Protocol Title	The treatment of menstrually-related mood disorders with the gonadotropin releasing hormone (GnRH) agonist, depot leuprolide acetate (Lupron)
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Total requested accrual

100 Patients (separately describe patient groups)

0 Volunteers

Human Research Protections Program Investigator and Staff Training:

"Just in time" human subjects protection training courses are required for investigators and staff participating on this protocol: None

Project Uses Ionizing Radiation: ☒ No ☐ Yes (attach *RSC/RDSC documentation)*

- ☐ Medically-indicated only
- ☐ Research-related only
- ☐ Both

IRB Panel: ☐Blue ☐White

IND/IDE ☐ No ☒ Yes *(attach FDA documentation)*
Drug/Device/# 34,662 Leoprolide acetate 3.75 mg
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 _____

Samples are being stored ☐ No ☒ Yes

Flesch-Kinkaid reading level of consent form:

Combined: 11.0

Genetics: 9.5

Precis

This protocol is designed to accompany Clinical Protocol # 81-M-0126, The Phenomenology and Biophysiology of Menstrually-Related Mood and Behavioral Disorders. Its original purposes were as follows: 1) to evaluate the efficacy of the gonadotropin releasing hormone (GnRH) agonist depot leuprolide acetate (Lupron) in the treatment of menstrually-related mood disorders (MRMD) by determining whether mood and behavioral symptoms are eliminated when the cyclic secretion of both gonadotropic hormones and gonadal steroids is suppressed, and 2) to determine the possible relevance of gonadal steroids to affective state by sequentially replacing estradiol and progesterone during continued GnRH suppression in those patients whose premenstrual symptoms remit following administration of the GnRH agonist. We observed that GnRH agonist induced ovarian suppression was an effective treatment compared to placebo in women with MRMD. Additionally, women with MRMD but not asymptomatic controls (participating in companion protocol # 92-M-0174) experienced a recurrence of mood and behavioral symptoms when either estradiol or progesterone (but not placebo) was added back. These data suggest that women with MRMD have a differential sensitivity to the mood destabilizing effects of gonadal steroids (1).

Having established that women with MRMD show a differential behavioral response to estrogen and progesterone, we now hope to identify the underlying mechanisms and physiologic concomitants of the differential behavioral sensitivity by performing studies (described in companion protocols) under the three hormonal conditions created by this protocol, and comparing results obtained with those seen in normal controls (Protocol # 92-M-0174). Planned studies include the following: cognitive testing, , brain imaging (3D PET, FMRI, MRS) and genetic studies including induced pluripotent cells.

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Abbreviations:

MRMD – menstrually related mood disorders

GnRH – Gonadotropin releasing hormone

1) Background and Scientific Justification

Menstrual-related mood disorder (MRMD) is a condition characterized by affective, behavioral, and somatic symptoms that are temporally linked to the luteal phase of the normal menstrual cycle (2). The pathophysiologic role of the luteal phase in MRMD is currently unclear, as are the roles of the ovarian steroids, estrogen and progesterone. In a previous study (3), truncation of the luteal phase with high doses of the progesterone receptor antagonist mifepristone (RU 486) failed to alter the course or severity of the symptoms of MRMD: Both affective and somatic symptoms typically associated with MRMD developed during hormonal conditions similar to the follicular phase. This study suggested the lack of causal relevance of mid - late luteal phase biology in MRMD. In contrast ovarian suppression with GnRH agonists resulted in the remission of MRMD symptoms in some (4-13) (but not all (14)) trials, suggesting the importance of changes in ovarian hormones in the development of MRMD symptoms. As stated previously (3), these data are consistent with MRMD representing a disorder of mood state that is triggered by hormone-related events occurring prior to the mid - late luteal phase of the menstrual cycle.

In this study (1), we sought to answer three questions: 1) Does ovarian suppression eliminate the symptoms of MRMD; 2) Will exogenous administration of estradiol or progesterone reverse the effects of ovarian suppression on mood; and 3) Do women lacking a history of MRMD respond similarly to women with MRMD during manipulation of gonadal steroid levels? Our findings to date confirm several previous studies reporting the therapeutic efficacy of ovarian suppression in women with MRMD (4-12). Second, our data suggest that women with MRMD experience an acute symptom precipitation when ovarian steroids are added back under conditions of leuprolide-induced ovarian suppression, similar to the observations of

Muse et al. (15) and Mortola et al. (9). Third, and of greatest interest, controls showed no perturbation of mood in response to either ovarian suppression or to exogenous hormone administration. Given the identical nature of the hormonal manipulations performed in patients and controls, it would appear that the mood changes seen in MRMD patients represent an abnormal response to otherwise unremarkable hormonal changes.

Efficacy of Leuprolide vs. Placebo: We observed significant reductions in MRMD symptom severity during leuprolide treatment for all symptoms measured on both daily rating scales except for the symptom of increased appetite. We observed that only six of ten (double blind) and ten of 18 women (open label or double blind) with MRMD receiving leuprolide met our criteria for response. Thus, we observed response rates between 45 and 60% employing relatively conservative criteria. The reported response rates to selective serotonin reuptake inhibitors (SSRI) in women with MRMD (40-60%) (16-25) appear similar to the rates observed in trials of GnRH-agonists. Thus, although the majority of studies would support the short term use of GnRH agonists or selective serotonin reuptake inhibitors in this condition, symptomatic response is not uniform, and predictors of response are lacking.

The relatively brief period of GnRH agonist administration was well tolerated by both women with MRMD and controls. Despite the occurrence of hot flushes during the leuprolide alone conditions, no woman dropped out of the study due to intolerable side effects. Indeed, we observed no adverse mood or behavioral effects during the hypogonadal state induced in this protocol, nor did we observe significant changes in weight or blood pressure recordings. In fact, women with MRMD rated themselves on the visual analogue scale ratings as feeling significantly better than the controls when they were hypogonadal and less symptomatic than during their baseline (pretreated) condition. We did not observe any woman with MRMD to

meet our criteria for response on placebo, and symptom rating scores were significantly decreased in the sample receiving leuprolide compared to those receiving placebo.

Effects of Hormone Addback: In the second phase of the study we observed the recurrence of typical MRMD symptoms, both physical and emotional, in women with MRMD during hormone addback. Symptom recurrence was observed within one to two weeks of exposure to either estradiol or progesterone given separately. The doses of estradiol or progesterone resulted in plasma hormone levels comparable to the levels observed during the normal menstrual cycle, and there were no differences in plasma hormone levels between women with MRMD and controls.

