Ovarian Hormone Withdrawal and Suicide Risk: An Experimental Approach

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Principal Investigator: Tory Eisenlohr-Moul, Ph.D.

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LIST OF ABBREVIATIONS

E2	17-beta-estradiol
P4	progesterone
RCT	Randomized controlled trial
SITBI	Self-Injurious Thoughts and Behaviors Interview
UWRAP/LRAP	University of Washington Risk Assessment and Management Protocol/Linehan Risk Assessment and Management Protocol
EXP	Experimental
PL	Placebo
EMA	Ecological Momentary Assessment
CESD	Center for Epidemiologic Studies Depression Scale
BHI	Beck Hopelessness Inventory
STAI	State-Trait Anxiety Inventory
UPPS-P	An Impulsivity Scale measuring Urgency, lack of Premeditation, lack of Perseverance, Sensation seeking, and Positive Urgency.
SSES	State Self-Esteem Scale
FT-IRAP	Future-Thinking Implicit Relational Assessment Procedure (Hopelessness Task)
SAR-IAT	Social Acceptance and Rejection Implicit Association Test (Negative Social Appraisals Task)
SST	Stop-Signal Task (Inhibitory Control Task)
DPT	Dot Probe Task (Threat Sensitivity Task)
SCID	Structured Clinical Interview for Diagnosis
IDS	Investigational Drug Services

STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with the ICH E6, the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46), and the NIMH Terms of Award. The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

PROTOCOL SUMMARY

Title: Ovarian Hormone Withdrawal and Suicide Risk: An Experimental Approach

Précis: 60 female outpatients with suicidal ideation but minimal[1,2] imminent risk for attempt will complete behavioral tasks measuring *hopelessness, social appraisals, inhibitory control, and threat sensitivity* as well as clinical interviews measuring suicidality in each of three conditions (A, B, C: order randomized across three menstrual cycles): (**A**) **perimenstrual E2/P4 withdrawal** (under placebo), (**B**) **perimenstrual P4 withdrawal** (exogenous stabilization of E2 only), and **(C) perimenstrual E2 withdrawal**

(exogenous stabilization of P4 only). A washout cycle will separate conditions. Analyses will compare the perimenstrual trajectories of symptoms and suicidality across the three conditions.

Objectives:

Test the hypothesis that key suicide risk pathways will be heightened during conditions involving P4 withdrawal (conditions A and B above) relative to the E2 withdrawal condition (condition C), and that this greater risk will be mediated by a steeper slope of withdrawal from ALLO during P4 withdrawal and natural (placebo) E2/P4 withdrawal conditions relative to E2 withdrawal condition (where P4 is stabilized). We will also examine the role of inflammatory gene expression in explaining the associations between E2, P4, ALLO, and suicidality.

Endpoint

Primary:

(1) Mean Within-Person Condition Differences (7 final placebo days – 7 final stabilization days) of SITB Suicidal Ideation subscale score (operationalization of suicidal *desire)* across the three conditions

(2) Mean Within-Person Condition Differences (7 final placebo days – 7 final stabilization days) of SITB Suicidal Planning subscale score (operationalization of suicidal *action capacity*) across the three conditions

Secondary:

(1) Mean Within-Person Condition Differences (7 final placebo days – 7 final stabilization days) of BHI Hopelessness Score

(2) Mean Within-Person Condition Differences (7 final placebo days – 7 final stabilization days) of CES-D Depression Total Score

(3) Mean Within-Person Condition Differences (7 final placebo days – 7 final stabilization days) of UPPS-P Lack of Premeditation Impulsivity subscale

(4) Mean Within-Person Condition Differences (7 final placebo days – 7 final stabilization days) of PROMIS Anxiety Total Score

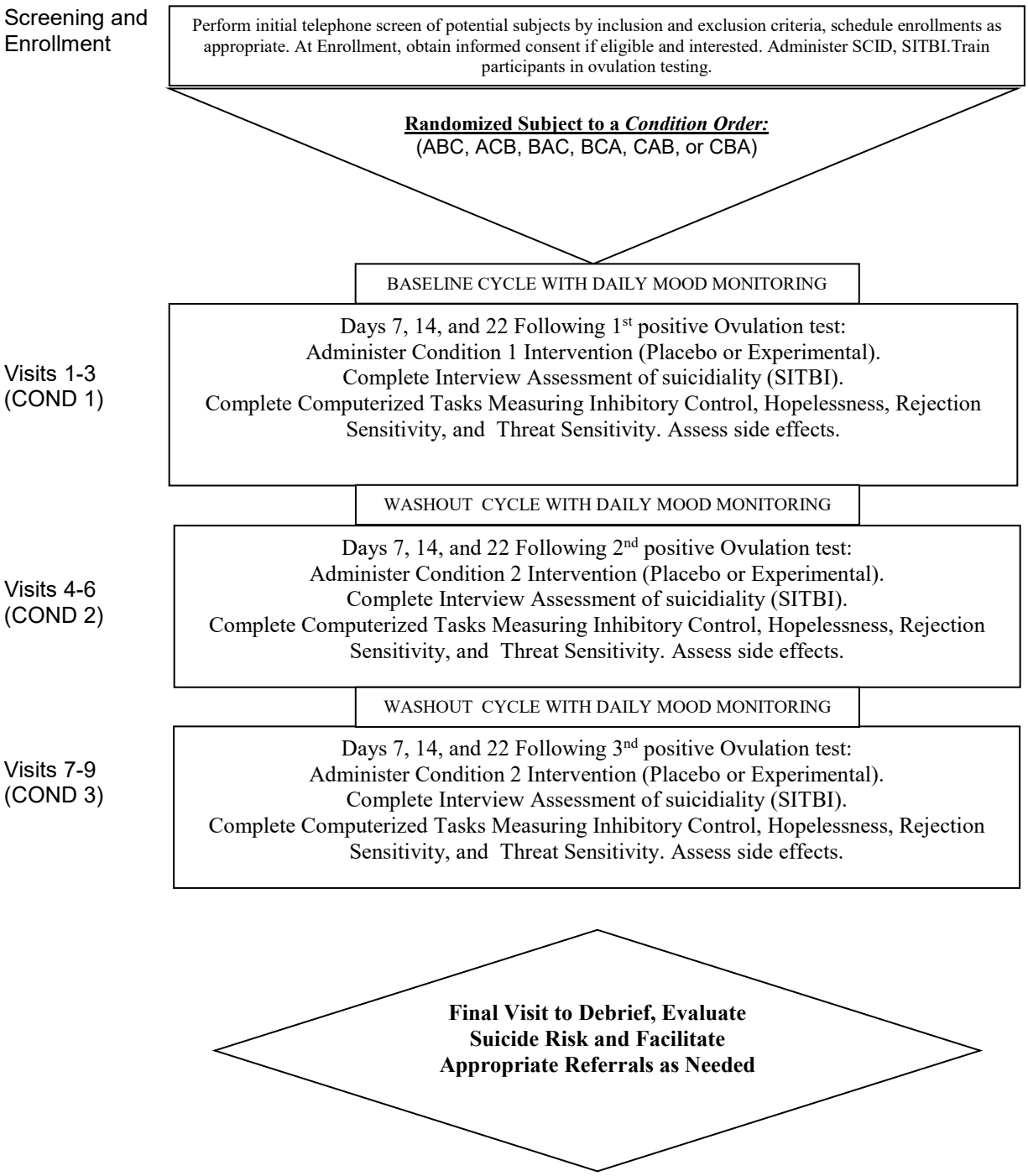
(5) Mean Within-Person Condition Differences (7 final placebo days – 7 final stabilization days) of SSES Social Evaluation/ Rejection Sensitivity subscale

Population: 60 female outpatients with suicidal ideation but minimal[1,2] imminent risk for attempt

