroxine levels and self-reported depression scores in the postpartum period, suggesting that low antenatal thyroid status is a risk for developing PPD.

Preliminary studies also indicate a possible association of HPA axis dysregulation and the development of postpartum depression. There is one study suggesting that symptoms of depression during pregnancy are associated with lower levels of CRH (59). Our own preliminary data indicate a significant study phase effect on integrated cortisol secretion (increased during addback compared with the withdrawal phase) and a trend for a diagnosis by hormone condition interaction, reflecting increased cortisol in women with a history of PPD compared to controls during the addback phase (60).

Other hormones such as prolactin, androgens, beta-endorphin, vasopressin, oxytocin, and growth hormone have marked fluctuations during pregnancy and parturition. Association between these hormones and psychiatric symptoms have not been substantiated.

Despite these observations, there are only a few systematic studies of the role of gonadal steroids in the etiology of PPD. This probably reflects a number of factors, including the low prevalence of PPD in the community, the presence of numerous psychosocial and medical stressors during pregnancy and the puerperium, and the difficulties involved in recruiting and evaluating women who are either pregnant or lactating and nursing an offspring. To avoid these problems, we propose to study women with a history of PPD compared with women without a history of PPD, in order to answer the questions, 1) "Are women who develop PPD specifically susceptible to the hormonal events of pregnancy and the puerperium?"; and 2) Is it the abrupt withdrawal of gonadal steroids or the prolonged exposure to gonadal steroids that is associated with mood symptoms in these women?

Preliminary results of this study demonstrate that acute withdrawal of estradiol and progesterone is associated with a recurrence of mood and behavioral symptoms in women with a history of PPD but not in women lacking such history (61). These findings document a differential sensitivity to changes in gonadal steroid levels in women with a history of PPD, similar to what is seen in women with premenstrual syndrome. However the specific role of hormonal withdrawal is unclear as we observed that women with PPD were also significantly more symptomatic than controls during the addback phase of hormonal treatment (though less robustly than during the withdrawal phase). It is possible, therefore, that the duration of exposure to gonadal steroids rather than their withdrawal was the critical determinant of symptom precipitation; i.e., continued exposure to estradiol and progesterone replacement in our study might have been associated with equal levels of mood symptoms. Accordingly, we propose to mimic the hormonal events of pregnancy by randomly assigning patients and volunteers to either a withdrawal or continued replacement group (details in Clinical Assessment and Procedures). Furthermore, in order to establish PPD as a distinct phenotype, we will study a

group of women with histories of major depression occurring outside of the perinatal period to test whether the mood and HPA axis effect of this paradigm are specific to postpartum depression or a nonspecific response to any major depressive episode.

Certain forms of affective illness are distinguished by their appearance during periods of reproductive endocrine change, such as during pregnancy and the postpartum/puerperium. Unlike depression accompanying endocrinopathies (e.g., hypothyroidism), these peripartum mood disorders have been shown in our prior work to represent differential sensitivity to an otherwise normal reproductive endocrine stimulus (62). As such, these disorders offer a unique opportunity to identify underlying mechanisms of affective dysregulation: i.e., in this protocol we have identified the trigger, but the underlying susceptibility that transforms a normal endocrine signal into an abnormal response is undetermined. One approach we have undertaken is to study women with a history of peripartum mood disorders (PPMD) to determine whether blinded exposure to supraphysiologic levels of reproductive steroids will result in abnormal neural network activation in affective regulating and reward circuitries. These studies, will not, however, explain the underlying cellular/neural differences that transform a normal signal into an abnormal response. For that purpose, we propose to opportunistically collect blood samples from women already diagnosed with PPMD in order to generate human induced-pluripotent cells (iPSCs), neural progenitors, and possibly human induced serotonergic and dopaminergic neurons for transcriptomic analysis that can be compared with those from controls under baseline conditions and following stimulation with ovarian steroids (i.e., estradiol and progesterone). This strategy has been successfully employed by our group in a related disorder, premenstrual dysphoric disorder (PMDD), albeit with lymphoblastoid cell cultures (LCLs) rather than h-iPSCs. h-iPSCs/neural progenitors offer the advantage of being a neural tissue and, therefore, physiologically more relevant to these conditions than the immune tissue represented by the

LCLs. Given, however, observation of immune dysfunction in women with postpartum depression (women with PPD have a higher than expected rate of postpartum thyroiditis), we will also create LCLs to determine the following: 1) whether clear differences in genomic expression can be identified that may explain the observed relationship between immune dysregulation and PPD; and 2) whether the differential transcriptional responses to ovarian steroids observed in women with a history of PMDD – a putative biomarker of hormone sensitivity – can be observed in women with a different disorder (PPD), albeit one characterized by susceptibility to reproductive hormone-induced affective dysregulation.

Cellular models, involving neuronal cells, lymphoblastoid cell lines (LCLs), and more recently neural progenitor cells (63) have many advantages, but also distinct limitations for the study of brain diseases (64), (65), (66). Studies employing both LCLs and neural progenitor cells have been employed to investigate several forms of both inherited diseases (67), (68) and neuropsychiatric conditions (69), (64), (70), (71), (72). Despite limitations of peripheral blood cell-derived cell lines, these cellular models offer the opportunity to identify biochemical characteristics related to specific disease phenotypes. Additionally, recent evidence supports the use of LCLs for identifying both local and long-distance regulatory elements involved in immune-mediated disease states (73).

2: Specific Aims

Aim 1: Determine whether cellular substrates of differential hormone sensitivity can be identified through creating neuronal progenitors exposed to high dose estradiol (E) and progesterone (P) (100nm each x 3 days) and withdrawal (3 days), with transcriptomic analyses conducted after each treatment.

Aim 2: a) Determine whether transcriptional response in immune-relevant pathways to hormone response and withdrawal in LCLs will, in these long term cultured cells, distinguish women with PPD from controls and, therefore, further implicate differential immune responses in the pathophysiology of PPD; and b) replicate differences in the polycomb repressor complex under both basal and E- and P-stimulated conditions that we previously observed in premenstrual dysphoric disorder (PMDD).

Hypotheses: H1) After exposure to combined E and P, neural progenitor cells from women with PPD will reveal a transcriptional pattern that differs from that in controls; H2) Similar to PMDD, LCLs from women with PPD will demonstrate a differential basal and hormone-stimulated transcriptional response in network and pathways analyses(e.g., Weighted Genome Correlation Network Analysis (WGCNA), Gene Set Enrichment Analysis (GSEA), and Database for Annotation, Visualization and Integrated Discovery (DAVID)).

Experimental Plan: This component of our protocol will build on access to unique samples of women with peripartum mood disorders as follows: 1) to create human-induced pluripotent cells
(h-iPSCs) and neuronal progenitor cells whose transcriptional responses to ovarian steroids in vitro will be assessed; and 2) to create lymphoblastoid cell lines (LCLs).

Participants: PPD- Peripheral blood samples will be obtained from women with PPD and control women (matched for age, race and parity) who met the following criteria: 1) participated in the GnRH agonist and E/P replacement-then-withdrawal paradigms conducted at the NIMH IRP; and 2) clearly demonstrated recurrence of depression (in the women with past PPD but not controls) after E/P withdrawal. This group of women with past PPD and controls therefore will represent a highly selected phenotype in which we have demonstrated behavioral ovarian steroid

sensitivity (in past PPD) or the absence of this phenotype (in controls). All women will be otherwise healthy, and medication free.

h-iPSCs: h-iPSCs will be generated from Sendai virus-transformed T cells (74), a procedure already successfully accomplished in the LNG laboratory, and then differentiated to neuroprogenitor cells (75) (also successfully achieved by LNG), serotonergic (76) (experiments underway in LNG) and dopaminergic neurons (77), (69). Following differentiation, cells will be cultured in a sex steroid-free growth medium which is phenol red-free media to reduce possible exposure to steroid-like activity prior to experimental exposure.

LCLs: EBV-transformed lymphoblastoid cell lines will be made from peripheral blood as described previously (78), and cells from women with PPD and matched controls will be cultured under identical conditions. As for IPSCs, 5 days before hormone exposure, LCLs will be cultured in a sex steroid-free growth medium of phenol red-free media supplemented with 15% knock out serum (KOSR) (estrogen-depleted).