Our data are consistent with two previous studies by Muse et al. (15) and Mortola et al. (9) reporting the return of both physical and emotional symptoms in women with MRMD when estrogens or progestins were added back under conditions of GnRH-agonist induced ovarian suppression. We observed significant (or at least a trend level of significance) worsening of MRMD symptoms for all symptoms measured in all scales during hormone addback except for the symptoms of food cravings and increased appetite. No differences in symptom severity measures were observed between women with MRMD and controls in any symptom during leuprolide-induced hypogonadism (with the exception of women with MRMD feeling more happy and more relaxed than controls). Investigators have reported correlations between plasma estradiol and/or progesterone levels and symptom severity in women with MRMD (26-30) and, additionally, have observed the following differences in plasma hormone levels in samples of women with MRMD compared to controls: no differences in levels of both estradiol and progesterone (31, 32), high levels of estradiol and low levels of progesterone (29, 33), and high levels of estradiol and no differences in progesterone (28). Our data are consistent with the suggested relationship (based on correlative rather than direct data) between MRMD symptom

severity and ovarian steroid secretion. Additionally, the proposed lag (three to seven days) (27-29) in the correlation between progesterone levels and symptom onset is also consistent with our observations of MRMD symptom recurrence occurring approximately one to two weeks after the administration of estradiol or progesterone. Several women experienced a remission of their symptomatic state after 7-10 days of symptoms despite being maintained on the same dose of estradiol or progesterone. This suggests that the onset of MRMD may be triggered by ovarian steroids, but the "switching out" of the MRMD state is independent of plasma hormone levels. Finally, the variable association between either estradiol or progesterone and symptom return, within individual women with MRMD, may provide an explanation for otherwise discrepant correlative relationships observed in studies with small sample sizes, since it is unlikely that a sample of women with MRMD would uniformly display a link between the same ovarian steroid and mood symptoms.

Our data extend the findings of our previous study employing RU 486 (3) in which we demonstrated that MRMD symptoms occur independent of the mid - late luteal phase of the menstrual cycle. The observations from this earlier study suggested that MRMD may represent either an autonomous mood disorder linked to, but not caused by, menstrual cycle hormones, or, alternatively, that hormonal events occurring prior to the mid - luteal phase of the menstrual cycle triggered the onset of MRMD symptoms. The data from the present study of a selected sample of leuprolide responders support the latter relationship and is consistent with the proposals of other investigators (27, 28) who have suggested that periovulatory changes in either estradiol or progesterone secretion may be critical to the onset of MRMD symptoms.

MRMD Patients Compared with Controls: The most striking finding in this study is the differential symptom response in women with MRMD compared to controls. Women with

MRMD show alleviation of symptoms with ovarian suppression and precipitation of symptoms with hormonal addback, while controls show no perturbation of mood with either hormonal manipulation. These observations converge with the demonstration of normal reproductive endocrine function in women with MRMD to suggest that normal levels or changes in gonadal steroids can trigger an abnormal response - i.e., mood state deterioration - in a susceptible population. Our data suggest that MRMD symptoms occur in a context within which normal levels of ovarian steroids (or their neurosteroid metabolites (34)) similar to those measured across the normal menstrual cycle may interact with neuroregulatory systems to alter behavior in a susceptible group of women. Identification of the mechanisms underlying the susceptibility to these effects will not only assist in the development of more specific treatments for individual women with MRMD but additionally will provide a context within which to address the relationship between mood dysregulation and changes in reproductive endocrine function in conditions such as postpartum depression and depression occurring in the context of the perimenopause.

Background and Justification for Brain Imaging Studies

Recent brain imaging studies in asymptomatic women (i.e., women without MRMD) confirm for the first time in humans that physiologic levels of ovarian steroids have the capacity to modulate the neurocircuitry thought to be involved in both normal and pathological affective states. First, Berman et al. performed cognition-activated O^{15}PET scans in women during conditions of GnRH agonist-induced hypogonadism and gonadal steroid replacement. They observed the elimination of Wisconsin Card Sort-activated regional cerebral blood flow (rCBF) in the dorsolateral prefrontal cortex as well as an attenuation of cortical activation in the inferior parietal lobule and posterior inferior temporal cortex (bilaterally) during GnRH agonist-induced

hypogonadism (35). The characteristic pattern of cortical activation re-emerged during both estradiol and progesterone addback. Additionally, they observed a differential pattern of hippocampal activation with estradiol increasing and progesterone decreasing activation relative to hypogonadism. This was the first demonstration that ovarian steroids have activational effects on rCBF during cognitive stimulation in the brain regions (i.e., PFC) implicated in disorders of affect and cognition. Two recent studies using fMRI and ^{1}HMRS respectively, have extended these findings in young women under conditions of GnRH agonist-induced ovarian suppression. Compared with baseline, ovarian suppression was associated with decreased activation in the prefrontal cortex during verbal memory encoding (but not retrieval) (36). In the second study with ^{1}HMRS, ovarian suppression was associated with an increase in choline concentration (a proxy for neuronal metabolism/turn-over) in the dorsolateral prefrontal cortex (37). Second, Goldstein et al. (38) observed an increase in amygdalar activity and arousal (as measured by fMRI and skin conductance, respectively) during the late follicular phase of the menstrual cycle (higher estradiol levels) compared to the early follicular phase (characterized by relatively low estradiol levels). Third, Protopopescu et al. (39) employed an affective pictures task in an fMRI study and observed increased OFC activity (a region that in some studies exerts inhibitory control over amygdalar functioning) during the luteal compared with the follicular phase. Moreover, preliminary data from these same investigators in women with MRMD (Silbersweig, DC, personal communication) suggest a relative loss of OFC activity (decreased inhibition) in women with MRMD during the luteal phase. Notwithstanding the caveat that decreased cortical "activity" also could reflect more efficient or optimal function, these data suggest that a reduction in OFC inhibition of amygdalar function during the luteal phase is associated with MRMD symptoms. Finally, Dreher et al. (40), have initiated an event-related

fMRI study of reward processing across the menstrual cycle in women with MRMD and controls. The paradigm employed disentangles transient reward error prediction (PFC) from sustained response to reward uncertainty (ventral striatum). Preliminary data in the controls demonstrate, for the first time in humans, that ovarian steroids modulate reward system function, with increased follicular phase activation of the OFC and amygdala during reward anticipation and of the midbrain, striatum, and left ventrolateral PFC during reward delivery. New analytical approaches will allow for testing the hypothesis that the hormonally-induced alteration in function includes changes in interregional neural interactions. These findings then suggest that cognitive and affective information processes may serve as probes to identify candidate circuits for the mediation of gonadal steroid-dependent affective dysregulation. Additionally, these neuroimaging studies in women suggest that ovarian steroids can influence many neural processes and systems relevant to MRMD including arousal, stress-responsivity, and reward processing.