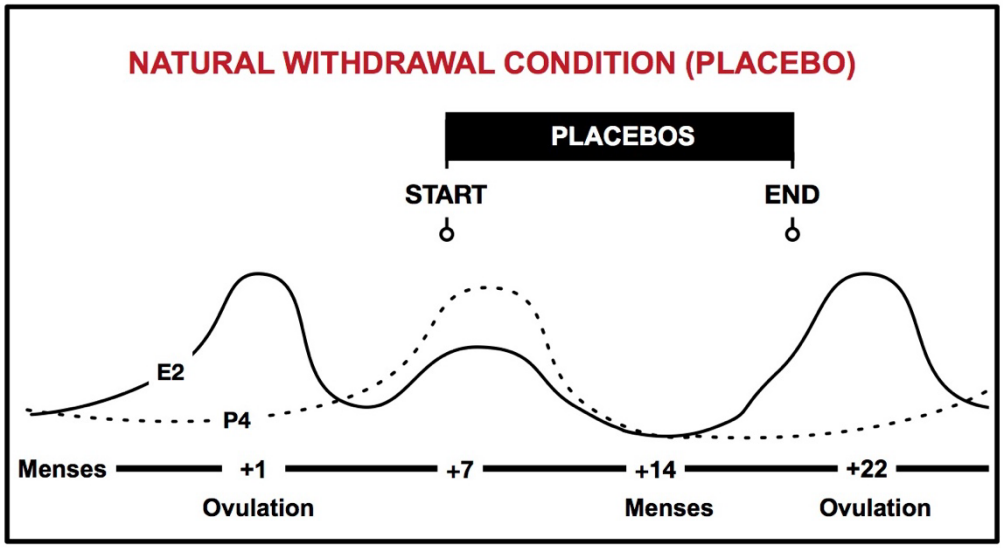
Phase: II

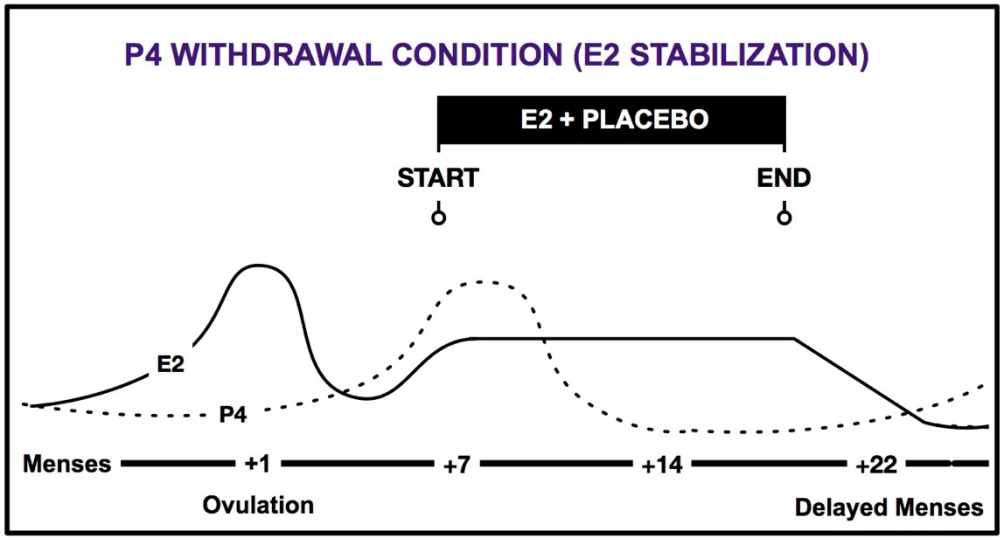
Number of Sites enrolling participants:	1
Description of Study Agent :	(1) Transdermal Estradiol .1mg/24hr weekly for two weeks, (2) Oral Micronized Progesterone 200mg (100mg each morning, 100mg each evening) for two weeks
Study Duration:	24 months
Participant Duration:	~3 active menstrual cycles (1 menstrual cycle lasts, on average, ~28 days) plus 1 baseline cycle and 2 washout cycles between conditions (i.e., 6 menstrual cycles total). Therefore, average participant participation will last roughly 6-7 months.

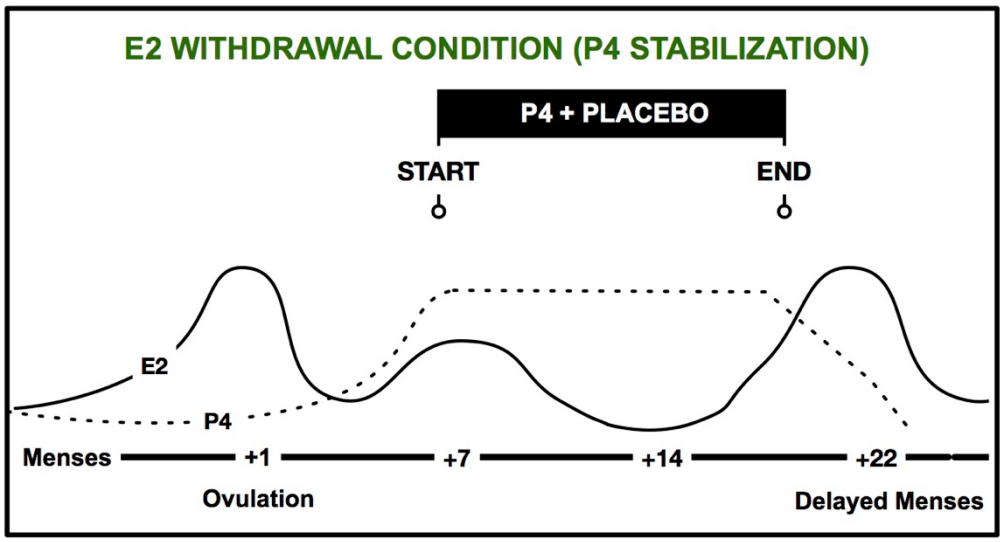
SCHEMATIC OF STUDY DESIGN



Overview of the Three Within-Person Counterbalanced, Crossover Study Conditions







1 KEY ROLES

Tory Eisenlohr-Moul, Ph.D. **– Primary Investigator**, Illinois-Licensed Clinical Psychologist, Clinician Responsible for Suicide Risk Assessment and Management; University of Illinois at Chicago, 912 S Wood St., Rm 335, Chicago, IL 60612. Email: *t.eisenlohr.moul@gmail.com*; moul@med.unc.edu. Phone: 859-317-0503.

Melissa Wagner-Schuman, M.D./Ph.D. (Primary Medical Expert) – **Medical Monitor, Supervising Physician Psychiatrist Regarding Hormone Administration**; University of Illinois at Chicago, Women's Mental Health Research Program, 912 S Wood St., Chicago, IL 60612. Email: mwagner9@uic.edu. Phone: (414) 303-0495

Madeline Divine, Project Manager – University of Illinois at Chicago, Women's Mental Health Research Program, 912 S Wood St., Chicago, IL 60612. Email: mdivine@uic.edu. Phone: (314) 315-3848.

2 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 BACKGROUND INFORMATION

Suicide is the second leading cause of death among females of reproductive age, yet we cannot reliably predict *when* an individual will attempt suicide. Suicide accounts for 13% of all deaths among reproductive-age American females[21]. Although existing models of suicide risk provide information about long-term, stable risk stemming from factors such as depression, interpersonal sensitivity, anxiety, or impulsivity, ***little is known about when these factors will translate into acute risk for attempt***[19,22,23]. By *directly studying suicidal individuals*[24] *and* focusing on biologic triggers and cognitive/behavioral mediators of acute changes in suicide risk, the proposed research responds to public calls from the U.S. President, Congress, Surgeon General, and the NIMH-co-sponsored Suicide Research Prioritization Agenda[23], to identify strong predictors of acute suicide risk[19]. Such work is imperative in developing targeted interventions for stabilizing and reducing suicidal behavior, and will improve the precision and effectiveness of critical efforts to block suicide attempts.

Perimenstrual (around menses) withdrawal from estradiol (E2) and progesterone (P4) may represent a time-varying biological mechanism of acute suicide risk. The perimenstrual period, which is characterized by rapid withdrawal from E2 and P4, is associated with more **hospitalization for suicide attempts**[1-3,25]**, more lethal attempts**[2], and **suicide deaths**[4]**.** *The rationale for studying perimenstrual hormone withdrawal* as a predictor of suicidality also comes from work showing that changes in normal levels of E2/P4, rather than *absolute* levels, precipitate affective symptoms and impulsivity in susceptible individuals[26,27]. Preliminary results of the PI's recently-completed similar experiment, described in the preliminary data section below), have provided the first evidence that perimenstrual E2/P4 withdrawal is responsible for perimenstrual exacerbation of suicide risk.

Theoretical Underpinning for Mediating Constructs. Selection of mediating constructs (Fig 1) through which ovarian steroid withdrawal is most likely[28,29] to influence suicide risk was informed by the Interpersonal Theory of Suicide[8,30], which posits that suicidal behavior arises from two acute risk factors: **Suicidal Desire**—a wish to die, and **Suicidal Action Capacity**—a *readiness to act* on urges. *Suicidal desire* is strongly predicted by hopelessness and negative social appraisals (thwarted belongingness, perceived burdensomeness)[8,31]. S*uicidal action capacity* is predicted by poor inhibitory control[32] and threat sensitivity—a tendency toward detection and reactions to threats (fostered by extreme stress exposure)[10,32]. *Because this is an integrative theory, the constructs measured here will allow testing of a variety of theories* (e.g., Neurocognitive Theory[7]).

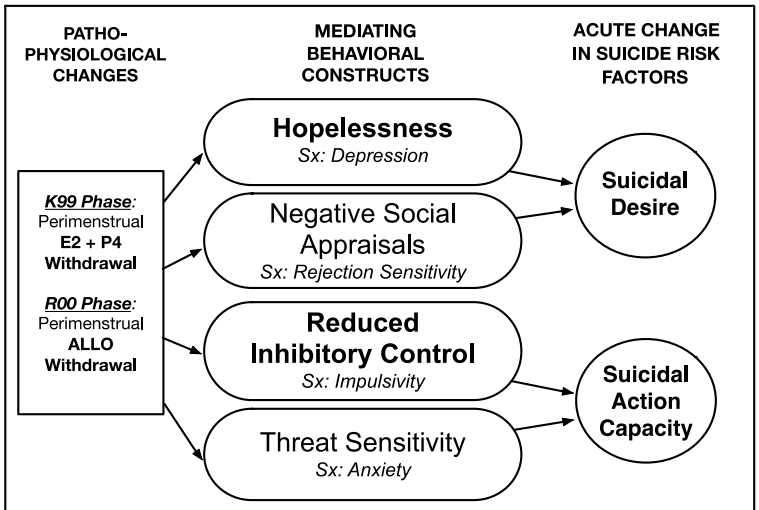


Figure 1. Pathophysiologic Model of Increased Perimenstrual Suicide Risk

Ovarian Steroid Changes Robustly Modulate Key Mediators of Suicidal Desire and Action Capacity

1. ***Hopelessness*** repeatedly emerges as ***the most robust*** prospective predictor of suicidal desire in inpatients[33] and outpatients[6]. Furthermore, hopelessness[5] and suicide[7,9,34] share functional alterations of the dorsomedial prefrontal cortex, supporting a central role of hopelessness in suicidality.