PPD and control cells will be cultured and collected at baseline, after exposure to combined E/P at supraphysiologic doses of 100nm each (in DMSO) for 3 days, and after withdrawal to vehicle only (DMSO alone) or continued E/P treatment for 3 days (to control for latency following initial hormone exposure).

RNA sequencing: Total RNA will be isolated from the cell pellets and then treated with DNase to remove any traces of genomic DNA contamination. Quality assessment of the RNA is done by an Agilent® 2100 Bioanalyzer. Whole-transcriptome RNA sequencing will be performed using an Ion Torrent NGS Sequencer, the Ion Proton. Total RNA will be polyAAA selected and used to prepare cDNA libraries using the Ion Total RNA-Seq Kit v2, barcoded with Ion Xpress™ RNA-Seq Barcodes.

Analysis of RNA sequencing data: Reads will be mapped to reference genome Hg19 and normalized. Using edgeR analyses (or other similar platforms), performed in either CLC Bio Workbench 11 or "R" programming environment (or other appropriate environment), differentially expressed genes will be computed between conditions and multiple comparisons will be corrected using the False Discovery Rate (FDR, Benjamini Hochberg) method. For each gene of interest, normalized expression between untreated and hormone treated/withdrawn cell cultures will be further analyzed by 2-way ANOVAs, with hormone condition (i.e., untreated, hormone exposure, hormone withdrawal) as the within-groups factor and diagnostic group (i.e., PPP or PPD versus Controls) as the between-groups factor. The appropriate post-hoc tests will be performed should significant interactions be detected.

Pathway/Network analysis of RNA sequencing data: Pathway/Network analyses (e.g., Gene Set Enrichment Analysis (GSEA)) will be performed on differentially expressed genes to identify significant biological processes, molecular functions and cellular components for samples in the baseline (untreated), E/P-treated condition, and after withdrawal. Additional analyses that could be performed include WGCNA to examine modules of differentially expressed genes between cells from women with PPD and controls under each hormone condition. Finally, we will subject all differentially expressed genes to other bioinformatics tools, such as the Database for Annotation, Visualization and Integrated Discovery (DAVID)to identify functionally related groups and networks, which can help to detect impacted biological, molecular and cellular functional domains.

Validation of RNA-sequencing: mRNA expression analysis using qRT-PCR will be used to quantitate differentially expressed genes at baseline, after E/P exposure, and after E/P withdrawal. TaqMan qRT-PCR assays will be carried out according to the assay developer's

recommended conditions on a 7900HT Fast Real-Time PCR System. A two-tailed, unpaired Students t-test will be performed for each comparison.

Additional Analyses: it is possible that whole exome sequencing will be performed in these samples, and evaluations of the methylome will be performed on repeated DNA samples across each of the hormonal conditions established in the clinical protocol (i.e., baseline, add-back, withdrawal and follow-up).

This study will be done in collaboration with Dr. David Goldman at the NIAAA.

c. Medications Employed in this Study:

In order to avoid fluctuating hormonal levels with subsequent menstruation, and to ensure reduction of gonadal steroids to hypogonadal levels during the withdrawal phase, suppression of ovarian function will be induced with the GnRH agonist leuprolide acetate (Lupron). As described in detail in protocol # 90-M-0088, Lupron is a synthetic nonapeptide that functions as an agonist that is 80 to 100 times more potent than natural GnRH in inducing the release of LH (79). During the first week of Lupron therapy, there is an initial increase in the pituitary release of LH and FSH leading to a transient increase in levels of the gonadal steroids estrone and estradiol in premenopausal females. However, within two to four weeks after initiation of treatment, there is a subsequent decrease in the number of GnRH receptors and an inhibition of pituitary elaboration of gonadotropins, leading to levels of estradiol similar to those observed in post-menopausal women.

Following suppression with Lupron, estradiol and progesterone will be administered in oral micronized form. The administration of 17 beta estradiol via an oral route using micronized E2 was chosen for this study because it has several advantages. First, it delivers the primary ovarian estradiol, into the circulation at a rate resulting in sustained levels of estradiol and

constant rates of excretion of estradiol conjugates (80). Second, at doses of 3-6 mg/day, it delivers sufficient estradiol into the circulation to raise estradiol plasma concentrations to levels similar to those of women around the ovulation phase of their menstrual cycle (80), levels that while far from reaching full pregnancy levels would give sustained supraphysiologic estradiol levels compatible with early pregnancy. Third, micronized estradiol has a short half-life, thus facilitating rapid reduction in plasma levels. Finally, the dose can be easily adjusted so that the presence of minor side effects such as nausea may not require complete cessation of the medication.

Orally administered micronized progesterone is biologically active and at doses of 600-1600 mg/day will result in plasma levels of 30-40 ng/ml (81,82). Progesterone in suppository form has been approved by the IRB for administration to patients with menstrual related mood disorders (MRMD) and controls under NIMH protocols # 92-M-0174, 90-M-0088.

2. Study Objectives**:**

There are several lines of evidence suggesting a potential role for the hormonal events that occur during pregnancy and parturition in precipitating mood and behavioral changes in a subgroup of women:

1) Gonadal steroids modulate the activity of various neurotransmitter systems,
2) Gonadal steroids may be efficacious in the treatment of PPD;
3) Women with a history of PPD are vulnerable to the development of recurrent episodes of PPD during subsequent pregnancies.

In our previous findings in this protocol, the specific role of hormonal withdrawal was unclear as we observed that women with PPD were also significantly more symptomatic than controls during the addback phase of hormonal treatment. It is possible, therefore, that the duration of exposure to gonadal steroids rather than their withdrawal was the critical determinant of

symptom precipitation. Thus, in this current protocol, we propose to mimic the hormonal events of pregnancy and randomly assign patients and volunteers to either a withdrawal or continued replacement group. Furthermore, in order to establish PPD as a distinct phenotype, we will study a group of women with histories of major depression occurring outside of the perinatal period to test whether the mood and HPA axis effect of this paradigm are specific to postpartum depression or a nonspecific response to any major depressive episode.

3. Subjects**:**

a) Description of Study Populations:

This study will enroll -40 asymptomatic women with a past postpartum depression, 20 asymptomatic women with a past major depression (not related to the postpartum), and 40 asymptomatic women with no past Axis I psychiatric illness.

b. Inclusion Criteria:

A. Group 1: Women with a history of postpartum depression

1) A history of DSM-IV major depression or hypomanic/manic episode that occurred within three months of childbirth (as determined by a SCID interview);

2) has been well for a minimum of one year;

3) a regular menstrual cycle for at least three months;

4) age 18-50;

5) not pregnant, not lactating and in good medical health;

6) medication free (including birth control pills);

7) no history of puerperal suicide attempts or psychotic episodes requiring hospitalization.

B. Group 2: Women with a history of Major Depressive Disorder

1) A history of DSM-IV major depression episode(s) occurring outside of pregnancy and not within three months postpartum;

2) has been well for a minimum of one year;

3) a regular menstrual cycle for at least three months;

4) age 18-50;

5) not pregnant, not lactating and in good medical health;

6) medication free (including birth control pills);

7) no history of suicide attempts or psychotic episodes requiring hospitalization.