Background and Justification for Induced Pluripotent Cell Study

We wish to collect an additional sample of venous blood in women who have participated (or who currently are participating) in protocols 90-M-0088, titled "The treatment of menstrually-related mood disorders with the gonadotropin releasing hormone (GnRH) agonist, depot leuprolide acetate (Lupron)," and 92-M-0174, titled "The central nervous system effects of pharmacologically induced hypogonadotropic hypogonadism with and without estrogen and progesterone replacement." Previous findings from these protocols have demonstrated a differential behavioral response (i.e., a recurrence of typical mood and behavioral symptoms experienced premenstrually) to physiologic levels of the ovarian steroids estradiol and progesterone in women with premenstrual dysphoria (PMD) compared with asymptomatic

controls. The observed differential response appears to be tissue specific since the effects of ovarian steroids at several other target tissues does not differ in women with and without PMD (e.g., endometrium, pituitary, breast). The biological basis for this differential behavioral response to ostensibly the same physiologic stimulus is unclear; however, it is well established that tissue specificity in the response to sex steroids is mediated by factors at the level of cellular function. Receptors for both estradiol and progesterone are widely distributed throughout the brain, and recent neuroimaging studies have demonstrated that both estradiol and progesterone regulate the activation patterns in brain regions involved with the processes of affective adaptation and stress responsivity.

We hypothesize that the capacity for phenotypic differences between women with and those without PMD will be preserved in the cellular function of induced pluripotential cells (IPCs). Although the phenotypic difference in response to sex steroids is tissue specific, the latent capacity for differential expression in response to steroids should be preserved in IPCs and expressed in the differentiation of the IPCs into neural elements. Thus, we plan to obtain T-lymphocytes from peripheral blood (47) in order to convert these cells into IPCs to establish neuronal and glial cell lines in both women with PMD and controls. This study will be done in collaboration with Dr. David Goldman at the NIAAA. The goal of this project will be to develop neurons and glial cells from participants and to characterize their gene expression patterns, morphological properties, and cell signaling pathways in response to exposures to physiologic levels of estradiol and progesterone. These experiments, therefore, will allow us to evaluate the nature of the differential behavioral response by examining protein expression and changes in cellular behaviors associated with the exposure to physiologic levels of either estradiol or progesterone across the two different behavioral phenotypes. The two behavioral phenotypes

will be constituted by those women with PMD whose symptoms remit during ovarian suppression and whose symptoms recur during physiologic addback of either estradiol or progesterone. This assures the phenotypic fidelity of the subjects and avoids the false positives that would otherwise be generated if diagnoses are established solely on the basis of baseline behavioral ratings. The selection of each woman will occur after completion of the six month study to ensure both the proper characterization of the behavioral phenotype and to include comparable numbers of women in whom symptoms recur after estradiol and progesterone exposure. To recruit sufficient numbers of women in each group we will contact women who have previously participated in these protocols, have met our baseline criteria for PMD or asymptomatic controls, and have further demonstrated either the elimination of symptoms during GnRH agonist and return of symptoms upon re-exposure to estradiol or progesterone (PMD) or the absence of negative behavioral symptoms during both GnRH agonist and ovarian steroid addback phases (controls). Each of these women who is interested in participating will be re-consented for this study.

We hypothesize that the T-lymphocytes obtained from women with PMD and those from asymptomatic controls can be converted into IPCs and differentiated into model neuronal and glial cell lines. The production of these pluripotential cell lines will retain genetic (or epigenetic) variations that will help us understand the differential behavioral response at a cellular level in this condition. a) Medications Used in Protocol

GnRH is a decapeptide produced by the hypothalamus and causes the anterior pituitary to release follicle stimulating hormone (FSH) and luteinizing hormone (LH). Leuprolide acetate (Lupron) is a synthetic nonapeptide that functions as an agonist that is 80 to 100 times more potent than synthetic native GnRH in inducing the release of LH (48, 49). 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, with longterm administration, Lupron decreases the number of GnRH receptors and inhibits pituitary secretion of gonadotropins, leading to levels of estrogens similar to those observed in post-menopausal women. These decreases in gonadal steroid levels occur within two to four weeks after initiation of treatment. Lupron is not active when given orally; however, following a single injection of depot Lupron, mean peak leuprolide acetate plasma concentration was almost 20 ng/ml after four hours and .36 ng/ml at four weeks, levels associated with effective gonadotropin suppression. The plasma half-life of depot Lupron is not known; however, the half-life in the non-depot form is approximately three hours.

The administration of 17 beta estradiol via a transdermal system was chosen for this study because it has several advantages as described by Cedars and Judd (50). First, it delivers the primary ovarian estrogen, estradiol, into the circulation at a constant rate and results in sustained levels of estradiol, constant rates of excretion of estradiol conjugates, and estrone/estradiol ratios less than one (as seen in the pre-climacteric period of life). Thus, transdermal estradiol is considered to result in a more physiological form of estrogen replacement than occurs with oral estrogens (51). Second, it delivers sufficient estradiol into the circulation to raise estradiol plasma concentrations to levels similar to those of women in the early follicular to mid-follicular phases of the menstrual cycle (50, 52-55), levels reported by some investigators as the minimum necessary for the relief of menopausal symptoms, in particular hot flushes. Finally, the system can be applied and removed without difficulty, allowing for easy adjustment of the dosage in the presence of minor side effects such as nausea that may not require complete cessation of the medication. With the application of the estrogen

patch system twice weekly, patient compliance has been reported to be excellent, and only occasional local irritation has been observed (52). Further, transdermal estradiol can provide adequate amounts of estrogen to suppress hot flashes, correct vaginal atrophy, and reduce urinary calcium excretion (52) without increasing synthesis of renin substrate. An increase in renin substrate has been suggested to accentuate or initiate the development of high blood pressure in susceptible women with other predisposing factors (56), and has been implicated as a factor contributing to the association of hypertension with administration of oral conjugated estrogen (57).