Ovarian steroid withdrawal may increase hopelessness *(and associated symptoms of depression)*. In animals, ovarian steroid withdrawal increases depressive behavior via GABAergic[75] and serotonergic[76,77] mechanisms, whereas E2 specifically is known to exert antidepressant effects.[78]

2. ***Negative social appraisals predict suicidal desire.*** Relationships are central to our ability to survive and thrive[35]. Perceptions of social rejection are experienced as painful[36,37] and increase suicidal desire[8]—particularly in reproductive-age females[38,39]. Additionally, suicidality is associated with altered function of brain regions associated with experiences of *social exclusion* and *rejection*[7].

Ovarian steroid withdrawal may increase negative social appraisals *(and associated symptoms of rejection sensitivity)***.** In animals, E2 and P4 facilitate social motivation and behavior[40-42], and ovarian steroid withdrawal

precipitates social withdrawal[15]. In females, elevated luteal P4 correlates with social motivation[43] and attention to social-affiliative opportunities[44]. The PI's longitudinal work indicates that ovarian steroid withdrawal predicts *increased* daily appraisals of social rejection in females at elevated suicide risk[12,16].

3. ***Poor inhibitory control*** enables suicidal desire to escalate into suicidal action[10,45]. Suicidal ideation alone is a poor predictor of suicidal behavior[19]; however, the addition of poor inhibitory control robustly predicts suicide attempts, suggesting its relevance for suicidal action capacity[11,46,47]. Suicide attempters and victims show deficiencies in the functioning of inhibitory circuits[10,48-52].

Ovarian steroid withdrawal may reduce inhibitory control *(and increase associated symptoms of impulsivity).* Cyclical E2/P4 withdrawal predicts poorer inhibitory control, greater impulsivity,[12,53-55] and decreased frontal cortex inhibitory activity[56], while high, stable ovarian steroids increase frontal cortex inhibitory activation[57,58]. Cyclical reductions in inhibition are particularly marked in females with low prefrontal dopamine, which corresponds to the greater impulsivity found in suicide victims[7,59]. In animals, baseline impulsive behavior is a risk factor for greater sensitivity to ovarian steroid withdrawal[60,61].

4. ***Threat sensitivity,*** (defined as increased *attention* and *reflexive responses* to threats), **predicts suicidal behavior**[10,39,62]. Repeated engagement of physiological stress systems also leads to HPA axis dysregulation, which prospectively predicts suicide attempts among chronically suicidal individuals (preliminary work by PI under review)[63]. Other research in suicide victims indicates that ***generalized*** threat sensitivity and reactivity to stimuli in daily life are acutely elevated prior to fatal suicidal behavior[62].

Ovarian steroid withdrawal may increase threat sensitivity *(and associated symptoms of anxiety)*. In animals, E2/P4 withdrawal increases threat sensitivity and reactivity[13] and causes threat-sensitizing alterations in associated brain circuitry, including the amygdala[64-66], the bed nucleus of the stria terminalis[66,67], and the periaqueductal grey[68,69], mediated by increased expression of $GABA_A$ receptor subunits that elicit anxiogenic effects of P4-derived neurosteroids[64,70-76]. In females, c*orrelates of suicidality* including social stress[77], social isolation[78], lifetime abuse[16], impulsivity[60], and PTSD [79,80], predict greater effects of ovarian steroid withdrawal on threat sensitivity. Cyclical reductions in E2/P4 correlate with morphological and functional changes in CNS fear and anxiety circuitry, especially amygdala sensitivity[28,81,82].

Despite robust animal and correlational human evidence that E2/P4 withdrawal modulates key mediators of suicide risk, **the PI's recently-completed, K99-funded experimental study (referred to as "the preceding study" throughout this document) was the first to experimentally test a pathophysiologic role for steroid hormone withdrawal on suicide risk factors in at-risk females.** The PI's recently-completed similar experiment (hypothesized model in Fig 1) tested a model in which ***perimenstrual E2/P4 withdrawal increases acute risk for suicidality*** by modulating mediating cognitive behavioral constructs (hopelessness, social appraisals, inhibitory control, threat sensitivity) and parallel clinical symptoms (depression, impulsivity, rejection sensitivity, anxiety) toward increased risk. To maximize both safety and generalizability, we studied females with suicidal ideation but minimal[17,18] imminent risk for suicide attempt. Preliminary results (n = 25) are outlined below. Additional planned analyses will delineate the within-person effects of ovarian steroid withdrawal on suicidal desire (via mediators hopelessness and social appraisals), and suicidal action capacity (via inhibitory control and threat sensitivity).

Safety and Feasibility Information. In a recently-completed similar experimental study by the PI (the K99-funded study), no serious adverse events have been recorded. Just two participants were withdrawn (by the study team; 0 dropouts) after randomization, supporting protocol feasibility. Missing data were minimal; participants completed 90% of daily symptom surveys, 97% of blood draws, and 100% of lab visits.

Results from Previous Experiment (Fig 2, above): <u>E2/P4 withdrawal causes perimenstrual worsening of daily self-reported suicidality in at-risk females, and EXPERIMENTAL STABILIZATION REDUCES THIS RISK</u>. Analyses of daily self-report data from the previous study (n = 30) indicates that perimenstrual hormone stabilization in the active condition *prevented* the robust perimenstrual increase in suicidal ideation observed under placebo (E2/P4 natural withdrawal). Results are identical for suicidal planning, suggesting the applicability of these results to suicidal <u>behavior</u> as well as suicidal thoughts. Further, as predicted, perimenstrual risk was *shifted* to the "withdrawal week" of the active condition (**not significantly different from natural perimenstrual levels of risk under placebo—i.e., the risk is not higher, but simply experimentally shifted to 2 weeks later**), further demonstrating the causal role of ovarian steroid withdrawal in perimenstrual worsening of suicidality. When results were standardized to reflect subject-specific means and standard deviations (as in Fig 2 at right), the average impact of stabilization was roughly a 1 person-standard-deviation condition difference in suicide outcomes. This suggests clinical significance. *Additional analyses using task and interview data are planned to identify the specific cognitive and behavioral constructs that account for experimental effects on suicidality outcomes*.

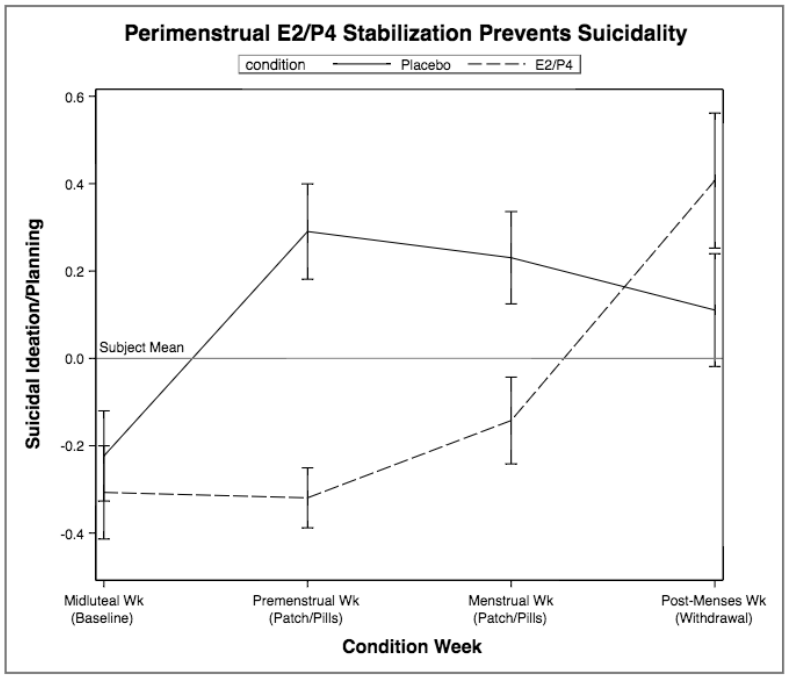


Fig 2: Preceding Study - Experimental Effects on Suicidality

2.2 RATIONALE

The **<u>rationale for the upcoming experiment</u>** is to build on the results of the previous experiment to **disentangle the relative effects and mechanisms of E2 and P4 withdrawal on suicide risk,** testing a mediational model in which P4 withdrawal is the primary driver of perimenstrual changes in behavioral constructs relevant to suicide risk, and ultimately, suicidal desire and action capacity, due to associated rapid withdrawal from ***P4-derived neuroactive steroids, particularly allopregnanolone (ALLO)***[83-87]***.*** ALLO, via positive allosteric modulation of $GABA_A$ receptors, exerts anxiolytic, antidepressive, and anticonflict effects[88,89]. P4 from the corpus luteum is the primary source of ALLO flux across the cycle. This fluctuation has important implications at the $GABA_A$ receptor, which is composed of a responsive combination of subunits that are up- or down-regulated to maintain homeostasis in the face of changing ALLO[70-72,90,91]. This ability of the $GABA_A$ receptor to change composition is important in times of considerable ALLO flux (e.g., perimenstrually[72,92,93]). Insufficient plasticity or maladaptive changes to the $GABA_A$ receptor in response to ALLO flux can cause paradoxical anxiogenic, despressogenic effects[71,75,90,91,94].

Therefore, the rationale for considering ALLO specifically stems from the following evidence: blocking conversion of P4 to ALLO ameliorates mood symptoms, including *hopelessness*[87], by blocking ALLO fluctuations and associated changes in $GABA_A$ receptor subunit expression[83]. ALLO also facilitates social behavior[42] and downregulates negative appraisals relevant to social cognition[95]. Finally, in a recent landmark paper from Dr. Leslie Morrow's lab, ALLO has recently been found to regulate inflammatory gene expression, a process that has been demonstrated to be heightened among suicide victims.