C. Group 3: Normal Controls

1) Controls will meet all criteria specified except they must not have any past or present Axis I diagnosis or evidence of menstrually related mood disorders.

c.) Exclusion Criteria for all Study Subjects:

Patients will not be permitted to enter this protocol if they have important clinical or laboratory abnormalities including any history of the following:

- endometriosis;
- undiagnosed enlargement of the ovaries;
- liver disease;
- breast cancer;
- a history of blood clots in the legs or lungs;
- undiagnosed vaginal bleeding;
- porphyria;
- diabetes mellitus;
- malignant melanoma;

- gallbladder or pancreatic disease;
- heart or kidney disease;
- cerebrovascular disease (stroke);
- cigarette smoking;
- a history of suicide attempts or psychotic episodes requiring hospitalization;
- recurrent migraine headaches;
- pregnancy (patients will be warned not to become pregnant during the study and will be advised to employ barrier contraceptive methods;
- pregnancy-related medical conditions such as hyperemesis gravidarum, pretoxemia and toxemia, deep vein thrombosis (DVT) and bleeding diathesis;
- any woman with a first degree relative (immediate family) with premenopausal breast cancer or breast cancer presenting in both breasts or any woman who has multiple family members (greater than three relatives) with postmenopausal breast cancer will also be excluded from participating in this protocol;
- any woman meeting the Stages of Reproductive Aging Workshop Criteria (STRAW) for the perimenopause (83) will be excluded from participation. Specifically, we will exclude any woman with an elevated plasma FSH level (> 14 IU/L) and with menstrual cycle variability of > 7 days different from their normal cycle length.
- subjects who are unable to provide informed consent.
- NIMH employees and staff and their immediate family members will be excluded from the study per NIMH policy.

4. Study Design and Methods

a.) Study Overview:

This is a mechanistic study designed to investigate the pathophysiology of postpartum depression. The experimental model used in this protocol has been a benchmark in understanding the neurobiology of PPD, and a similar design is employed in our companion studies of severe premenstrual dysphoria to refine the hormone-related phenotype of these affective disorders. Women undergo extensive assessments with standard measures to establish their individual patterns of mood symptoms in relation to the hormonal conditions induced in this study. In addition to standard rating scales and interviews the study includes routine lab workThis study lasts five months plus two months of follow-up during which time the subject may have multiple out-patient visits in addition to completing daily mood assessments.

b.) Recruitment:

Patients will be referred from community physicians and sought by advertising for women who suffered moderate postpartum-related, mood disturbances in the past. NIH Employees and staff will not be directly recruited by or through their supervisors or co-workers to participate in this study.

All recruitment materials will be approved by the IRB before use.

Advertisements, both free and paid may be used to recruit subjects for this study. The written advertisements will be used in color as submitted, or may be printed in black and white. The color of the ads may vary. Color changes will not be used to change the emphasis of an ad.

The size of the ads may also vary, but all parts of the ads, including fonts and pictures, will be changed proportionately to the rest of that ad. Disproportionate changes in size will not be used to change the emphasis of an ad.

Flyers of various sizes, both with and without tear-off labels may be used in the following ways:

- On bulletin boards on the NIH campus, at coffee shops, grocery stores, bookstores, libraries, fitness centers, community centers, or placed in venues, such as at advocacy group offices, in doctor's office waiting rooms, and retail establishments with approval of the venue or in accord with their policy.
- Made available at outreach exhibits, speaking engagements, support group meetings, parent groups, professional meetings or conferences, association/trade meetings with approval of the venue or in accord with their policy.
- May be given directly to those requesting study information.
- May be sent using commercially-available mailing lists via direct mail. If used for direct mail, the flyer will identify the source of the mailing list. The flyer may be posted electronically on websites such as NIH or NIMH websites, advocacy/support group websites such as Postpartum Support groups, publications' websites such as Washington Parent, or Gazette.

Print ads will also be used as paid advertisements in local and national magazines with targeted advertising (e.g., Washington Parent, Bethesda Magazine, Washingtonian, More, Washington Parent, Washington Woman, Oprah, Women's Health) as well as in local newspapers (e.g., The Washington Post, Express, Washington Examiner, Gazette, Washington Jewish Week, Military papers).

IRB approved ads will be used on websites and e-newspapers. All will link to the NIMH.NIH.gov landing page for this study. Websites include advocacy websites such as Mental Health Association of America (MHA), MHA Montgomery County, NAMI, and DBSA. They may also appear on the websites of vendors that run print or other paid ads, such as newspapers and magazines. Twitter ads may be sent from Mental Health NIMH @NIMHgov, the NIMH extramural Office of Communications Twitter account.

IRB-approved Listserv ads will be posted on listservs with the permission of the moderator and IRB-required statement on how the receiver was identified. Listservs may include those of parenting organizations such as NIH Parenting Listservs and DC Urban Moms, women's health organizations such as Postpartum Support Virginia and Postpartum support Maryland and professional groups, such as the Greater Washington Society of Social Workers and the Greater Washington Women's Mental Health Consortium.

c.) Screening:

Subject eligibility for this protocol is determined in protocol 81-M-0126 at the NIH Clinical center.

d.) Study Procedures:

Those selected will be personally interviewed to confirm the inclusion criteria by a psychiatrist, psychiatric nurse, or social worker to obtain a description of the following: 1) the nature of the relationships between mood disorders and the puerperium, and development and evolution of the disorder; 2) type, severity and duration of symptoms; 3) premorbid psychiatric history; 4) medical and medication history, including medication taken for mood problems; 5) psychiatric history.

Prior to treatment, a complete physical and neurological examination will have been performed by a physician on the research team and the following routine laboratory data obtained:

A. Blood

Complete blood count; thyroid function tests; renal function tests, such as BUN and creatinine; electrolytes; glucose; liver function tests; and serum β-HCG (test for pregnancy).

B. Urine

Routine urinalysis.

C. EKG

D. Results of Pap smear performed not longer than one year prior to onset of treatment will be obtained. Subjects who will not be able to perform a Pap smear privately or whose results seem to be a reason for concern will be sent for a gynecologic consultation. All subjects will be required to use non-hormonal forms of birth control (e.g., barrier methods) to avoid pregnancy during this study.

E. Subjects will be excluded if there is evidence of significant renal, cardiac, hepatic, or other serious medical disorder.

Procedures:

Initial screening lasting two to four months is completed under protocol 81-M-0126. Clinical Assessment: The amount of time assigned to evaluations may include a screening process time of up to two months. All women will complete a four-item visual analogue self-rating to confirm the absence of clinically significant mood and behavioral symptoms across the menstrual cycle. This log also will serve to confirm the presence of regular menstrual cyclicity. After the first screening visit all participants will return to the clinic after approximately 4-5 weeks to review the quality of their self-ratings and to address any problems about the ratings that they might have encountered. After we receive the completed set of ratings (2 months), the ratings are reviewed by staff to determine the absence of clinically significant changes in mood symptoms throughout the menstrual cycle.

Participants also will have a psychiatric history taken which includes diagnostic interviews such as the Structured Clinical Interview for Axis I DSM-IV Disorders (SCID) and

the minor depression module of the SADS-L. Urinary HCG will be determined to rule out pregnancy. Subjects who meet the inclusion criteria and who are medication free may be asked to return for follow-up interviews. During these interviews, phenomenological assessments may be performed by instruments such as the Premenstrual Tension Syndrome Scale (Steiner-Carroll Scale) (84), Beck Depression Inventory (85), Spielberger State-Trait Anxiety Inventory (86), Hamilton Rating Scales for Depression and Anxiety (87), Edinburgh Postnatal Depression Scale (88)) and related psychological measures. Additionally, subjects will be asked to fill out symptom self-rating scales, a modified version of the Daily Symptom Rating Form (Halbreich and Endicott) (89) and visual analogue scales (90), on a daily basis. During screening in protocol 81-M-0126 all subjects will receive a careful medical screening and will be excluded if there is evidence of significant renal, cardiac, hepatic, or other medical disorder. All patients will receive routine physical and laboratory examinations such as urinalysis, hematocrit, hemoglobin, BUN, creatinine, liver function tests, electrolytes, HIV, and a serum pregnancy test. Additionally, all women will have a normal EKG, and a normal PAP and gynecologic exam within the past year.

Subjects will be seen at the outpatient clinic on a regular biweekly basis. (Total = three baseline and 15 visits during treatment and follow-up.) During clinic visits, subjects will be monitored and managed for side effects, and blood samples will be drawn.

Research Procedures: Following the baseline period patients will receive 3.75 mg of the gonadotropin releasing hormone (GnRH) agonist leuprolide acetate (Lupron) during the early follicular phase of the menstrual cycle via intramuscular injection on a monthly basis in our clinic for five months. During the first two months of GnRH agonist administration, all subjects

will receive placebo progesterone and placebo estradiol tablets and will be told that at some point the placebo pills will be switched to active medication.