Progesterone is widely prescribed in gynecologic settings, with proved indications including dysfunctional uterine bleeding, endometriosis, mastodynia, galactorrhea and precocious puberty (58). Further, progesterone suppositories have been approved by the IRB for administration to patients with MRMD under NIMH protocol # 82-M-0193.

2) Study Objectives

The results of this protocol and its companion protocol (92-M-0174) demonstrate that during conditions of GnRH agonist-induced ovarian suppression women with MRMD experience a differential behavioral response to physiologic levels of ovarian steroids compared with controls. Our current objectives are to employ this hormone manipulation paradigm to investigate the underlying substrates for this differential behavioral response as well as the mechanisms mediating the differential risk. The current study objectives are as follows:

1) To investigate possible differences in the substrate mediating the observed differential response, we will employ multimodal imaging paradigms (O^{15} PET, MRI) to examine the neurocircuitry regulated by ovarian steroids in MRMD and controls under the

hormone conditions established in this protocol. Additionally, we will examine hormone-related differences in HPA axis responsivity between women with MRMD and controls.

2) To investigate possible mechanisms underlying the observed differences in behavioral response to ovarian steroids we will examine the profile of neurosteroid metabolites in plasma using a metabolomics platform to determine if the enzymatic metabolism of behaviorally relevant steroids differs in women with MRMD and controls. Additionally, we will employ functional genomics studies in-vitro cell cultures to examine potential differences in cellular function in women with MRMD and controls after exposure to physiologic levels of ovarian steroids.

3) Subjects

a) Description of Study Population: The subjects of this study will be women who meet the criteria for MRMD as described in Protocol # 81-M-0126, "The Phenomenology and Biophysiology of Menstrually-related Mood and Behavioral Disorders." All patients in this protocol will have already participated in Protocol # 81-M-0126 and will have a prospectively confirmed and predictable relationship between their mood disorder and the premenstrual phase of the menstrual cycle; i.e., a 30% change in severity of symptom self rating scales, relative to the range of the scale employed, during the seven days premenstrually compared with the seven days post-menstrually in two out of three months of study. We intend to recruit 100 women with MRMD to complete the current study objectives. Participants who drop out may be replaced.

b) Inclusion criteria:

- History within the last two years of at least six months with menstrually-related mood or behavioral disturbances of at least moderate severity--i.e., disturbances that are distinct in appearance and associated with a notable degree of subjective distress;

- Symptoms should have a sudden onset and offset;
- Age 18-50;
- Not pregnant and in good medical health;
- Medication free.

The Schedule for Affective Disorders and Schizophrenia will be administered to all patients prior to study entry. Any patient with a current axis I psychiatric diagnosis will be excluded from participating in this protocol.

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

A. Blood

Complete blood count; thyroid function tests; cortisol; renal function tests, such as BUN and creatinine; electrolytes; glucose; liver function tests.

B. Urine

Routine urinalysis; urine pregnancy test.

GnRH agonist will not be administered to any subject with significant clinical or laboratory abnormalities. The blood tests and urinalysis will be repeated two weeks after GnRH agonist administration to rule out any evidence of acute renal, hepatic or hematologic toxicity.

Additional tests

Results of Pap smear performed within one year of the onset of treatment will be obtained.

c) Exclusion Criteria: The following conditions will constitute contraindications to treatment with hormonal therapy and will preclude a subject's participation in this protocol:

- Current Axis I psychiatric diagnosis

- History consistent with endometriosis;
- Diagnosis of ill-defined, obscure pelvic lesions, particularly undiagnosed ovarian enlargement;
- Hepatic disease as manifested by abnormal liver function tests;
- History of mammary carcinoma;
- History of pulmonary embolism or phlebothrombosis;
- Undiagnosed vaginal bleeding;
- Porphyria;
- Diabetes mellitus;
- History of malignant melanoma;
- Cholecystitis or pancreatitis;
- Cardiovascular or renal disease;
- Pregnancy;
- Any woman meeting the Stages of Reproductive Aging Workshop Criteria (STRAW) for the perimenopause (59). Specifically, we will exclude any woman with an elevated plasma FSH level ($\geq$ 14 IU/L) and with menstrual cycle variability of > 7 days different from their normal cycle length.

Subjects taking birth control pills will be excluded from the study. Subjects taking diuretics, prostaglandin inhibitors, or pyridoxine (putative treatments for MRMD) will similarly be excluded from the study, as will patients taking psychotropic agents (e.g., lithium carbonate, tricyclic antidepressants). All subjects will be required to use non-hormonal forms of birth control (e.g., barrier methods) to avoid pregnancy during this study.

- 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 outpatient study consists of a baseline period (three months), followed by three months of GnRH administration (phase I) and three months of estradiol and progesterone replacement (phase II). Subjects who experience remission of symptoms in Phase I will advance to Phase II of the protocol. Those who do not experience symptom relief in Phase I may opt to enroll in another treatment protocol or receive open treatment for a time-limited period. Throughout the study participants will have weekly or biweekly visits to the outpatient clinic for blood draws and ratings, will complete daily self ratings, and will be scheduled for additional procedures and tests as described below.

b) Recruitment

Patients will be sought by advertising for women who suffer severe menstrually-related mood disturbances.

- Flyers will be produced with tear-offs. Posters will not have tear-offs.
- Flyers/poster will be used in color as submitted, or may be printed in black and white. The color of the flyer may vary. However, color changes will be made proportionally throughout the ad and will not be used to change the emphasis of the flyers.
- The size of the flyers/posters may vary, but all parts, including fonts and pictures, will be changed proportionately to the rest of that flyer. Disproportionate changes in size will not be used to change the emphasis of the flyer.
- Flyers/posters may be posted on bulletin boards on the NIH campus, and 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.