<u>In sum, the rationale for the experiment</u> is based on the need to disentangle effects of E2 and P4 withdrawal on suicide risk, and to identify a pathophysiological mediators (i.e., ALLO withdrawal, inflammatory gene expression) of the deleterious effect of perimenstrual E2/P4 withdrawal on suicidal risk in vulnerable females,

thereby beginning to clarifying the pathophysiology of acute suicidality. The long-term impact of this work is to identify targets for pharmacological and behavioral interventions to prevent suicide.

Design Overview: 40 female patients (up to 60 enrolled to allow for dropout) with recent suicidal ideation but minimal[17,18] imminent risk for suicide attempt will complete behavioral tasks and clinical risk assessments in **three** conditions: **(A) perimenstrual E2/P4 withdrawal** (under placebo), **(B) perimenstrual P4 withdrawal** (during exogenous perimenstrual stabilization of E2 only) and **(C) perimenstrual E2 withdrawal** (during exogenous perimenstrual stabilization of P4 only). Mediation by degree of perimenstrual ALLO withdrawal will be explored for significant condition contrasts.

CENTRAL HYPOTHESIS: Test the hypothesis that key suicide risk pathways will be heightened during conditions involving P4 withdrawal (conditions A and B above) relative to the E2 withdrawal condition (condition C), and that this greater risk will be mediated by a steeper slope of withdrawal from ALLO during P4 withdrawal and natural (placebo) E2/P4 withdrawal conditions relative to E2 withdrawal condition (where P4 is stabilized).

JUSTIFICATION OF E2/P4 DOSING AND SCHEDULE

Chosen formulations are bioidentical to human E2 and P4 and were selected to mimic luteal phase concentrations or E2 and P4. Results from the PI's recently-completed similar experiment indicate that these doses were effective in stabilizing (rather than increasing) luteal phase levels.

Rationale for Route and Dose of E2 and P4. We will use Climara® 7 day transdermal E2 patches. Peak levels of E2 with Climara (~45 pg/mL), achieved within 24 hrs., correspond to luteal E2 levels[102]. No transdermal P4 is available. Although transdermal synthetic progestins are available, these molecules have a high affinity for androgen and mineralocorticoid receptors, limiting their usefulness for testing hypotheses. Thus, oral prometrium will be dosed b.i.d. to achieve luteal P4 levels (~13 ng/dL)[102] to create **steady-state levels**.

Rationale for Duration of E2/P4*.* Administration for 14 days starting 7 days after ovulation will span the range of time in which natural perimenstrual withdrawal would occur and prevent natural withdrawal of *either E2 or P4*.

Compliance: Pills are dispensed in a double-blind manner. Patches will be placed by CTSA staff at UIC. All study staff will remain blinded. Analyses of blinding data from the previous experiment revealed that the blinding was successful; participants were not more likely to report believing they were on active treatment in the active condition relative to the placebo condition *(p* = .58). Participants will: **1)** be educated on the importance of compliance; **2)** be asked about compliance on each daily call; and **3) have *steroid levels*** measured to confirm compliance.

Route and Dose of Estradiol (E2). By avoiding the first-pass metabolic effects of oral estrogen, **transdermal E2** creates more stable blood levels and a more physiologic profile of E2 relative to its metabolites estrone and estriol[120-122]. Transdermal E2 also has a superior safety profile than oral estrogen for thromboembolic and metabolic risk[123-125]. The use of a 7 day transdermal system provides significantly more stable concentrations and fewer patch adherence problems than twice weekly patches[126]. Climara® 7 day patches will be employed because the adhesive layer of the matrix patch (vs. reservoir systems) consists of polymeric acrylate or vinyl acetate in which the E2 molecules are distributed to continuously releases E2, which is transported across the skin leading to sustained circulating levels of E2 during each 7-day period. Levels of E2 achieved in pharmacological studies with Climara patches (around 45 pg/mL) corresponds roughly to normative luteal levels of E2 (reference range: 40-200 pg/mL) of E2[102]. Climara achieves peak levels of E2 within 24 hours.

Route and Dose of Progesterone (P4). No transdermal P4 is available. Although transdermal synthetic progestins are available, these molecules have a high affinity not only for the progesterone receptor but also for the androgen and mineralocorticoid receptors, limiting their usefulness for testing our hypotheses. Therefore, **oral micronized progesterone** will be dosed daily to achieve luteal levels P4 (about 13 ng/dL)[102] given on a b.i.d. schedule to create more steady-state levels and minimize the greater variability (peaks and troughs) that can occur with the oral route. Prometrium peaks within 3 hours and declines throughout the next 24 hours.

Duration of E2/P4 Administration in experimental conditions. Administration of either E2 or P4 for 14 days starting 7 days following ovulation will span the range of time in which natural perimenstrual withdrawal from E2 and P4 naturally occur, and will therefore prevent natural perimenstrual withdrawal from E2 or P4.

2.3 POTENTIAL RISKS AND BENEFITS

2.3.1 KNOWN POTENTIAL RISKS

1) Psychological Symptom Assessment. Clinical interviews and self-report assessments contain questions regarding sensitive personal information, including severe psychological symptoms such as suicidal and other impulsive behaviors (e.g., substance abuse). As a result, participants may become upset or embarrassed when discussing current or past distressing life events and behaviors. On the other hand, recent evidence suggests that research questions pertaining to potentially distressing topics such as psychiatric symptoms, suicidality, and sexual or physical abuse do not significantly increase distress or acute suicide risk in females who report these issues[24,116]. The chance of increased distress is necessary in order to assess how symptoms change in response to perimenstrual hormone withdrawal (vs. stabilization).

2) Effects of the Ovarian Hormone Stabilization Protocol on Mood/Anxiety and Behavior.

Mood/Anxiety. As the population being studied in this protocol will have elevated psychiatric symptoms, and as ovarian hormone changes are implicated in depressive symptoms in some females[27,97], it is possible that adverse mood reactions may occur (1) during the administration of ovarian hormones in the stabilization condition or (2) naturally during the placebo (natural) perimenstrual withdrawal condition. ***However, because the proposed hormone manipulation mimics (i.e., stabilizes) the luteal hormone levels that females naturally experience***[102]***, the proposed study is not expected to pose any greater risk to participants than what they experience in daily life.*** Furthermore, although the slow taper at the end of the withdrawal prevention, luteal hormone stabilization conditions could elicit negative mood changes, these would not be expected to be any more severe than those that would otherwise have arisen from normal perimenstrual hormone withdrawal—*that is, we will have simply delayed the normal withdrawal process*.

Suicidality. As detailed above, the population will be recruited to achieve a population of females that is simultaneously: (1) experiencing **recent suicidal ideation**, yet (2) at **acceptably low**[17,18,100] **current risk for suicidal crisis/attempt** based on an assessment conducted during a screening visit (see **Table 1**). Because ovarian hormone changes are implicated in psychiatric symptoms in some females[27,97], and because such symptoms may increase suicide risk, it is possible that increases in suicidality may occur (1) during the administration of ovarian hormones in stabilization conditions or (2) naturally during the placebo (natural) perimenstrual withdrawal condition. ***However, because the proposed hormone manipulations mimic (i.e., stabilizes) the normal physiologic range of luteal hormone levels***[102] ***that females naturally experience, the proposed studies are not expected to expose participants to greater risk for suicidality than what they experience in daily life.*** This notion is borne out by the results of the preceding experiment by the PI, which suggest a beneficial effect of the active hormone stabilization condition on symptoms. The results also indicate that the "shifted" risk during active hormone patch/pill withdrawal does not represent an net increase in risk, because (1) typical perimenstrual increase in risk is prevented, and (2) the experimental withdrawal-related symptoms are not significantly different in magnitude from the natural withdrawal-related symptoms that are prevented earlier in the condition (as observed under placebo).

3) Hormone Side Effects. We do not expect any serious adverse mood or other side effects associated with the hormonal manipulations for the following reasons: First, we will be administering the physiologically relevant steroid hormones (estradiol and/or 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[127-130]. Second, prior exogenous hormonal studies (or E2, P4, or a combination) conducted in Dr. Rubinow's lab at the NIH that induced substantially more elevated hormone

concentrations and a more precipitous hormone withdrawal. In those studies in females with histories of reproductive mood disorders (whom are excluded in the present study), although hormone induction and withdrawal was associated with increased mood symptoms, symptoms were transient and no subjects experienced a mood-related adverse event defined by meeting criteria for MDD or developing imminent risk for suicide. **During the the PI's recently-completed similar study, no physical or mental adverse events were sufficient to lead the medical supervisor to recommend unblinding or termination of participation.**

Possible Side Effects of Estradiol. Nausea is the most common side effect of estrogen administration. At conventional hormone doses, this complaint seldom interferes with eating, and no weight loss has been reported as a result of nausea. Breast engorgement 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 extremely small. 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. In the upcoming experiment, however, the length of E2-only administration in the P4 withdrawal condition (see Research Strategy) is only 14 days, which is not sufficient to cause endometrial hyperplasia[131]. There is an increase in thromboembolism in females receiving non-contraceptive estrogen therapy[132-135], but this risk is most evident in obese females and in those who smoke (exclusionary factors in the present research).