After the second month of Lupron-alone treatment, active estradiol and progesterone replacement will be initiated. Subjects shall be randomly assigned to one of two groups, and treatment shall be administered as follows:

Group 1 ("withdrawal" group) - while continuing to receive monthly Lupron injections, all women in this group will be started on 2 mg B.I.D. of 17 beta-estradiol and 200 mg B.I.D. of progesterone for a period of eight weeks. After four months of GnRH agonist with placebo (two months) and estradiol/ progesterone replacement (two months), placebo will be substituted for the active medication while subjects continue on Lupron for another month. Participants will then be followed for an additional two months while unmedicated. Total duration of treatment with Lupron shall be five months.

Group 2 ("continued replacement" group) - while continuing to receive monthly Lupron injections, all women in this group will be started on 2 mg B.I.D. of 17 beta-estradiol and 200 mg B.I.D. of progesterone for a period of 12 weeks. Subjects shall receive Lupron for a total of five months, with placebo (two months) and estradiol/ progesterone replacement (three months). Participants will then be followed for an additional two months while unmedicated. Total duration of treatment with Lupron shall be five months.

The difference between these groups is, therefore, the duration of treatment with estradiol and progesterone - eight weeks of hormonal addback in the withdrawal group and 12 weeks of hormonal addback in the continued addback group. The fifth month of Lupron, then, provides the comparison between the effects of hormonal withdrawal and hormone continuation.

During the active medication phase of the trial, blood levels of progesterone and estradiol will be determined and the doses will be titrated accordingly by an unblind co-investigator. The

blood levels that we expect to achieve and sustain in each woman will be approximately 500 pg/ml of estradiol and 30-40 ng/ml of progesterone. Doses will not exceed 10 mg of estradiol and 1600 mg of progesterone. By maintaining a progesterone: estradiol plasma level of approximately 50:1, we expect to prevent the side effects associated with unopposed estradiol (Leon Speroff, personal communication). As outlined in the "Hazards and Precautions" section of this amendment our collaborative gynecologist will be consulted should any such need arise. Serum pregnancy tests will be performed in all women prior to the administration of the GnRH agonist to exclude pregnancy. No pregnant woman will be entered into the study. Subjects will be seen at the outpatient clinic on a regular biweekly basis. (Total = three baseline and 15 visits during treatment and follow-up.) During clinic visits, subjects will be monitored and managed for side effects, and blood samples will be drawn.

All blood samples (50 ml per visit, 50 ml for genetics on one occasion and 10 ml for the IPCs on only one occasion) will be drawn at the 4th floor outpatient clinic. Fifty cc of blood will be drawn at each clinic visit (n = 15) in the 28 week study. This blood will be analyzed for several hormonal (e.g., neurosteroid metabolites and metabolomics profile), biochemical, and metabolic measures that could distinguish either women with from those without PPD or the symptomatic state in women with PPD.

Additionally, participants will be asked to continue daily self-ratings for mood and behavioral changes throughout the trial and will also have more extensive periodic assessments of mood, behavior and neuropsychological changes. Mood and behavioral assessments include the following measures: repeat SCID interviews lasting approximately 30 minutes (psychological testing), Quality of Life Questionnaire (91) and Social Adjustment Scale (92)self- report scales requiring 15 minutes to complete

Symptom ratings, diagnostic interviews, and other questionnaires will be collected through an online system using a subject-specific log in and password to protect confidentiality (see Appendix I). Both participants and investigators will input data into CTDB/CTSS. Participant entered data will be reviewed during the clinic visit. Women will also be invited to participate in an additional optional research procedures as follows: Peripheral Blood Samples for IPCs

Women who participate in this protocol will be approached to obtain consent for a blood sample as part of the core consent documents. Additionally, we wish to contact women who have previously participated in this study in order to obtain their consent to obtain an additional blood sample. Previous participants will be asked to provide consent using the revised genetics consent form. All women will be asked to provide a 10 ml blood sample (93) to study functional genomics of neuronal and glial precursors. If we are unable to produce cell lines from the initial blood sample obtained then participants will be re-contacted to allow us to take an additional 10 ml blood sample. Risks and potential adverse reactions from a second blood sample should be similar to those of the initial sample.

e.) <u>End of Participation</u>

After the completion of this trial, all participants will be evaluated for the presence of clinically significant mood symptoms. Additionally, the results of symptom ratings and plasma hormone levels will be reviewed with each participant. If negative mood symptoms are present, we will discuss therapeutic options prior to referral of each participant back to her community health care provider. The options for follow-up in those women who report persisting negative mood symptoms include no treatment with follow-up by community health care provider or initiation of standard antidepressant therapy such as selective serotonin uptake inhibitors with follow-up in community. Our experience in this protocol suggests that in the majority of women depressive symptoms that emerged during this study were of mild to moderate severity and time-

limited. In those women in whom depressive symptoms persisted and were accompanied by distress, standard antidepressant therapy led to a remission of symptoms.

5. Management of Data and Samples**:**

a. Storage

Samples of plasma are being stored for future analysis. These samples are stored, in coded vials in secured freezers located in the Fourth Floor Clinic in Building 10 of the NIH campus. Samples are inventoried by codes we assign. Blood samples will be stored in locked freezers on the NIH campus. The key to the code will be kept in a separate, secure area and will only be accessible to the PI. These samples will be used for the study described in this consent form**.**

The inventory of samples and mood ratings are maintained on the Branch server and database. Upon the completion of the study, plasma samples will be retained. Any loss or destruction of samples will be reported to the IRB.

Data from structured diagnostic interviews and symptom ratings are kept in secure research files and electronically on the Branch server space or within the CTDB database. Access to these research files is only available to study investigators. Symptom ratings, diagnostic interviews, and other questionnaires will be collected through an online system using a subject-specific log in and password to protect confidentiality (see Appendix I).

b. Data and sample sharing plan

This protocol is subject to the Genomic Data Sharing (GDS) policy. Data and samples will be shared with dbGaP, and The Perinatal Psychiatry Genetic Consortium - Postpartum depression: Action towards Causes and Treatment (PACT), which is headquartered at The University of North Carolina at Chapel Hill.

Data and samples may also be shared with collaborating laboratories at NIH or outside of NIH and/or submitted to NIH-designated repositories and databases if consent for sharing was obtained. Repositories receiving data and/or samples from this protocol may be open-access or restricted access.

Samples and data will be stripped of identifiers and may be coded ("de-identified") or unlinked from an identifying code ("anonymized"). When coded data is shared, the key to the code will not be provided to collaborators, but will remain at NIH. Data and samples may be shared with investigators and institutions with an FWA or operating under the Declaration of Helsinki (DoH) and reported at the time of continuing review. Sharing with investigators without an FWA or not operating under the DoH will be submitted for prospective IRB approval. Submissions to NIH-sponsored or supported databases and repositories will be reported at the time of Continuing Review. Submission to non-NIH sponsored or supported databases and repositories will be submitted for prospective IRB approval.

Required approvals from the collaborating institution will be obtained and materials will be shipped in accordance with NIH and federal regulations.

The proposed research with our collaborators will be in accord with the purpose of the study stated in the consent that participants signed from the start of the protocol in 1995 to present. Starting in the 2008 consent forms, subjects were given the option whether they wanted to share their samples or not.

The ability to perform a meta-analysis of data will move the field forward. The plan is to focus on investigating the heterogeneity of Perinatal Depression (PND) in order to identify clinical subtypes.

Human tissue samples are being shared with collaborators of the PACT Consortium with a pending Material Transfer Agreement from the Office of Technology Transfer.

All personally identifiable information will be removed from the data and data will be coded with a research identifier that is not derived from or related to information about the subjects. The codes will be stored in a secure database in the Section database and server.