- Flyers/posters may be made available at outreach exhibits, speaking engagements, support group meetings, parenting groups, professional meetings, and association/trade meetings with approval of the venue or in accord with their policy.
- Flyers/posters may be given directly to persons requesting study information.
- Flyers/posters may be posted electronically on websites such as such as NIH or NIMH websites, advocacy/parenting group websites such as PTAs, athletic groups or neighborhood groups, publications' websites such as Washington Parent, or Gazette.
- Flyers/posters may be sent electronically to persons requesting study information
- Flyers/posters may be sent electronically to listserv administrators. SBE will not post directly to Listservs. Rather, an email with the material attached, including the following statement will be sent to the administrator of the listserv:

You are receiving the email because your email address is included on this listserv. The purpose of this message is to inform you of an NIMH study that is recruiting subjects. The administrator of this listserv has permitted its use for this distribution.

The administrator has the option of sharing the information with their list. Examples of types of Listservs to be contacted include professional groups, parenting/family, school, sport groups, women's health and fitness. SBE will retain copies of all correspondence with the administrator of each listserv and submit as requested to the IRB.

- IRB-approved advertisements may be placed in local and national print publications of newspapers, magazines and support or health care organizations, such as The Washington Post, The Express, Washington Parent, Gazette, Washingtonian, Washington Jewish Week and others.
- NIH Record announcements will appear as text in the NIH Record.

- NIH employees and staff will not be directly recruited by or through their supervisors or co-workers to participate in this study.

c) Screening

All patients in this protocol will have already participated in Protocol # 81-M-0126 and will have a prospectively confirmed and predictable relationship between their mood disorder and the premenstrual phase of the menstrual cycle; i.e., a 30% change in severity of symptom self rating scales, relative to the range of the scale employed, during the seven days premenstrually compared with the seven days post-menstrually in two out of three months of study. Initial assessments may be performed by instruments such as the Premenstrual Tension Syndrome Scale (Steiner-Carroll Scale) (60), Beck Depression Inventory (61), State-Trait Anxiety Inventory (62), Hamilton Depression Scale (63), and related psychological measures. Additionally, subjects will be asked to fill out symptom self-rating scales (as described in screening protocol 81-M-0126), the daily symptom rating scale (Halbreich and Endicott) (64) and visual analogue scales (65), on a daily basis. 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. All subjects meeting criteria 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, and a serum pregnancy test. Blood specimens will also be collected on interview days by routine venipuncture and will be assayed for a number of endocrine measures such as estrogen, progesterone, sex hormone binding globulin, testosterone, prolactin, cortisol, T4, T3, and TSH.

d) Study Procedures

Baseline

All subjects will have a three month baseline period during which mood and behavioral ratings will be obtained. Urinary pregnancy tests will be performed in all women prior to administration of the GnRH agonist to exclude pregnancy. No pregnant women will be continued in the study.

Phase I

Following the baseline period patients will receive 3.75 mg of GnRH agonist via intramuscular injection on a monthly basis in our clinic for a maximum of twenty-four weeks (see Figure I). Subjects will fill out symptom self-rating scales on a daily basis preceding, during, and following treatment with GnRH agonist. Additionally, subjects will be administered the following ratings during weekly clinic visits: Beck Depression Inventory, Spielberger State-Trait Anxiety Inventory, the Rating Scale for Premenstrual Tension (PMTS) (self and rater administered versions) and a side effect checklist. Following the third month of GnRH agonist treatment, the results of the trial will be discussed with each patient. In those subjects who continue to evidence cyclical mood and behavioral changes comparable to their menstrually-related mood disturbances, GnRH agonist administration will be discontinued, and these subjects will be offered participation in an alternate treatment protocol. Those patients who experience a complete remission of mood and behavioral disturbances during the treatment with the GnRH agonist will be entered into the second phase of this study.

Phase II

Phase II of the study consists of the sequential replacement of estradiol and progesterone under double blind (subject and rater) conditions to determine whether these hormones are critical in the determination of the patient's premenstrual mood state. These patients will continue to receive GnRH agonist for another 12 weeks. While continuing on the GnRH agonist, patients will be randomized into one of two groups as follows: group I will receive 0.1 mg/day of 17ß- estradiol via skin patch (Estraderm) for a period of five weeks. During the fifth week, progesterone suppositories (200 mgs twice daily) will be added to provide progesterone withdrawal-induced shedding of the endometrium and menses in order to prevent prolonged

exposure of the endometrium to unopposed estrogen. Following the four weeks of estradiol administration and the week of combined estradiol and progesterone, there will be a two week washout period when placebo patches and suppositories will be substituted for estradiol and progesterone. Patients will then be administered progesterone (200 mg) suppositories twice daily for a period of five weeks following which no progesterone will be taken. Group II is identical to group I except the order of administration of hormones is reversed: progesterone suppositories (200 mg BID) will be administered for the first five weeks, followed by a two week washout, and then estradiol 0.1 mg/day for five weeks, with the addition of progesterone during the fifth week of estrogen treatment. To maintain double blind conditions all patients will use both patches (active or placebo) and suppositories (active or placebo) daily for the 12 weeks of phase II.

Blood Drawing

Following the administration of the GnRH agonist, in addition to the completion of daily mood and behavioral rating scales, blood samples (50 ml) will be drawn bi-weekly, if possible, for 24 weeks of this study (total = 660 ml). All blood sampling will be obtained in the following fashion. Subjects will arrive at the 4th or 9th floor outpatient clinic; they will be seated or placed at bed rest, a scalp vein needle will be placed in the forearm or hand via venipuncture and approximately 50 ml of blood will be drawn. This blood will then be analyzed for several measures such as gonadotropins, estradiol, and progesterone to confirm creation of the intended hormonal conditions. Additionally, blood samples will be assayed for hormonal/metabolic measures that could distinguish either the symptomatic state in women with MRMD or women with from those without MRMD.

Related Studies

I. Brain Imaging

In an earlier study, we demonstrated that the expected pattern of cognitive task - activated cerebral blood flow was eliminated during Lupron-induced hypogonadism and normalized with hormone addback. These group data did not permit us to determine the following: 1) whether the disappearance of the normal response during hypogonadism reflects recruitment of new neural areas of simple increased variance, and 2) whether women with MRMD differ in their activated response from controls. Subjects in this protocol will be asked to participate in brain imaging (as outlined in protocols # 90-M-0014, 91-M-0124) studies during each of the different hormonal conditions of this study. The use of 0^{15} PET and FMRI will enable us to investigate the effects of gonadal steroids on cerebral blood flow on an individual basis.