It may be important to note that there were numerous concerns about increased risk for breast cancer and cardiovascular events related to the use of hormone replacement therapy (estrogen plus a progestin) that stemmed from the initial results of the Women's Health Initiative (WHI), which randomized 27,000 postmenopausal females to HRT or placebo. Critical reviews subsequent to the initial WHI reports showing an increased incidence of non-fatal breast cancer and cardiovascular events have argued that the discordant findings between the WHI and the observational studies finding benefit of HRT for all cause and cardiovascular mortality reflected problems in the design of the WHI, the most important of which may have been the age and condition of the study subjects[136]. As the risk of breast cancer, heart disease and metabolic dysregulation increase with age; the likelihood of adverse events upon exposure to E2 should also increase with age. Recent reports from the North American Menopause Society[137] and the Endocrine Society[138] confirm that the risk of CVD in the WHI was observed in older but not younger females. Additionally, data suggest that the formulation of E2 (conjugated equine estrogen) used in the WHI is associated with an increased profile of risk compared with transdermal E2, and the progestin used in the WHI (medroxy progesterone acetate) is particularly antagonistic (among progestins) to the beneficial cardiovascular effects of E2[139,140]. The Endocrine Society concludes that "Data from the various Women's Health Initiative studies, which involved participants of average age 63, cannot be appropriately applied to calculate risks and benefits of menopausal hormone therapy in females starting shortly after menopause"[138]. In fact, there is substantial evidence for the safety of E2, particularly if administered to females within 10 years of their final menstrual period and if administered as the transdermal form (e.g., lower risk of VTE)[137,138].

Given our plans to administer transdermal E2 to participants: (1) for only 14 days, (2) no older than 45 years of age, (3) who are currently regularly menstruating and medically healthy, and (4) who are at no greater than average risk for CVD, breast cancer or VTE, the **risk of serious adverse events is exceedingly low.**

Possible Side Effects of Progesterone. Progesterone and the synthetic progestins are widely prescribed, with indications including dysfunctional uterine bleeding, endometriosis, mastodynia, galactorrhea, and precocious puberty[131]. In a recent study, a dose of 300 mg of oral micronized progesterone (100mg higher than the dose proposed) was given to a sample of females with premenstrual mood disturbance and was well tolerated by this sample[141]. Side effects reported included lightheadedness and fatigue; these were very mild and caused no dropouts.

4) Confidentiality. A breach of confidentiality could indicate to others a participants' history of suicidality.

5) Venipuncture. Standard risks associated with venipuncture are present during the laboratory testing sessions.

2.3.2 KNOWN POTENTIAL BENEFITS

This is a mechanistic study to determine the role of ovarian hormone changes in constructs that are relevant to suicidality. <u>There is no direct benefit to the participants</u> other than the benefit of knowing that they are contributing to research on the causes of suicidality.

3 OBJECTIVES AND PURPOSE

The purpose of this mechanistic study is to experimentally test a pathophysiologic mediational model in which late luteal (perimenstrual) ovarian steroid withdrawal increases suicidal desire (via increased hopelessness and negative social appraisals) and suicidal action capacity (via reduced inhibitory control and increased threat sensitivity). We predict that <u>natural</u> P4 withdrawal (compared to the two conditions in which P4 is stabilized) will increase reports of suicidal desire (partially mediated by task performance indicating increased hopelessness and negative social appraisals) and action capacity (partially mediated by task performance indicating decreased inhibitory control and increased threat sensitivity). Therefore, we predict that administration of P4 will reduce suicide risk by buffering the deleterious impact of perimenstrual withdrawal from P4. We predict that administration of E2 will have some mild protective effects, but not to the same extent as P4.

4 STUDY DESIGN AND ENDPOINTS

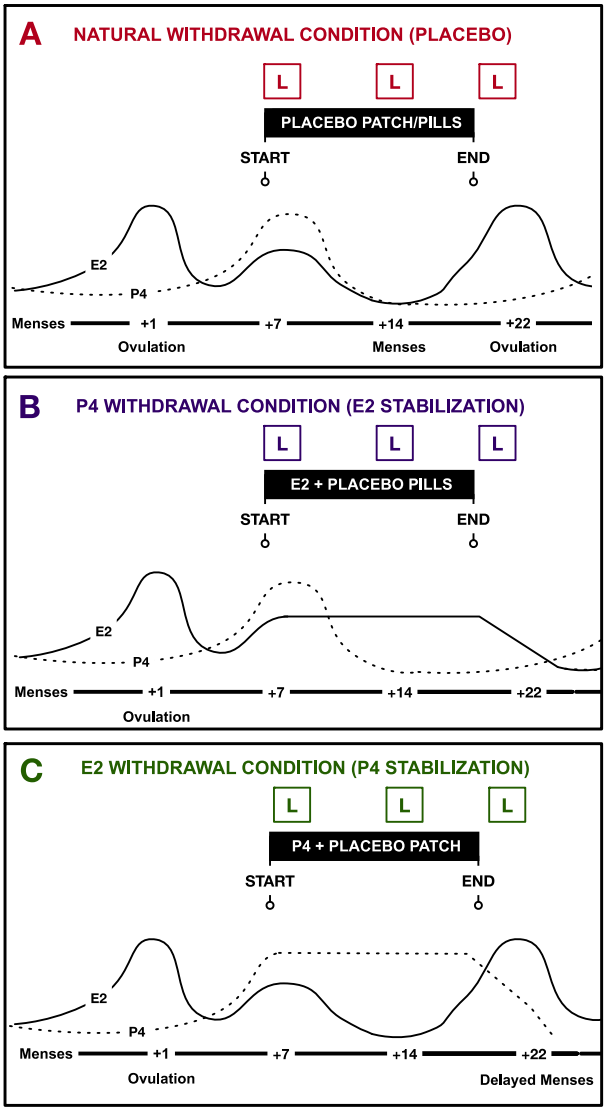
4.1 DESCRIPTION OF THE STUDY DESIGN

This is a single-center study; there is no phase designation because it is a mechanistic experiment with agents (E2,P4) that is commonly used in clinical practice.

<u>Participants:</u> 60 female outpatients (recruited to achieve a targed N of 40 completers) with a with past-month suicidal ideation but **low**[17,18] **imminent suicide risk** (see **Human Subjects**) receiving treatment as usual. Participants must: be 18 to 45 years of age, have normal menstrual cycles (21-35 days), not pregnant, breastfeeding, or trying to get pregnant, not taking hormones, no history of serious medical illness, normal weight (BMI 18-29.99), and low risk for thrombotic events (factor V Leiden mutation based on family history). *Most outpatients with suicidality take medications; therefore, to increase feasibility/generalizability, medication use will be measured and covaried in analyses (as in the similar study recently completed by the PI).*

Psychiatric Diagnoses. The SCID-I and SCID-II. *History of manic episode, psychosis, or substance abuse disorder will be exclusionary due to risk of suicidal crises*[17,100]*.* Additionally, self-report history of postpartum depression or premenstrual dysphoric disorder are exclusionary, since reproductive mood disorders may be characterized by a different pattern of sensitivity[101]. Based on data from the preceding study, we expect 90% of our sample to have lifetime depressive disorder, 50% to meet criteria for BPD, and 50% to have lifetime anxiety disorder.

Study Design (Fig 2). THIS IS NOT A CLINICAL EFFICACY TRIAL. However, an RCT design with transdermal 17β-E2 and oral micronized P4 will investigate the unique roles of perimenstrual withdrawal from E2 and P4 in acute suicidality (***Fig 1***). Participants will be trained in the protocol, including urine ovulation testing (LH surge preceding ovulation 24h), daily phone assessment, and patch/pill schedule. Using a double-blind, counterbalanced, **within-subject cross-over design**, participants will receive each of three, 2-week perimenstrual phase conditions (**see Fig 3 at right**) with washout cycles in between: (CONDITION A): E2/P4 withdrawal during placebo patches/pills, (CONDITION B): P4 withdrawal during E2 stabilization with active E2 patch and placebo pills, and (CONDITION C): E2 withdrawal during P4 stabilization with active P4 pills and placebo patch. The UIC Investigational Drug Service will manage randomization and dispensing. During the stabilization conditions, participants will wear weekly E2 patches (Climara®) delivering 0.10 mg E2 per day for seven days and will take oral progesterone (Prometrium®) at a dose of 100mg b.i.d. These doses were tolerated by 100% of participants in the preceding similar experiment. These doses will achieve (stabilize) luteal E2 or P4 levels[102].



Timing of Hormone Administration. Based on urine testing, participants will begin medications seven days after ovulation (+7 in Fig 3) to coincide with a time in the cycle when E2/P4 are elevated. The goal of the active E2 or P4 manipulations are to **extend and maintain the mid-luteal phase hormone profile** of either E2 or P4 beyond the point in the cycle that would normally be associated with natural withdrawal (as in Condition A under placebo). In the active conditions, the luteal phase hormone profile of *either* E2 (Condition B) or P4 (Condition C) will be maintained for 14 days by applying a new patch on day +14 (extended luteal phase levels spanning +7 to +21), preventing natural withdrawal and stabilizing E2 or P4 in physiologic[102] luteal range. In the prior study, this dosing schedule resulted in a stabilized, slower hormone taper as both endogenous hormones were naturally falling during the perimenstrual experimental time period, with exogenous hormone slowing/buffering this decline during the active phase. Of note, the placebo condition uses the same patch/pill schedule.

Assessment of Outcomes. In addition to daily surveys, participants will complete phone measures starting at ovulation and ending 5 days following the last patch/pill. Three lab sessions will assess serum E2, P4, and ALLO, and behavioral outcomes.