6. Additional Considerations**:**

Depot leuprolide acetate (TAP, pharmaceuticals) is administered in this protocol under the IND # 163205 sponsored by NIMH (Maryland Pao, MD is the official representing the Institute). Estradiol, progesterone, and placebo capsules will be manufactured by either NIH Pharmacy, Knowles Pharmacy or Pine Pharmaceuticals. Knowles Pharmacy, a PCAB-accredited compounding pharmacy located at 10400 Connecticut Avenue #100, Kensington, MD, will prepare the estradiol, progesterone, and placebo capsules on a per-prescription basis under FDA 503a and USP <795> regulations. Pine Pharmaceuticals, Inc. is located at 100 Colvin Woods Parkway, Tonawanda, NY. Pine Pharmaceuticals, Knowles Pharmacy and the NIH Pharmacy will produce these capsules under current Good Manufacturing Practice (cGMP) regulations. The medications administered in this protocol are dispensed, stored, and monitored by the NIH Pharmacy Investigational Drug Management and Research Section.

7. Risks and Discomforts**:**

Potential discomforts in this study could arise from the medications administered in this study, the repeated venipuncture (i.e., bruising or pain at site of needle entry), the completion of rating scales, and the behavioral and endocrine challenge studies.

Medications: We do not expect any adverse side effects associated with the hormonal manipulations outlined in this protocol for the following reasons: First, we will be administering the physiologically relevant steroid hormones (estradiol and progesterone) and not the substituted steroids (such as ethinyl estradiol or norethindrone) present in many oral contraceptives and which have been reported to have a potentially more serious profile of side

effects. Second, the doses of Lupron, estradiol and progesterone, and the duration for which they will be administered in this protocol, will result in plasma hormone levels comparable to those commonly used for in vitro fertilization (IVF) protocols lasting 1-3 months. Therefore, based on current IVF procedures (80 ,94-96) (Leon Speroff, personal communication) we do not anticipate any adverse incidents arising from the proposed doses of estradiol and progesterone. Third, no adverse reactions or events were encountered in the 25 women who completed the study to date. Finally, comparable extended, uninterrupted gonadal steroid treatment (such as oral contraceptives) for 6 to 12 weeks has been shown to be well-tolerated (97).

Lupron: The most frequent adverse effect of Lupron is hot flushes (flashes) reportedly occurring in 4-89% of patients receiving the drug. Lupron-induced hot flushes have ranged in severity from occasional mild flushing to frequent sweating. Episodes of flushing appear to decrease with continued therapy in most patients receiving Lupron; however, in at least one study, the incidence of hot flushes did not appear to decrease with continued therapy. In a recently completed study of 400 women of reproductive age with either uterine fibroids or endometriosis who each received 3.75 mgs depot Lupron every month for a period of six months, the most common side effects reported to occur were as follows: 1) hot flashes of mild to moderate intensity (89%), 2) headache (22%), 3) nervousness or irritability (11%), and 4) insomnia (10%). Local irritation at the injection site was complained of in less than 10% of the patients in this sample, and there was a mean decrease in bone density, as measured by bone densitometry, of 3.4 to 4.0%, which totally reversed after the medication had been discontinued for six months. Approximately ten patients of the original sample of 400 found the side effects to be severe enough to discontinue therapy. In the majority of women regular menstrual cycle function returned within two months following the last injection of depot Lupron (Tapp Pharmaceuticals, personal communication). Blurred vision, myalgias, lethargy, memory

disorder, and numbness have been reported in less than 3% of patients receiving the drug. Thrombophlebitis, pulmonary embolus, and congestive heart failure have occurred rarely in patients receiving Lupron, but a causal relationship to the drug has not been established. Adverse GI effects occurring in 2% or more of patients receiving Lupron include nausea and/or vomiting, constipation, and anorexia. Diarrhea and a sour or unusual taste in the mouth have been reported less frequently. Other adverse effects of Lupron occurring in less than 3% of patients include decreased hematocrit and hemoglobin concentration, fatigue, fever, facial swelling, rash, hives, hair loss, and itching. From our experiences with 20 PMS patients and 20 controls, Lupron is well tolerated (no dropouts) with the most common side effects being hot flushes and a decrease in libido. Limited information is available on the acute toxicity of Lupron. Following subcutaneous administration of Lupron in rats at dosages 250-500 times the usual human dosage, dyspnea, decreased activity, and local irritation at the injection site were observed; however, there is no evidence to date that overdosage in humans produces similar adverse effects. Lupron dosages up to 20 mg daily for up to two years have not produced unusual adverse effects in humans. There has been one report of an anaphylactic reaction in a patient following administration of a GnRH agonist. Recent longitudinal follow-up studies of girls and boys receiving GnRH agonists as a treatment for precocious puberty report the development of normal reproductive function, skeletal growth, and fertility (98,99).

Estradiol: Nausea is the most common side effect of estrogen administration. At conventional replacement doses, higher than those employed in this protocol, this complaint seldom interferes with eating, and no weight loss has been reported. Breast engorgement, endometrial hyperplasia and bleeding are also common side effects of estrogen administration. Pre-existing fibroid tumors of the uterus may enlarge under the effects of estrogen; however, at the dosage and for the duration of estrogen administration in this protocol this risk is small.

The relationship between estrogen, both endogenous and exogenous, and the development of endometrial carcinoma has been suggested by several different lines of investigation (100). Numerous retrospective case control studies published since 1975 have indicated that post-menopausal exposure to unopposed estrogens for more than one year results in a two to 12 fold increased relative risk for endometrial cancer. A relationship between the dose and duration of estrogen use and the risk for endometrial cancer has also been shown, the risk being increased after one to four years of estrogen use and rising also with the dosage employed. However, the addition of progesterone to estrogen replacement therapy appears to decrease the risk of endometrial hyperplasia and endometrial cancer to equal or below that of women receiving no hormonal treatment. Recent studies suggest that the optimal regimen to prevent hyperplasia during long term ERT and thus, inferentially, the risk of carcinoma, consists of 12 to 13 days of progesterone treatment each month when estrogens are administered (101). There is an increase in thromboembolism in women receiving non-contraceptive estrogen therapy (102-106). Additionally, some but not all studies report an increase in risk of stroke (107,108) in older women taking estrogen therapy. However, these complications are unlikely at the dose and duration of estrogen replacement employed in this protocol, and in the younger age group of women who participate in this study. One study (94) reported no effect of the estrogen patch on the four clotting indices previously shown to be altered by oral contraceptive use (101,109,110). Blood pressure, on average, appears to be unaffected by estrogen therapy, although both increases and decreases have been reported. In observational studies, post-menopausal estrogen therapy has been observed to lower the relative risk of cardiovascular disease in some but not all studies (107,111). In contrast, recent randomized controlled trials in older postmenopausal women (e.g., Women's Health Initiative [WHI]) report an increased risk of cardiovascular disease (112). Emerging data suggest that these disparities in findings may be

related to the timing of initiation of estrogen therapy in relation to the proximity of menopause. Subgroup analyses of the combined estrogen and progestin (EPT) arm of the WHI demonstrated a significant interaction between coronary heart disease (CHD) risk and time since initiation of EPT, with an increased risk in the early years following initiation and a decreased risk in later years. Additionally, the increased risk of CHD was observed in older but not younger perimenopausal women (113-118). High doses of oral estrogens have been reported to elevate hepatocellular enzyme levels and, less commonly, cause cholestatic jaundice. The risk for gall stones and hepatocellular adenomas has been reported to be increased in association with oral contraceptive use, and although uncommon these complications may also occur with the use of replacement doses of estrogen (119,120). Estrogen therapy also may increase the risk of urinary incontinence in older postmenopausal women (121-123). Further, most studies have suggested an increased relative risk of breast cancer after four or five years' use (124-136), similar to the risk expected if the onset of menopause was delayed for a comparable length of time.

Due to the publicity surrounding the cancellation of the treatment arm of the Women's Health Initiative study (137) that involved the administration of combined conjugated estrogens and medroxyprogesterone acetate (Prempro), we have included the following statement in the consent documents:

Adverse Events Related to Combined Hormone Replacement and the Results of the Women's Health Initiative (WHI):

The WHI study demonstrated that continuous administration of one form of estrogen (conjugated estrogens) in combination with one form of progesterone (medroxyprogesterone acetate) is associated with an increased risk of dementia, heart attacks, stroke, blood clots, and breast cancer. Estradiol, the form of estrogen that we use in this study, is administered as a sole agent (with the exception of one week's combination with progesterone) and, consequently, we

do not expect that it will pose the increased risks observed with the chronic combination of the conjugated estrogens and medroxyprogesterone administered in the WHI study. Indeed, while the estrogen alone arm of the WHI trial was shown to be associated with an increased risk of stroke, no increased risk of either heart disease or breast cancer was observed (137,138).