II Genetic Studies

This protocol offers an unparalleled opportunity to identify potential genetic contributions to the differential sensitivity to gonadal steroids underlying MRMD because it identifies relatively pure phenotypes; i.e., women with estrogen - or progesterone - precipitated mood disorders and women for whom the same manipulation of gonadal steroids is without effect on mood. A specific hormonal precipitant for the mood disorder is identified or disconfirmed in each subject, thus, dramatically increasing the likelihood of detecting any genetic contribution to the differential behavioral response to the same hormonal signal. Two genetic strategies will be employed: candidate gene studies examining polymorphisms in the receptors modulating the hormone signal (i.e., estrogen, progesterone, and androgen receptors); and establishment of cell lines for genome scanning in the future when polymorphism mapping

microarray technology becomes widely available. Wherever possible, internal control genotypes will be acquired (affected family-based control method).

Induced Pluripotent Cells. Samples derived from from peripheral blood cells (47) may be utilized for procedures such as special stains, immunohistochemistry, direct immunofluorescence or immunoelectron-microscopy for localization of immune deposits, PCR, or establishment of cell cultures. In our protocol, the samples will be used to grow a pluripotent cell lines. e) End of Participation

At the completion of this study, a member of the research team will review the results of the study with each woman. Women with MRMD will be offered participation in other evaluation and treatment protocols as indicated or, alternatively, referred for standard treatment to a caregiver in the community.

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

a) Storage

Samples and data will be stored using codes that we assign. Data will be kept in password-protected computers. Samples will be kept in locked storage. DNA samples and cell lines will be stored in locked freezers in David Goldman's laboratory (NIAAA) on Fischer's Lane in Rockville. Only study investigators will have access to the samples and data.

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 (Including Genomic Data) and Sample Sharing Plan:

This protocol is not subject to the Genomic Data Sharing (GDS) policy. The sharing of Genomic data according to the GDS policy is covered under the screening protocol 81-M-0126. Data and samples may be shared with dbGaP, 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. Data will not be shared from those who completed participation prior to Amendment 32 (FF).

6) Additional Considerations

a. Research with investigational drugs or devices N/A

7) Risks and Discomforts

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. Regular menstrual cycle function returned within two months following the last injection of depot Lupron (Tapp Pharmaceuticals, personal communication). In our experience to date, menstrual cycle irregularity has persisted up to six months after the last injection. Blurred vision, lethargy, memory disorder, and numbness have been reported in less than 3% of patients receiving the drug. Thrombophlebitis and/or 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. 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 (86-88). The FDA recently published a caution about the effects of GnRH agonists in elderly males; however, the safe use of these agents in younger adults and children was not questioned in the FDA statement.

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 (89). 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 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 and thus, inferentially, the risk of carcinoma, consists of 12 to 13 days of progestin treatment each month when estrogens are administered (90). There is an increase in thromboembolism and stroke in women receiving estrogen therapy (91-98) (and, possibly, ovarian and lung cancer (99-102)); however, these complications are unlikely at the dose and duration of estrogen therapy employed in this protocol and in the younger age of the women participating in this trial. One study (52) reported no effect of the estrogen patch on the four clotting indices previously shown to be altered by oral contraceptive use (90, 103, 104). Additionally, recent studies (105, 106) observed that an increased risk of venous thromboembolism was associated with oral but not transdermal estrogen compared with nonusers (odds ratios = .42 [95% CI, 1.5 to 11.6] and 0.9 [95% CI, 0.4 to 2.1] respectively (107)). Blood pressure, on average, appears to be unaffected by estrogen therapy, although both increases and decreases have been reported. Post menopausal estrogen therapy has been observed to increase the relative risk of cardiovascular disease in some (95, 108) but not all studies (109-111). Indeed recent analyses of the Women's Health Initiative demonstrate that the adverse effects of estrogen therapy on cardiovascular outcomes were largely confined to older women compared with younger perimenopausal women (98, 112-120). 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 (121-123). Further, most studies have suggested an increased relative risk of breast cancer after four or five years' use (98, 120, 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 (58). Side effects reported in women taking progestins are uncommon but may include breakthrough bleeding, edema, change in weight (increase or decrease), cholestatic jaundice, rash (with or without pruritus), depression of mood, easy fatigue, 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.

Side effects observed in patients receiving combined oral contraceptives include nausea, breast soreness, vaginal discharge, fluid retention, hypertension, and clotting abnormalities, which have been associated with the estrogen 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 estrogen 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.

Symptom Production

Preliminary observations in this protocol (1) suggest that some women may experience a recurrence of their typical MRMD symptoms during the hormone addback phase. These symptoms have been similar in severity to those experienced prior to study entry. However, if symptoms are intolerable we will discontinue the study.

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, but the patient's continued involvement with our clinic will not be jeopardized. Similarly if menopausal symptoms occurring secondary to GnRH agonist treatment are intolerable, drug treatment will be discontinued.

Blood Drawing

One discomfort of this study may occur due to the venipuncture and multiple blood sampling. Total blood withdrawal (660 ml) falls within NIH guidelines (550 ml. per eight week period).

Potential discomforts in this study could arise from the repeated venipuncture (i.e., bruising or pain at site of needle entry) or the completion of rating scales. The latter should take between 5-10 minutes each day and in our experience have not been a source of discomfort in the women with whom we have worked.

Risks Related to Additional Procedures

Genetic testing

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.

Brain imaging

The risks related to MRI and PET scanning are described in the consent forms associated with the protocols 90-M-0014, and 91-M-0124 under which they will be performed.

Rating Scales, interviews and cognitive Testing

No discomfort is expected to be associated with the rating scales, or 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.

8) Subject Safety Monitoring

Subjects are evaluated by one of the associate investigators. Medical history and physical assessments occur at each clinic visit including interviews, symptom assessments, vital signs, and laboratory testing when clinically indicated. After completing the study, subjects will either be discharged from this protocol with a referral to the community, or will be referred to other NIMH studies.

Patients will be warned not to become pregnant during the study and will be advised to employ barrier contraceptive methods. Observations from our previous study (1) suggest that some women may experience a recurrence of their typical MRMD symptoms during the hormone addback phase. These symptoms have been similar in severity to those experienced prior to study entry. However, if symptoms are intolerable we will discontinue the subject's participation in the study (or the study-specific procedure).