Lab Construct Measures. *Each computerized task is scored using within-task comparisons, controlling for training-related changes in response time.* ***Hopelessness*** will be measured via the Future Thinking Implicit Relational Assessment Procedure (FT-IRAP)[103]; participants respond with *true* or *false* to pairings between "I expect" and "I don't expect" with sets of negative (e.g., worry, sadness) and positive (e.g., enjoyment, happiness) events. Greater latency to optimistic/hopeful, and lower latency to pessimistic/hopeless pairings indicate implicit hopelessness[103]. Performance predicts clinical hopelessness and depression[103]. Negative ***Social Appraisals*** will be measured using the Social Acceptance-Rejection Implicit Association Test (SAR-IAT)[104], in which participants respond to pairings of *self* words (e.g., I, me) and *other* words (e.g., them, you) with words indicating social acceptance (e.g., included) or social rejection (e.g., rejected). Response times to pairings indicate the strength of implicit self-with-acceptance or self-with-rejection associations. Performance predicts explicit social

appraisals and negative affect in social interactions[104]. ***Inhibitory Control*** will be measured using the Stop-Signal Task (SST)[105]; participants withhold a prepotent response. Performance predicts prefrontal cortex activation and lower impulsive clinical outcomes[105]. ***Threat Sensitivity*** will be measured as attentional bias for threat pictures on a Dot Probe Task (DPT)[106]. Attentional bias to threat on this task is linked to anxiety disorders[107] and multimodal indices of threat sensitivity in daily life[106,108]. ***Parasympathetic*** influences on the heart will be quantified using electrocardiogram, which will be collected and cleaned according to established evidence-based protocols. ***Respiratory Sinus Arrhythmia (RSA)*** will measure of parasympathetic (vagal) influence, and will be calculated using the well-validated peak-valley method. Greater RSA values reflect greater parasympathetic activity. RSA will be evaluated for a 10-minute baseline. ***Concurrent Suicidal Desire*** and ***Action Capacity*** will be estimated with the Suicidal Ideation, Thoughts, and Behavior Interview (SITBI)[112]. ***Validity*** will also be examined with a wide variety of well-validated self report laboratory measures of the above constructs; all items of these measures are provided in the appendix.

Daily Online Symptom Measures*: A variety of items intended to capture a wide array of symptoms related to the above constructs; all items are provided in the appendix.*

Daily Safety and Suicidality Telephone Call*:* **Suicidal desire and action capacity** will be measured with a daily telephone call during active condition days using a shortened version of the SITBI[112]. Items are provided in the appendix.

Participant Safety: As in the preceding experiment, emergent changes in suicidality will be managed using the structured University of Washington Risk Assessment and Management Protocol (UWRAP)[24,116]. ***However,*** *because the manipulation mimics luteal E2/P4 levels that females* ***naturally*** *experience,* the manipulation is not expected to pose greater risk than *a normal*[102] *cycle—the risk will simply be shifted during P4 stabilization. This is supported by the results of the prior experiment.*

Primary Windows of Comparison for Hypothesis Testing. To test whether prevention of P4 withdrawal (in Condition C) reduces the perimenstrual increase in suicide risk, **primary** hypothesis tests will compare degree of suicide risk (as measured with self-report, tasks, and interviews) during the perimenstrual weeks of each condition (days +7 to +22)**. Secondary** analyses will examine Condition C, testing whether tapered withdrawal from exogenous P4 around days +16 to +22 causes "shifted"/"postponed" perimenstrual risk.

Hypotheses/Analytic Plan. It is hypothesized that Condition A (E2/P4 withdrawal under placebo) and Condition B (P4 withdrawal under E2 stabilization) will BOTH be associated with heightened perimenstrual suicidality, but that Condition C (E2 withdrawal under P4 stabilization) will significantly reduce perimenstrual increases in suicidality. We also hypothesize that a steeper slope of ALLO withdrawal, calculated across the lab ALLO measures in each condition, will mediate the more deleterious effects of P4 withdrawal conditions. Multilevel models will be used (repeated observations nested within individuals) to predict daily or weekly repeated suicidality and mediator outcomes from a within-person condition factor (and, if appropriate, the interaction of condition and day). Mediation by degree of ALLO withdrawal will be tested by estimating a 95% CI for the indirect effect of condition (focal predictor) on suicide risk outcomes (dependent variable) via the slope of ALLO change across the perimenstrual phase (mediator). ***Power Analysis****.* For primary hypothesis tests, 80% power to detect a conventionally medium-sized effect of condition (f = .25) is achieved with 24 participants[117]. To allow for attrition and improve generalizability and power, we propose to include 30 participants.

4.2.1 PRIMARY ENDPOINT

The primary endpoints (described below) of this mechanistic study are suicidal ideation and planning score differences between placebo condition and stabilization conditions on the SITBI measure. These were chosen given their strong predictive validity in epidemiological studies.

(1) Mean Within-Person Condition Differences (7 final placebo days – 7 final stabilization days) of SITB Suicidal Ideation subscale score

(2) Mean Within-Person Condition Differences (7 final placebo days – 7 final stabilization days) of SITB Suicidal Planning subscale score

4.2.2 SECONDARY ENDPOINTS

The secondary endpoints of this study include the daily telephone measures as follows:

(6) Mean Within-Person Condition Differences (7 final placebo days – 7 final stabilization days) of BHI Hopelessness Score

(7) Mean Within-Person Condition Differences (7 final placebo days – 7 final stabilization days) of CES-D Depression Total Score

(8) Mean Within-Person Condition Differences (7 final placebo days – 7 final stabilization days) of UPPS-P Lack of Premeditation Impulsivity subscale

(9) Mean Within-Person Condition Differences (7 final placebo days – 7 final stabilization days) of PROMIS Anxiety Total Score

(10) Mean Within-Person Condition Differences (7 final placebo days – 7 final stabilization days) of SSES Social Evaluation/ Rejection Sensitivity subscale

4.2.3 EXPLORATORY ENDPOINTS

None.

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 PARTICIPANT INCLUSION CRITERIA

In order to be eligible to participate in this study, an individual must meet all of the following general criteria:

- Provision of signed and dated informed consent form
- Stated willingness to comply with all study procedures and availability for the duration of the study
- Ability to take oral medication and be willing to adhere to the medication regimen

To be eligible for participation in the proposed study, an individual must meet all of the following criteria.

1. Must be biologically **female** and between the ages of **18-45 years**.
    - **Justification**: Females younger than 18 or older than 45 may have inconsistent menstrual cycles or may be postmenopausal and therefore no longer cycling.

2. Must have **normal menstrual cycles** between 21 and 35 days.
    - **Justification**: Abnormal menstrual cycle lengths or variability in lengths could introduce error variance into the experiment, and could reduce confidence regarding the timing of the experimental manipulation to cycle events.

3. Must be under the **current care** of an outpatient mental health provider, whom they report seeing at

least once every 3 months.
    - **Justification**: Because we intend to recruit females with current suicidality, engagement with a mental health provider is required as a protective measure to ensure that participants have access to treatment if needed.

4. If the woman has children, she must be at least **1 year postpartum**.
    - **Justification**: The postpartum period can be characterized by altered hormone levels and changes; therefore, postpartum females may introduce error variance into the experiment.

5. Must **not be pregnant, breastfeeding, or trying to become pregnant**. Pregnancy status will be confirmed using urine pregnancy test at the enrollment visit and again at the first visit of the second condition. Participants will be encouraged to use a barrier method of birth control during the study.
    - **Justification**: Administration of ovarian hormones to a pregnant woman could negatively influence the pregnancy in a variety of ways, including risks to the health of the fetus.

6. Must not be taking any form of **exogenous hormones** or IUD, and must have ended previous use of hormonal preparations at least one month prior to the study.
    - **Justification**: The use of additional exogenous hormones poses safety risks and would undermine experimental control of hormones.

7. Must be of **normal weight** (BMI between 18.00-29.99 kg/m2); measured at the enrollment visit.
    - **Justification**: Responses to doses of hormones may vary by BMI and risk for thromboembolic events with hormone use increases in obese females.

8. Must report no personal history of any **chronic medical condition**, including but not limited to metabolic or autoimmune disease, epilepsy, endometriosis, cancer, diabetes, cardiovascular, gastrointestinal, hepatic, renal, or pulmonary disease, and no personal or first degree family history of thromboembolic events.
    - **Justification**: Administration of exogenous hormones to females with these chronic medical conditions may increase the risk of an adverse health-related response to hormones. Further, the purpose of the present study is to determine the impact of hormonal stabilization (vs. natural withdrawal) in otherwise physically *healthy* females.

9. Must not report 2 or more first degree relatives with hormone dependent cancers (ovarian, uterine, breast, colon, prostate) or a known genetic predisposition (BRCA1, BRCA2, PTEN, or Tp53) to those cancers
    - **Justification**: Administration of exogenous hormones might increase cancer risk in individuals with genetic predisposition.

10. Must not currently smoke **cigarettes**.
    - **Justification**: Administration of ovarian hormones to females who smoke may increase the risk of thromboembolic events.

11. Must not report a history of clinical diagnosis of postpartum depression or premenstrual dysphoric disorder (Note: PMDD diagnosis must have been made based on prospective daily ratings).
    - **Justification**: Some evidence would suggest that the pattern of perimenstrual symptoms that have been linked with these reproductive mood disorders *could* differ meaningfully[27] from that which we be expect in the more general clinical population of females with suicidality[12]. Because these distinctions are not yet clear, females with a self-reported history of clinical diagnosis with these disorders will be excluded.

12. Must not report any history of manic episode, psychotic symptoms, or substance use disorder.
    - **Justification**: These factors are known sources of variability in suicidality and would therefore introduce error into our models as well as increasing the risk that a participant would experience

a suicidal crisis during the study[17,100]. Use of opioids, methamphetamine, and cocaine will be screened at the enrollment visit to validate self-report measures of substance use.

13. Must report **at least some recent suicidal ideation (in the past month)** at enrollment.

- **Justification**: As reviewed in the research strategy, the purpose of the study is to examine the impact of natural perimenstrual hormone withdrawal (vs. prevention of either E2 or P4 withdrawal) on within-person changes in suicide risk. The present sampling strategy **ensures generalizability**[24] of our findings to clinical populations of interest and will also **prevent a floor effect** (i.e., zero-inflation of daily outcome measures and associated limitations in power to detect effects) on primary daily outcome measures (e.g., mediating behavioral constructs, suicidality).