Estrogens may precipitate migraine headaches, and depression has also been reported to occur with the use of estrogens. In general, considering the dose and duration of treatment that we propose to use in this protocol, the risk of developing such side effects is negligible.

Progesterone: Progesterone and the synthetic progestins are widely prescribed, with indications including dysfunctional uterine bleeding, endometriosis, mastodynia, galactorrhea, and precocious puberty (139). Side effects reported in women taking progestins may include breakthrough bleeding, edema, change in weight (increase or decrease), cholestatic jaundice, rash (with or without pruritus), depression, easy fatigue and sedation, lack of initiative, and chloasma. Since progestins are often used in women with antecedent menstrual irregularity, it is not clear whether the breakthrough bleeding represents an effect of the medication or refractoriness to treatment. In the large majority of patients, menstruation occurs predictably following withdrawal of progestins and is usually more regular than in spontaneous cycles. In a recent study, an average dose of 1750 mg of oral micronized progesterone was given to 59 women with PMS for a period of three months and was well tolerated by this sample. The side effects reported on progesterone were lightheadedness, fatigue, forgetfulness, and headaches. These were very mild and caused no dropouts (Ellen Freeman, personal communication).

For the sake of completeness we will also describe the side effects reported when estradiol and progesterone are combined in the form of oral contraceptives. Side effects observed in patients receiving combined oral contraceptives include nausea, breast soreness,

vaginal discharge, fluid retention, hypertension, and clotting abnormalities that have been associated with the estradiol component of the oral contraceptive. Thromboembolic disorders including thrombophlebitis, pulmonary embolism, and cerebral and coronary thrombosis appear to occur with greater frequency in women taking oral contraceptives. While the increased incidence of these disorders has been associated with the estradiol component of the oral contraceptives, it is now believed that the progestogen component may, to a lesser extent, contribute to the increased risk. There are relatively few reports associating oral contraceptives with the development of carcinomas (vaginal, uterine, hepatic, and mammary) despite the vast use of these agents, although this may reflect the latent period needed for cellular transformation. Finally, several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies.

Any patient experiencing clinically significant side effects such as nausea, hypertension, vomiting or extreme fluid retention from the medication will have the dose titrated to achieve relief of the symptoms. If adequate relief cannot be achieved in this manner, drug treatment will be discontinued. Similarly if menopausal-like symptoms occurring secondary to GnRH agonist treatment are intolerable, drug treatment will be discontinued.

Venipuncture:

One discomfort of this study may occur due to the venipuncture and multiple blood sampling. The total blood withdrawal is estimated to be 50 ml per clinic visit for a total of 750 ml for the 28 week study (i.e., 15 visits). An additional 60 ml sample of blood will be obtained for genetics (50ml) and IPCs (10ml) that will be timed to coincide with one of the regular visits in those women currently participating in this protocol. Total blood withdrawal over 28 weeks of study duration - 1,080 ml) and falls within NIH guidelines

(550 ml per eight week period). The total blood volume collected will be 200 ml every eight weeks (4 visits= 200 ml = 200 ml) plus one 10 mL additional sample for IPCs and one 50 ml sample for genetics.

Psychological and Cognitive tests:

The risks and discomforts of the symptom evaluations and baseline exams are minimal. No discomfort is expected to be associated with the physical examination or the clinical interview other than potential stress of answering personal questions. All clinical assessors have extensive experience in clinical psychiatric assessment and will make every effort to implement protocol procedures in a sensitive and supportive manner. Research interviews will be interrupted if subjects become distressed or object to answering questions. Other measures to minimize risks include the careful assessment of each subject before the study, and close clinical scrutiny during all aspects of the study.

Intravenous catheter insertion may be momentarily painful and there is a slight risk of infection or inflammation at the site of the catheter insertion. Some people faint when needles are inserted, so this procedure will be performed with the subject seated.

History and Physical Exam and Laboratory Tests: It is possible that during the evaluation portion of the study a subject may be found to have a medical problem of which they were previously unaware. In that event, the participant will be given the results and an appropriate referral made as indicated by the condition identified.

Donation of blood for genetic material:

Under some circumstances, it can be a risk for genetic information about an individual to be known. Variation in some genes is known to be directly related to risk for certain illnesses. Other genes may be shown at some point in the future to be related to illness. Since the results of these genetic tests may allow prediction of risk of illness in some cases, it is possible this

information could be used against a subject participating in the study. Although there are no physical risks associated with participation in genetic studies, apart from those routinely associated with phlebotomy, psychological and social/economic risks associated with genetics studies of the kind described here are difficult to define, and remain the subject of heated controversy in the ethics community. The major risk, to the degree that any exists, is that a breach of confidentiality regarding genetic studies that resulted in third parties finding out genetic information about a person could theoretically place a person at risk for loss of insurance, loss of employment, etc. because of genotype-based discrimination. To our knowledge, no person has ever suffered harm for the reasons just described as a result of participating in a genetics research study. Regardless, our written informed consent process will go over these risks carefully. In addition, subjects will have the right to "withdraw" from the research by having their DNA sample destroyed. Finally, all research records containing any subject-identifying information will be stored under lock and key, or in secured computing environments by the CTDB. Personal identifiers are never associated directly with genotypes in the same data file - all genotype information is indexed only to de-identified subject codes. With these safeguards in place, we are confident the research is virtually without psycho-social-economic risk to subjects.

8. Subject Safety Monitoring**:**

Subjects, both patients and healthy controls, are monitored by one of the associate investigators during all study procedures. Medical history and physical assessments occur at each clinic visit including interviews, symptom assessments, vital signs, and laboratory testing when clinically indicated.

As the population being studied in this protocol may be at high risk to develop subsequent depressions and as gonadal steroids have been implicated in the etiology of

postpartum depression, some depressive reactions may occur during this protocol. However, based on experience with Lupron and estradiol/progesterone addback in women with menstrually-related mood disorders and preliminary observations of 16 women participating in this protocol, we would expect mood symptoms to be of mild severity. Adverse mood symptoms will be monitored by administering the Beck Depression Inventory (BDI); if BDI scores of > 20 are observed at any period in the protocol then the Hamilton Rating Scale for Depression will be administered on consecutive days. Anyone scoring > 20 on the Hamilton for three or more consecutive days, or any subject with suicidal ideation, or anyone with concerns about their ability to continue in the study, will be considered to have severe mood symptoms and be discontinued from the protocol. In the event of the occurrence of severe mood symptoms, either the protocol will be terminated, or if symptoms occur during the withdrawal phase, hormone replacement will be re-instituted. Should this step prove to be unsuccessful, conventional medication will be prescribed as needed. Further, although we do not anticipate severe adverse reactions, we have arranged for inpatient hospital admission at the Clinical Center if symptoms are otherwise unmanageable.

Criteria for individual subject withdrawal:

Any subject requiring immediate treatment will be referred for private treatment. In the event that private treatment cannot be arranged promptly, the subject will be treated openly with an antidepressant until successful referral can be achieved.

Subjects presenting as “healthy” but found to have a psychiatric diagnosis will be discharged from this protocol and either be referred to the community for further evaluation and treatment or consented to another NIMH protocol. In the case a psychiatric diagnosis is unveiled; the assessment and disposition plan will be reviewed with a senior clinician who reviews the case with the Dr. Schmidt.

9. Outcome Measures**:**
Outcome measures will include the scores on several standardized cross-sectional rating scales (Beck Depression Inventory, Hamilton Depression and Anxiety Scales, and Edinburgh Postpartum Depression Scale), symptom rating scales completed daily, plasma hormone measures (including neurosteroid levels and metabolomic profile), induced pluripotent cells and immortalized lymphoid cells mRNA expression after exposure to estrogen and progesterone withdrawal, and the response to the o-CRH stimulation tests.