As we are administering doses of estradiol and progesterone designed to produce physiologic levels, we expect - and have seen - no unexpected adverse events. Similarly, we do not anticipate unexpected adverse events with the dose of Lupron that we propose to use. Nonetheless, we see subjects every two weeks during their clinic visit and advise them in the consent form that if they experience side effects, they should notify the investigator immediately. Any adverse events will be reported as per NIH policy.

9) Outcome Measures

Current outcome measures include symptom ratings, brain activation and connectivity, metabolomic and genomic measures.

10) Statistical Analysis

A responder to Lupron will be defined in the following way: 1) the absence of PMTS rating scale scores above ten during any week of phase I (this threshold reflects our clinical experience with this rating scale and represents the maximum follicular phase score seen in over 110 premenstrual syndrome patients followed longitudinally), and 2) the absence of two weekly mean scores on anxiety, depression, or irritability scales greater than 2.5 on a six point scale within a two month time period (1, 84). The effects of both estrogen and progesterone replacement on mood ratings will be analyzed using similar threshold criteria to define patients as responders (recurrence of mood syndrome) or non-responders. Previous estimates by Van Horn et al. (85) in CBDB, NIMH, suggest that 15 women with MRMD and 15 controls (recruited through protocol # 92-M-0174) would be required in the brain imaging component of this protocol to achieve statistical power of 80% and an alpha = .05. In our first study we observed a response rate to GnRH agonist-induced ovarian suppression of 60% and a recurrence rate of mood symptoms during addback of 100%. Therefore, to study 15 women in the brain

imaging component of this protocol we would need to admit 25 additional women to this protocol.

To recruit sufficient numbers of women in each group for IPCs we will contact women who have previously participated in these protocols, have met our baseline criteria for PMD or asymptomatic controls, and have further demonstrated either the elimination of symptoms during GnRH agonist and return of symptoms upon re-exposure to estradiol or progesterone (PMD) or the absence of negative behavioral symptoms during both GnRH agonist and ovarian steroid addback phases (controls). Each of these women who is interested in participating will be re-consented for this study.

11) Human Subjects Protection

a) Subject selection:

Subject selection will be equitable. We will select physically healthy adult female individuals. Male subjects will be excluded as this is a study about menstrually related mood disorders which does not apply to men. The proportion of ethnic minorities (vs. Caucasians) in the total sample, will be approximately consistent with the overall U.S. population proportions.

b) Justification for inclusion/exclusion of children:

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

c) Justification for inclusion or exclusion of other vulnerable subjects:

Pregnant women are excluded due to the risks associated with hormone administration. Cognitively impaired subjects and those unable to provide consent are excluded due to the rigorous requirements of daily ratings, cognitive testing and weekly study visits.

d) Justification of sensitive procedures:

N/A

e) Safeguards for vulnerable populations:

Protections for NIH employees, staff and family members participating in this study include 1) assuring that the participation or refusal to participate will have no effect, either beneficial or adverse, on the subject's employment or position at the NIH, 2) giving employees and staff who are interested in participating the "NIH Information Sheet on Employee Research Participation" prior to obtaining consent, and 3) assuring that there will be no direct solicitation of employees or staff.

f) Qualifications of Investigators:

Peter J. Schmidt, M.D., is an investigator with the Section on Behavioral Endocrinology, NIMH. He has over 20 years experience performing studies that examine the effects of reproductive hormones on mood and behavior in women with reproductive endocrine-related mood disorders. He will be allowed to obtain consent.

David R. Rubinow, M.D., is a special volunteer and collaborator within the Section on Behavioral Endocrinology, NIMH. He has over 30 years experience in reproductive endocrinology and psychiatry. He will not be involved in obtaining consent. He does not have access to personally identifiable information (PII).

Lynnette K. Nieman, M.D., is a senior investigator in the Reproductive Biology and Medicine Branch, NICHD and has extensive experience with clinical research studies in endocrinology and reproductive biology. She will not be involved in obtaining consent.

Pedro E. Martinez, M.D., is a staff clinician within the Section on Behavioral Endocrinology, NIMH and has performed endocrine studies examining the effects of aging and reproductive hormones on mood and behavior in both adults and children. He will be allowed to obtain consent.

Shau-Ming Wei, PhD has an extensive experience in experimental design, and she has designed and implemented both behavioral and neuroimaging experiments over the past several years. Her graduate training at the NIH/Brown Program included collaborative neuroimaging studies on the effects of genotype and reproductive hormones on brain function. She has worked with our program for more than ten years (as part of our ongoing collaborations with Dr. Karen Berman's group), first as a doctoral candidate, then as a postdoctoral fellow. Currently, she is a research fellow within our Branch and in the future will be offered a position as a staff scientist. Over the last decade Dr. Wei has worked closely with our clinical program and this protocol involving the administration of a GnRH agonist with and without ovarian steroid hormone replacement. She will be involved in imaging data collection and analysis, and manuscript preparation. She will not obtain informed consent.

The Principal Investigator has verified that all individuals working on this protocol required to take HRPP training under OHSRP SOP 25 (Training requirements for the NIH Human Research Protections Program) have completed all required training.

12) Anticipated Benefits

Menstrually-related mood disorders constitute a condition that causes significant distress to a potentially large number of women. The potential benefit of determining an effective and consistent treatment for menstrually-related mood disorders is quite significant, particularly since existing therapies successfully treat only two thirds of patients with this disorder. Further, the opportunity to explore potential biological mechanisms underlying both the menstrually-related mood disorders and their response to treatment offers the possibility of uncovering some etiopathogenic mechanisms involved in these and related mood disorders.

13) Classification of Risk

The overall risks for this study are more than minimal.

There are low risks to individual subjects in the use of medication and procedures under the conditions stated in this protocol. Patients stand to gain effective and scientifically validated pharmacologic treatment of a condition that is frequently associated with pronounced debilitation. Lupron is currently available for long term administration. Finally, patients who continue to experience cyclic mood symptoms during treatment with GnRH analogue will be unlikely to respond to further hormonal manipulation and would potentially profit from currently available psychotropic medication.