14. Must be categorized as having **acceptably low imminent risk for suicidal crisis/attempt** according to established clinical and research guidelines[100] (see Suicide Experimental Eligibility Table), which have been adapted for the K99/R00 studies (the preceding experiment and the present experiment) in collaboration with Dr. Prinstein, the suicide-focused co-mentor on the K99 training grant. As Dr. Prinstein notes in his 2014 methodological paper on research-based suicide risk assessment[18], evaluation of imminent risk of suicidal crisis/attempt is a complex clinical task, requiring integrative consideration of a variety of factors (including: general clinical presentation, ideation severity, frequency, recency, and associated consideration/intent; access to suicidal means; previous attempts and gestures; presence of suicidal planning, recent stressors, frequency of contact with mental health provider; and presence of social support). *Therefore*, our specific criteria for delineating whether a participant is eligible are based on an evidence-based determination[18,100] that the participant is at **acceptably low imminent risk of suicidal crisis/attempt**. These decisions will be based on responses to a variety of questions from the SITBI and Severity of Suicidal Ideation Interview (clinical interviews at the beginning of an enrollment visit).

- ***Justification:*** Individuals with current suicidality **are not necessarily at imminent risk for suicidal crises/attempt** (i.e., their risk is manageable on an outpatient basis through frequent clinical contact, such as the daily calls in the present study). Because the proposed hormone protocol simply prolongs the physiologic luteal hormone profile of E2 or P4, it is **not** expected to expose participants to additional risk of suicidal crisis beyond what they would naturally experience during their typical monthly menstrual cycles. However, out of an abundance of caution, we will **exclude females with elevated imminent risk for suicidal crisis/attempt** (determined by structured interview and established guidelines[18,100]). Individuals with histories of suicidality often have ongoing, habitual thoughts about death or suicide that are highly distinguishable from **imminent risk for suicide attempt**[17,100,118]. In further support of this notion and of the feasibility of recruiting this population, it should be mentioned that up to 1/3 of the American population experiences suicidal ideation at some point in their lives[118], yet less than .01% die by suicide each year[100,118]***.*** **Data from the preceding similar experiment (K99 experiment) provide clear evidence of the safety of this protocol; no participant was hospitalized for imminent risk or attempted suicide during the study.**

SPECIFICS OF SUICIDE RISK-RELATED ELIGIBILITY CRITERIA

The proposed study will **include only participants with current suicidal ideation** (past-month endorsement of suicidal ideation) in order to maximize generalizability to our clinical population of interest. We hypothesize that *natural* perimenstrual withdrawal from ovarian hormones will be associated with significant but **transient shifts in continuously-estimated suicide risk *that are not expected to escalate to the level of suicidal crisis/attempt*** (due to the exclusion of certain females at highest risk for attempt, as noted below). In order to minimize the risk of suicidal crises during the study, we will **exclude females who meet evidence-based criteria**[18,100] **for imminent risk of acute suicidal crisis/attempt**.

*The goal of these exclusion/inclusion criteria is to **minimize** the risk that a participating woman will demonstrate imminent risk of suicidal crisis/attempt during the study. The determination of acceptably low current risk of suicidal crisis/attempt will be carried out in multiple steps, as follows:*

1. First, potential participants must pass a phone screen in which they will report on their previous psychiatric diagnoses and recent history of suicide attempts. At that juncture, the following criteria will be applied:
    a. A woman will be excluded if she reports a suicide attempt or any concrete suicidal planning in the past 3 months, as the majority of repeated attempts occur within the first three months following an initial attempt[17,100].
    b. A woman will be excluded if she reports a history of more than one suicide attempt, as multiple attempts represent a uniquely potent risk factor for additional suicidal crises/attempts[17,100].
    c. A woman will be excluded (and referred) if she reports that she does not currently visit a mental health provider (psychiatrist, therapist) at least once every 3 months[17].
    d. A woman will be excluded if she reports any extreme external stressors (e.g., death of a loved one, loss of employment) in the past month, as extreme stressors drastically increase the risk of suicidal crisis[17,100].
    e. A woman will be excluded if she reports a history of clinical diagnosis with manic episode, psychotic symptoms, or substance use disorder, as these disorders reduce predictability and increase likelihood of suicidal crises/attempt[100].

2. Second, potential participants must pass an in-person eligibility screening at the beginning of an enrollment visit, in which they will complete a clinical interview designed to more thoroughly assess the risk factors described in 1a-1e above, as well as additional determinants of suicide risk outlined in **Table 1** below.
    a. Eligibility criteria in 1a-1e above will again be re-assessed and applied.
    b. Using a clinical interview based on the SITBI interview[112] and the Scale for Suicidal Ideation interview[119], the PI will evaluate each woman on a variety of risk factors for suicidal crisis and evaluate whether a woman has acceptably low[17,18,100] imminent risk of suicidal crisis/attempt using the decision rules described in the leftmost column in Table 1 and summarized below:
        - ***The evidence-based determination of acceptably low risk[17,18,100] of suicidal crisis/attempt will be based on the count of positive findings in each Row of Table 1 (see next page), as follows: No positive findings in Row 5; No greater than 1 positive finding in Row 4; No greater than 2 total positive findings in Rows 3 + 4; No greater than 3 total positive findings in Rows 2, 3, and 4.***

3. *Note that ongoing participant suicide risk monitoring and management is described in later sections.*

SUICIDE-RELATED EXPERIMENTAL ELIGIBILITY TABLE

The goal of the suicide-related exclusion/inclusion criteria (outlined below) for the proposed set of studies is to ***minimize*** *the risk that a participant will experience an acute suicidal crisis or attempt suicide during the study. Evidence-based criteria were developed in collaboration with mentors at UNC Chapel Hill During the K99 Phase of the Grant*[17,18,100]. History of mania, psychosis, or substance abuse disorder will also be exclusionary.
Table notes: Recent = In the past month. MHP = Mental Health Provider. [1]SITBI items 3a, 3b, 4a, 4b, 4c. [2]SITBI items 1a, 1b, 1c, 1f, 2c. [3]SITBI items 2a, 2b.

	Suicide Attempts[1]		**Suicidal Thoughts**[2]				**Recency of Last Suicidal Planning**[3]	Current Access to **Means**	Recent **Stressors**/ Loss	Current Frequency of **MHP Contact**	Current **Social Support**
ROW	**Number**	**Recency**	**Recency**	**Recent Frequency**	**Recent Severity**	**Recent Intent**					
1	Never	Never	In past year	Less than monthly	1f=1	2c=1	Never	No Access to Means	None	>1x/ month	Multiple Strong Supporters
2 *No more than 3 total positive findings allowed in Rows 2,3, +4*	Any gesture or NSSI, no attempt	In past 2 years	In past month	~1x/ month	1f=2	2c=2	>2 years ago		Extreme Stressors in past year	~1x/ month	One Strong Supporter or multiple supports
3 *No more than 2 total positive findings allowed in Rows 3 + 4*	1 aborted attempt	in past year	In past week	~1x/ week	1f=3	2c=3	1-2 years ago	Access to Nonspecific or Unfeasible means	Extreme Stressors in last 3 months	~1/ 2 months	Very Limited Support
4 *No more than 1 positive finding allowed in Row 4*	1-3 attempts	In past 6 months	Now	>1x/ week	1f=4	2c=4	3 months to 1 year ago	Access to Specific, Feasible Means	Extreme Stressors in last 1-2 months	~1x/ 3 months	No Support
5 *No positive findings allowed in Row 5*	>3 attempts	In past 3 months					<3 months ago		Extreme Stressors in last month	<1x/ 3 months	

5.2 PARTICIPANT EXCLUSION CRITERIA

Any individual meeting any of the following exclusion criteria at baseline will be excluded from the study:

1. Must **not be pregnant, breastfeeding, or trying to become pregnant**. Pregnancy status will be confirmed using urine pregnancy test at the enrollment visit and again at the first visit of the second and third conditions. Participants will be instructed to use a barrier method of birth control during the study.
    - **Justification**: Administration of ovarian hormones to a pregnant woman could negatively influence the pregnancy in a variety of ways, including risks to the health of the fetus.

2. Must not be taking any form of **exogenous hormones** or IUD, and must have ended previous use of hormonal preparations at least one month prior to the study. Potential participants who were previously taking oral contraceptives or other hormonal medications must have one normal menstrual cycle (menstrual period) prior to enrollment in the study.
    - **Justification**: The use of additional exogenous hormones poses safety risks and would undermine experimental control of hormones.
    - **Participants will be required to use licensed barrier methods of contraception during study participation; if they are unable to commit to this responsibility, they will not be eligible for participation**.

3. Must report no personal history of any **chronic medical condition**, including but not limited to metabolic or autoimmune disease, epilepsy, endometriosis, cancer, diabetes, cardiovascular, gastrointestinal, hepatic, renal, or pulmonary disease, and no personal or multiple first degree family history of thromboembolic events.
    - **Justification**: Administration of exogenous hormones to females with these chronic medical conditions may increase the risk of an adverse health-related response to hormones. Further, the purpose of the present study is to determine the impact of hormonal stabilization (vs. natural withdrawal) in otherwise physically *healthy* females.

4. Must not currently smoke **cigarettes**.
    - **Justification**: Administration of ovarian hormones to females who smoke may increase the risk of thromboembolic events.