10. Statistical Analysis**:**
Analysis of Data: A repeated measures design has four groups of ten subjects each for a total of forty subjects. Each subject is measured during the hypogonadal baseline, at the end of the two months of addback, and during the following month (i.e., continued addback in group 2 or withdrawal in group 1). The effect of hormonal addback and withdrawal in subjects and controls on mood and biological parameters will be calculated using ANOVA-R with post-hoc Bonferroni t-tests.

Sample Size Justification: Based on previous data (61), the between-subject standard deviation is 0.69 and the within-subject standard deviation is 0.28. This design achieves 67% power when an F test is used to test the Groups factor at a 5% significance level and the actual standard deviation among the appropriate means is 0.30 (an effect size of 0.43), achieves 81% power when an F test is used to test the Times factor at a 5% significance level and the actual standard deviation among the appropriate means is 0.16 (an effect size of 0.57), and achieves 60% power when an F test is used to test the group by time interaction at a 5% significance level and the actual standard deviation among the appropriate means is 0.13 (an effect size of 0.46).

11. **Human Subjects Protection**:

Subject selection:

All subjects must meet the inclusion and exclusion criteria listed in Section 3. We will select physically healthy adult female individuals. The proportion of ethnic minorities (vs. Caucasians) in the total sample, will be approximately consistent with the overall U.S. population proportions.

Justification for inclusion/exclusion of children:

We will exclude children or minors because the study population is women with postpartum depression.

Justification for symptom challenge procedure:

The justification for employing a symptom challenge is three-fold as follows:

a. The study of PPD is very difficult because of pharmacologic limitations and child care constraints. A hormonal model of pregnancy and parturition may help overcome some of these difficulties.

b. The development of mood and behavior symptoms in this study may predict psychiatric problems in future pregnancies and may suggest a subgroup of women with an affective disorder who are differentially susceptible to the hormonal changes of pregnancy and parturition.

c. This model may be helpful in understanding underlying biological mechanisms in depression and affective disorders in general.

Safeguards: As described above in the Subject Safety Monitoring: Adverse mood symptoms will be monitored by administering the Beck Depression Inventory (BDI); if BDI scores of > 20 are observed at any period in the protocol then the Hamilton Rating Scale for Depression will be administered on consecutive days. Anyone scoring > 20 on the Hamilton for three or more

consecutive days, or any subject with suicidal ideation, or anyone with concerns about their ability to continue in the study, will be considered to have severe mood symptoms and be discontinued from the protocol. In the event of the occurrence of severe mood symptoms, either the protocol will be terminated, or if symptoms occur during the withdrawal phase, hormone replacement will be re-instituted.

Participation of NIH Staff or family members of study team members

NIH staff and family members of study team members may be enrolled in this study as this population meets the study entry criteria. Neither participation nor refusal to participate as a subject in the research will have an effect, either beneficial or adverse, on the participant's employment or position at NIH.

Every effort will be made to protect participant information, but such information may be available in medical records and may be available to authorized users outside of the study team in both an identifiable an unidentifiable manner.

The *NIH Frequently Asked Questions (FAQs) for Staff Who are Considering Participation in NIH Research* will be made available. Please see section 12 for consent of NIH Staff.

This study collects sensitive information on medical and psychiatric diagnoses and drug and alcohol use. The PI will train study staff regarding obtaining and handling potentially sensitive and private information about co-workers through staff discussions and written branch/section procedures. Prior to enrollment, potential participants will be informed that this sensitive information will be in their NIH medical record.

Incidental and Secondary Findings

In this project, we are analyzing ribonucleic acid (RNA) information which could inform us whether particular genes or gene groups are being expressed in women with PPD. We will not be looking for incidental findings associated with each woman's specific DNA. We may have

incidental findings on a group basis that may be relevant to diseases other than PPD. These findings will not be reported on an individual basis but may be included in future publications.

12. Consent Documentation and Process**:**

Prospective participants will receive an explanation of the objectives, procedures, and hazards of this protocol that is appropriate to their level of understanding. The attached consent forms will be reviewed and written or electronic informed consent obtained. When a document that is in electronic format is used for the documentation of consent, this study will use the iMed platform, which is 21 CFR, Part 11 compliant, to obtain the required signatures. The participant will be selected in CRIS, and their identification will be confirmed prior to selecting the study consent in iMed. During the consent process, participants and investigators will view the same approved consent document simultaneously in the same location at the Clinical Center. After all questions have been answered, and if the subject would like to participate, they will both sign the electronic consent using a finger, stylus or mouse, and a timestamp will be generated and recorded on the consent form. This informed consent documentation will be included as part of the medical record maintained for each subject. The right of the subject to decline to participate or to withdraw from the study at any time will be made clear. Subject confidentiality will be assured in accordance with the Federal Privacy Act. Subject anonymity will be protected in any published form of the collected data. Separate consent forms are provided for genetic tests involved in the study. The consent forms contain all required elements.

Considerations for Consent of NIH staff, or family members of study team members:

Consent for NIH staff will be obtained as detailed above with following additional protections: Consent from staff members will be obtained by an individual independent of the staff member's team whenever possible. Otherwise, the consent procedure will be independently monitored by

the CC Department of Bioethics Consultation Service in order to minimize the risk of undue pressure on the staff member.

13. Data and Safety Monitoring**:**
a. Data and safety monitor
We will have an independent safety monitor (ISM) for the study. The Independent Safety Monitor (ISM) will be a sole physician with relevant expertise. William Regenold, M.D.C.M. will serve as the independent safety monitor (ISM) for this protocol for the Behavioral Endocrinology Branch. He is a psychiatrist, board certified in Adult and also Geriatric Psychiatry who is Senior Research Physician and Medical Director for the Noninvasive Neuromodulation Unit in the Experimental Therapeutics and Pathophysiology Branch of the Intramural Research Program of NIMH, working primarily with mood disordered patients. He provides clinical care, assists in consenting subjects for research protocols, conducts research procedures, assists in data analysis and administers and monitors noninvasive neuromodulation therapies including electroconvulsive therapy (ECT) and repetitive transcranial magnetic stimulation (rTMS), for treatment resistant depression. In addition, he was he was at the University of Maryland Department of Psychiatry for 24 years where he was Director of the Division of Geriatric Psychiatry, the Adult Inpatient Service and the Electroconvulsive Therapy Service. His research at the University of Maryland focused on mood disorder pathophysiology and electroconvulsive therapy. Dr. Regenold's primary responsibility will be to provide independent safety monitoring in a timely fashion. This will be accomplished by review of SAEs or unexpected adverse events, immediately after they occur, with follow-up through resolution. The ISM will evaluate individual and cumulative participant data when making recommendations regarding the safe continuation of the study. The ISM focus will be on SAEs or unexpected adverse events rather than *all* adverse events. The ISM will be provided with quarterly reports on safety and enrollment as well as all protocol revision and other pertinent documents relating to the study.

Such reports will include information on laboratory tests and SAEs or unexpected adverse events. The ISM will also be notified of SAEs within 24 hours. The ISM will not have direct involvement in the conduct of the study.

b. Data and safety monitoring plan

The PI will prepare a quarterly report on data and safety parameters for the Independent Monitor. The Independent monitor will provide a written monitoring report to be submitted to the IRB at the time of continuing review.

c. Criteria for stopping the study or suspending enrollment or procedures

The study will be stopped if there is any Serious Adverse Event related to the research. The PI/Independent Monitor and IRB will determine if changes are needed for the research to continue or if it will be closed. Any changes required as conditions for resuming the research must be submitted as an amendment and IRB-approved before the changes can be implemented.

14. Quality Assurance**:**

A. Quality assurance monitor

Quality assurance will be monitored by the PI, the research team and the Office of Regulatory Oversight (ORO) serving NIAAA, NIDA and NIMH.

B. Quality assurance plan

ORO monitors intramural research studies to ensure compliance with GCP, organizational policies and regulations. Audit frequency is determined by the ORO SOP based on the study level of risk. Results of ORO audits are provided to the PI, The Clinical Director and the IRB. As an IND study, this protocol will be subject to GCP audits at study initiation and after the first enrolled subject. Timing of subsequent review will be established by ORO but no less frequent than every other year.

15. Reporting for Unanticipated Problems, Adverse Events and Protocol Deviations**:**
Reporting will be in compliance with policy 801.