14) Consent Documentation and Process

Each patient will receive a verbal and written explanation of the purpose, procedure, and potential hazards of this project. A record of the communication of this information and of the consent to participate in this study will be placed in the medical record. The right of the subjects to withdraw from the study or to refuse any procedure will be made clear. Any patient whose symptoms become excessive during either the GnRH alone or estrogen/progesterone replacement phases will be offered another medication without completing the sixth month trial. Confidentiality of patients will be assured according to the laws of the State of Maryland. In case of published data resulting from the study, care will be taken to protect the anonymity of patients.

Consent for NIH employees and staff will not be obtained by coworkers.

15) Data and Safety Monitoring

A. Data and Safety Monitor

Data and safety will be monitored by the Principal Investigator.

B. Data and Safety Monitoring Plan

The PI will review data and safety parameters at least annually. The PI will document the data and safety review in the research records and at the time of continuing review.

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

The study (or the study-specific procedure) will be stopped if any patient experiences clinically significant, unexpected, side-effects from the medication that cannot be relieved with a dose reduction. The PI and IRB will determine if changes are needed for the research to continue or if it will be closed.

16) Quality Assurance

A. Quality Assurance Monitor

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

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 CNS 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.

17) **Reporting of Unanticipated problems, adverse events and protocol deviations**

The Principal Investigator is responsible for detecting, documenting, and reporting unanticipated problems, adverse events (AEs), including serious adverse events (SAEs), and deviations in

accordance with NIH policy, IRB requirements, and federal regulations. Relatedness to the research of all serious adverse events will be determined by the PI in consultation with the CD. Serious unanticipated problems and serious protocol deviations will be reported to the IRB and CD as soon as possible and in writing not more than 7 days after the PI first learns of the event. Not serious unanticipated problems and not serious deviations will be reported to the IRB and CD as soon as possible and in writing not more than 14 days after the PI first learns of the event. Written reports will be submitted in PTMS.

Deaths will be reported to the Clinical Director within 7 days after the PI first learns of the event.

It is anticipated that participants in this study will occasionally miss or fail to complete an assessment or procedure, such as a study visit, completion of a rating scale, blood draw or other laboratory test as a result of an issue by participant, research staff, lab, or phlebotomy. Omissions such as these will be considered expected events and not protocol deviations provided they are infrequent and do not include data needed to assess safety or the primary study outcome. Cumulative proportions of these missed events in the study population will be presented to the IRB annually. In addition, the rate of omissions will be monitored by the Investigators. If an individual misses more than 15% of the required assessments/procedures or if more than 15% of the participants miss completion of the same assessment or procedure, it will be considered a deviation and a deviation report will be sent to the IRB within 14 days.

All adverse events, deviations, and unanticipated problems will be summarized and reported at the time of Continuing Review.

18) Alternatives to Participation

Subjects do not receive any treatment in this study or forego any treatment in order to participate in this study. The alternative, therefore, is not to participate.

19) Privacy:

All research activities will be conducted in as private a setting as possible.

20) Confidentiality

Samples and data will be stored using codes that we assign. Data will be kept in password-protected computers. Samples will be kept in locked storage. Only study investigators will have access to the samples and data.

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.

21) Conflict of Interest

NIH guidelines on conflict of interest have been distributed to all investigators. There are no conflicts-of-interest to report.

22) Technology Transfer

There are no technology transfer agreements associated with this protocol

23) Research and Travel Compensation

Reimbursement of travel and subsistence will be offered consistent with NIH guidelines. Compensation will be prorated for parts completed if subjects do not complete the study. No escort fee will 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.

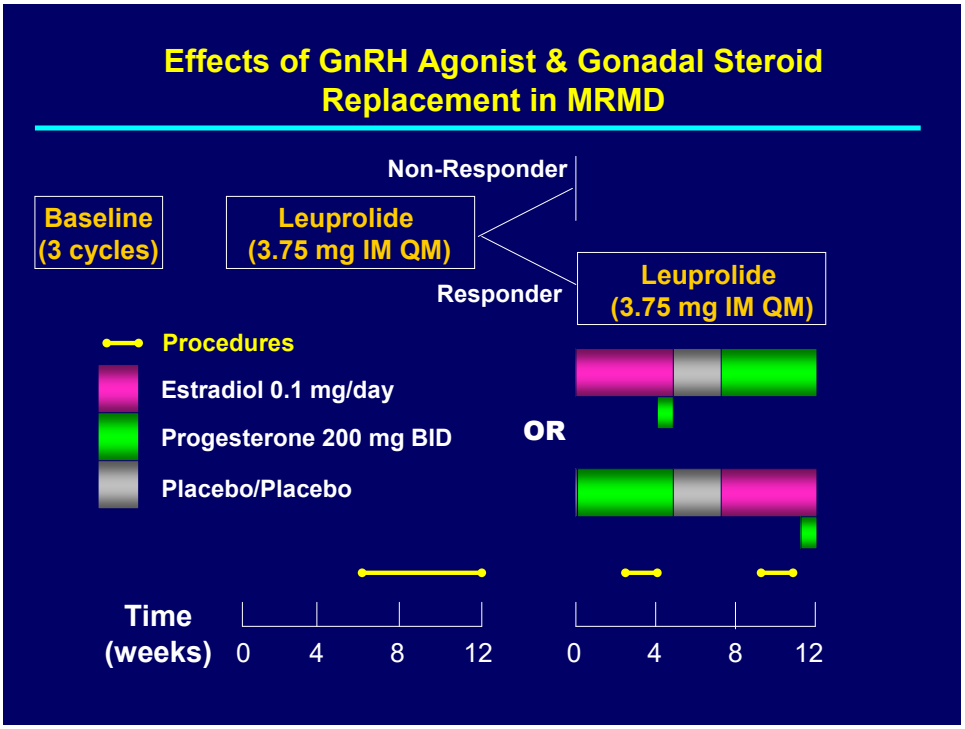
1) Core Protocol

Initial evaluation, physical exam (2 hours)	100.00
Screening phase	100.00
Clinic visits (weekly) 2 hour x 13	390.00
Multiple venipuncture x 13	260.00
Psychological testing x 5	100.00
Symptom rating scales x 6 months	150.00
Investigational drugs a) Depot Lupron injections 100.00 x 6 =	600.00
b) Skin patches daily x 3 months	400.00
c) Vaginal suppositories daily x 3 months	400.00
Total	2500.00

Blood Volume

Core protocol: 660 mls

24) Flow Sheet



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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.