16. Alternatives to Participation**:**
Patients may elect not to participate in this study without jeopardizing their participation in other studies at NIH.

17. Privacy**:**
All research activities will be conducted in as private a setting as possible.

18. Confidentiality**:**
Data and samples will have all personally identifying information removed and will be assigned a code. The key to the code will be kept separately and securely. The inventory of samples and mood ratings are maintained on the Branch server and database. Hard copies are stored in locked cabinets within a locked room, human tissues are stored in locked freezers within a locked room with pass key access, electronic data (including the sample inventory) is stored on the NIMH server and CTDB database in which access is restricted to members of the Behavioral Endocrinology Branch and CTDB staff.

This study collects sensitive medical information. The PI will train study staff regarding obtaining and handling potentially sensitive and private information about co-workers through staff discussions and written branch/section procedures.

19. Conflict of Interest Statement**:**
The NIH policy on conflict-of-interest has been distributed to all investigators. None of the investigators have a conflict of interest to report regarding this protocol. No NIH investigator involved in this study receives payments or other benefits from any company whose drug, product or device is being tested.

20. Technology Transfer:
Human tissue samples are being shared with collaborators of the PACT Consortium pending a Material Transfer Agreement obtained from the Office of Technology Transfer.

21. Research and Travel Compensation**:**

For this study, the subject may be compensated for research-related discomfort and inconveniences. Compensation will be in the form of a check payment. This will be given at the end of each study phase.

Volunteers will be paid in accordance with NIMH guidelines as follows:

Study 95-M-0097	
Clinic visits (15-18)	540.00
Repeated blood draws (16)	350.00
Mood and behavioral ratings	150.00
Investigational drugs	
a) Depot $100.00 x 5	500.00
b) Estrogen tablets	100.00
c) Progesterone tablets	100.00
Total Compensation for 95-M-0097 is up to:	$1740.00

Additional tests (separate consent)

Blood Sample for IPCs	3 x $20.00 per 10 ml sample	60.00

This study does not offer reimbursement for, or payment of, travel or meals. If an overnight stay is necessary, lodging may be provided.

Employees and staff who participate during work hours must have permission from their supervisor. NIH employees and staff must either participate outside of work hours or take leave in order to receive compensation.

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Appendix **I**

Clinical Trials Database - Security Overview

There are multiple aspects to the security framework for the Clinical Trials Database (CTDB) and Clinical Trials Survey System. The following features allow for the safe and secure collection of research variables:

- **Application Firewall**- The NICHD has recently upgraded their application firewall which protects both the front end web server and back end database server for the CTDB. Strict policies are in place which control exactly who has pre-defined, limited access to the application. This firewall is a state-of-the-art hardware solution which blocks access to everyone but authorized users for the CTDB system.
- **Data Encryption and SSL Certificates** - The CTDB system makes use of military grade encryption both for the session and the data storage. The CTSS collects de-identified self-reported data. Both systems protect information from interception by encrypting the data flow using SSL with a 1024 bit signed certificate. The entire communication session from the time a client requests a connection to the system to the time a user logs out is encrypted using a certificate from an industry recognized vendor. The result is a secure communications channel for our partners, providing data confidentiality and integrity. In further, the CTDB program encrypts data stored within the system, thereby providing an additional layer or security for the sensitive CTDB clinical data.
- **CTSS HIPAA Requirements** - Below is the list of identifiers to be removed for the de-identification of health information under HIPAA. This is found in 45 C.F.R. 164.514(a),(b)&(c)

  (A) Names;
  (B) All geographic subdivisions smaller than a State, including street address, city, county, precinct, zip code, and their equivalent geocodes, except for the initial three digits of a zip code if, according to the current publicly available data from the Bureau of the Census:
  (1) The geographic unit formed by combining all zip codes with the same three initial digits contains more than 20,000 people; and
  (2) The initial three digits of a zip code for all such geographic units containing 20,000 or fewer people is changed to 000.
  (C) All elements of dates (except year) for dates directly related to an individual, including birth date, admission date, discharge date, date of death; and all ages over 89

and all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 or older;
(D) Telephone numbers;
(E) Fax numbers;
(F) Electronic mail addresses;
(G) Social security numbers;
(H) Medical record numbers;
(I) Health plan beneficiary numbers;
(J) Account numbers;
(K) Certificate/license numbers;
(L) Vehicle identifiers and serial numbers, including license plate numbers;
(M) Device identifiers and serial numbers;
(N) Web Universal Resource Locators (URLs);
(O) Internet Protocol (IP) address numbers;
(P) Biometric identifiers, including finger and voice prints;
(Q) Full face photographic images and any comparable images; and
(R) Any other unique identifying number, characteristic, or code, except as permitted by paragraph (c) of this section

- **Logical Access Controls (Role/Privileges)** – Logical access controls are in placed using role based security for database access and application account access. Security controls are in place to detect unauthorized access attempts. The application is further protected by the NICHD firewall and NIH firewall. Inactive user accounts are monitored and removed when not needed, and users are disconnected after a specific period of inactivity. Encryption is used (1024bit SSL key) and data is HIPPA compliant. Access is monitored and apparent security violations are investigated when identified. Insecure protocols are disabled on all application servers. Guest and anonymous accounts/access is disabled.
- **Audit Trails** – Activity involving access to and modification of sensitive or critical files is logged and monitored for possible security violations. Access to these audit trails is strictly controlled and can be used to support after-the-fact investigations of how, when and why normal operations ceased should this occur. Off-line storage of audit logs is retained for a period of at least 1 year. Suspicious activity is investigated and appropriate action is taken when warranted.
- **Physical and Environmental Protection** – The servers are physically located in a secured NIH data center with controlled limited access. All work products from the system including Data backup tapes are rotated to off-site storage with must be authorize and are recorded. All visitors to sensitive areas are escorted with entry codes changed periodically. Fire prevention and suppression devices are installed and in working condition. All heating and air-cooling systems are periodically checked to ensure proper working condition.

- **Production Input and Output Control** – Audit trails are in place to record data changes. Only authorized system administrators are allowed access to this data, as well as any data backup tapes. Damaged media is sanitized or destroyed, and any hardcopy media is shredded when no longer needed.
- **Contingency Planning** – All critical data files, database files and web server files have been identified. A regularly scheduled data backup solution is in place with identified resources supporting critical operations. A comprehensive contingency plan has been developed and documented. This plan has been approved by key affected parties. The Contingency plan/Disaster recovery plan is regularly tested and adjusted as appropriate.
- **Hardware and Software System Maintenance** – Access is limited to the hardware and software infrastructure. Restrictions are in place as to who performs maintenance activities. Procedures are in place to monitor the use of system resources. All new and revised hardware and software are tested and approved before implementation. All system and application components are tested, documented and approved prior to promotion to production environment. Detailed system specification as prepared and reviewed by management. A version control system is in place for all key application and operating system files. The systems are actively managed to monitor and reduce vulnerabilities with unnecessary services eliminated.
- **Strategic framework** – The tactical security framework provides a mechanism whereby the Clinical Trials support personnel provide day-to-day operational support activities for the regular maintenance of the CTDB system. These initiatives incorporate application and software security. The CTDB application was designed using the latest Java technology. This allows the adaptation of the application to ever-changing business rules within the application. An Oracle 10g relational database provides the repository for the clinical data. The use of a robust, industry standard relational database provides a modular architecture design of CTDB which allows for the CTDB program to assign role based security to the participants in the system. This allows roles to be defined and implemented for different users- such as investigators, study participants, report writes, etc.- in order to secure database access and the application data stored within the system. This implies that the NICHD CTDB partners can implement custom roles and maintain their own clinical data with a high level of confidence that the data will not be compromised nor shared with non-participants. As technology evolves, this attention to the strategic framework allows us to address

individual software components and target them for enhancements or upgrades all while maintaining the integrity and confidentiality of the CTDB system. Another example of this strategic framework is the reporting interface. The modular design of CTDB allows provides the ability to upgrade to Cognos reporting with the result being more detailed comprehensive reporting abilities. The net result is a feature-enhanced system while maintain the strict security framework of the system.