5. Must not report a history of clinical diagnosis of postpartum depression or premenstrual dysphoric disorder (Note: PMDD diagnosis must have been made based on prospective daily ratings).
    - **Justification**: Some evidence would suggest that the pattern of perimenstrual symptoms that have been linked with these reproductive mood disorders *could* differ meaningfully[39] from that which we be expect in the more general clinical population of females with suicidality[8]. Because these distinctions are not yet clear, females with a self-reported history of clinical diagnosis with these disorders will be excluded.

6. Must not report any history of manic episode, psychotic symptoms, or substance use disorder.

- **Justification**: These factors are known sources of variability in suicidality and would therefore introduce error into our models as well as increasing the risk that a participant would experience a suicidal crisis during the study[1,149].

7. Must not test positive for opioid use, cocaine use, or methamphetamine use at the enrollment visit.
   - **Justification**: Use of these drugs are indicative of either a chronic medical condition or increased risk of suicidal crisis, both of which are exclusionary in the present study.[149]

5.3 STRATEGIES FOR RECRUITMENT AND RETENTION

Recruitment and Informed Consent

We will utilize the same strategies for recruitment as were used in the similar preceding experiment: (1) social media advertisements with links to a qualtrics eligibility survey, and (2) community flyers with contact information.

Potential participants will complete initial eligibility screening by telephone and a secure online questionnaire.

No more information will be asked of participants than necessary to obtain eligibility information and to contact those who appear to be eligible to schedule a screening/enrollment visit. At this initial visit, the PI (Dr. Eisenlohr-Moul) will obtain written informed consent for further screening regarding suicidality from those individuals who pass the initial telephone and questionnaire screenings and are interested in participating. This consent will inform participants regarding the nature of the questions to be asked. If the participant consents, the Self-Injurious Thoughts and Behaviors Interview[160] and the Scale for Suicidal Ideation Interviews[168] will be administered. If the participant is not eligible for the study following the screening (see details above), they will be dismissed and referred for treatment as necessary. If they are eligible, Dr. Eisenlohr-Moul will obtain additional written informed consent for participation in the larger study (i.e., the screening visit will become the enrollment visit). During the consenting process for the larger study, all of the applicable consent forms will be reviewed with each individual, and they will be given as much time as they would like to discuss their participation with their significant others and decide whether to participate.

INCLUSION OF WOMEN AND MINORITIES

Men will not be included in this study, given the stated purpose of examining ovarian hormone withdrawal as a mechanism of suicidal risk in females. With respect to ethnic and racial diversity, The University of Illinois at Chicago (UIC) Women's Mental Health Research Program (WMHRP) has a strong commitment to the enrollment of racial minority women in research projects, and this commitment will be reflected in the proposed work. The WMHRP has extensive experience with recruitment and retention of diverse samples in women's mental health studies that reflect Chicago demographics. We plan to recruit proportions of ethnic and racial minorities for the proposed work that are as consistent with the demographics of Chicago (per 2016 data) as possible. We have conservatively estimated our ability to recruit a sample (N=30) that is 60% Caucasian (n = 18), 32% Black or African American (n=9), 5% Asian (n = 2),

and with 3% identifying as more than one race (n = 1). With regard to ethnicity, we estimate that 25% (n = 7) will identify as Hispanic, and 75% (n = 23) will identify as non-Hispanic.

JUSTIFICATION FOR INCLUSION OF INDIVIDUALS WITH CURRENT SUICIDALITY

As reviewed in the study background and rationale, the purpose of the study is to examine the impact of natural perimenstrual hormone withdrawal (vs. prevention of hormone withdrawal/stabilization of E2 or P4) on within-person changes in suicide risk. The present sampling strategy **ensures generalizability**[3] of our findings to clinical populations of interest (i.e., not simply depressed females, but those with suicidality) and will also **prevent a floor effect** (i.e., zero-inflation of daily outcome measures and associated limitations in power to detect effects) on primary daily outcome measures (e.g., mediating behavioral constructs, suicidality).

Individuals with current suicidality **are not necessarily at imminent risk for suicidal crises/attempt** (i.e., their risk is manageable on an outpatient basis through frequent clinical contact, such as the daily calls in the present study). Because the hormone protocol simply prolongs the physiologic luteal hormone profile, it is **not** expected to expose participants to additional risk of suicidal crisis beyond what they would naturally experience during their typical monthly menstrual cycles. However, out of an abundance of caution, we will **exclude females with elevated imminent risk for suicidal crisis/attempt** (determined by structured interview and established guidelines). Individuals with histories of suicidality often have ongoing, habitual thoughts about death or suicide that are highly distinguishable from **imminent risk for suicide attempt**. In further support of this notion and of the feasibility of recruiting this population, it should be mentioned that up to 1/3 of the American population experiences suicidal ideation at some point in their lives, yet less than .01% die by suicide each year.

RETENTION OF INDIVIDUALS WITH SUICIDALITY

Although a variety of exclusion criteria will be applied, the PIs recent work has prepared her to recruit, retain, and maintain the safety of participants and potential participants with suicidal ideation but low imminent risk of attempt. Furthermore, the majority of individuals with suicidal ideation do not evidence imminent risk for suicide attempt, supporting the feasibility of recruiting from this population. With regard to retention, the PI has demonstrated the capacity to recruit and retain females with BPD with suicidal ideation, and participants with BPD in her recent study have completed 85% of daily email surveys; given larger incentives for shorter daily contacts, we expect a higher response rate. In the recently-completed experimental study, the PI and her team were successful in retaining 30 participants with suicidality through a similar 4-month study.

Empirically-supported strategies for retention will include study branding, frequent contact, monetary rewards, and graduated compensation schedules.

Study subjects will receive the following monetary incentives:

Participants who complete the protocol in full will receive $2650, paid in installments over 6-7 months of participation following the completion of the enrollment visit and folling each of three conditions.

Study participants will receive the following monetary incentives:

- $150 pay for one enrollment visit
- $150 pay for each laboratory testing session x 9 laboratory sessions (= $1350 total)
- $250 for completing daily online symptom surveys in each condition (=total $750)
- $100 for completing daily phone calls in each condition (=total $300)
- $100 for debriefing visit

Rationale for Pay: The compensation structure utilized here is identical to that used for the previous iteration of this experiment (the K99-funded experiment) at the PI's previous institution (UNC Chapel Hill). It is critical that the compensation structure remain identical in order to prevent any possible change to the protocol that may affect participant investment or attitudes toward completion of surveys and laboratory visits. The extended nature of the protocol (6-7 months) and the intensive requirements of participation coincide with the level of incentive we are providing. Given the intensive nature of the study and the long time-span over which compensation will be provided, we believe that this compensation structure is NOT likely to be coercive.

5.4 PARTICIPANT WITHDRAWAL OR TERMINATION

5.4.1 REASONS FOR WITHDRAWAL OR TERMINATION

Participants are free to withdraw from participation in the study at any time upon request. An investigator may terminate participation in the study if:

- Any clinical adverse event (AE), or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant (severe mood deterioration, severe changes in suicidality, severe hormonal side effects), as determined by Dr. Eisenlohr-Moul in collaboration with Dr. Wagner (or the DSMB).

- The participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.

5.4.2 HANDLING OF PARTICIPANT WITHDRAWALS OR TERMINATION

PROTOCOL FOR DISCONTINUATION AND REFERRAL

If a participant shows emergence of severe mood symptoms or greater than low risk of suicide attempt, the URWAP protocol for assessing and managing suicide risk will be closely followed (see Appendix for UWRAP). In particular, if a participant appears to need immediate treatment, our offices are in close proximity to UIC emergency department, and participants requiring immediate emergency care can easily be escorted there. The blind will be broken for the participant and their study participation will be discontinued. If it is revealed that the participant was currently in the placebo condition, they will simply be referred to their mental health provider (or, as necessary, to the emergency department) for treatment. However, if it is revealed that the participant was currently in an active hormone stabilization condition, the supervising physician (Dr. Wagner) will oversee a gradual tapering of the hormonal intervention and the patient will be referred to their mental health provider (or, as necessary, the emergency department) for treatment.

5.5 PREMATURE TERMINATION OR SUSPENSION OF STUDY

Administration of study agent will be halted when **three serious AEs* determined to be "probably related" (i.e., with new onset during study patches/pills) are observed**. The PI will immediately notify the DSMB when this occurs. The DSMB will notify the study sponsor and investigators immediately when the third severe event is reported and enrollment screens will stop accepting new study participants. **The DSMB will request the statistical analyses and tables that they wish to review, and these will be prepared by an unblinded biostatistician associated with the study.** All requested documentation will be provided to the DSMB. The DSMB will convene an ad hoc meeting by teleconference or in writing as soon as possible. The DSMB will provide recommendations for proceeding with or terminating the study to the PI and the NIH. The study sponsor will inform all appropriate regulatory agencies of the temporary halt and the disposition of the study (i.e., terminate, continue).

Statistically, the most likely examples of serious adverse events in the present study include development of a venous thromboembolism or a suicide attempt during an experimental phase of the study.

6 STUDY AGENT

6.1 STUDY AGENT(S) AND CONTROL DESCRIPTION

6.1.1 ACQUISITION

Both Climara patches (transdermal E2) and Prometrium pills (oral micronized P4) will be acquired through UIC Investigational Drug Services from their respective manufacturers.

IDS will order climara + prometrium as well as appropriate placebo materials.

6.1.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

Please see attached inserts for information about active Climara patches (transdermal E2) and Prometrium (oral micronized P4). Both transdermal E2 and oral micronized P4 are readily commercially available. Placebo patches and pills (both in stock at UIC IDS) are formulated and designed to be identical in appearance to these agents; all packaging and blinding will be accomplished via UIC Investigational Drug Services.

6.1.3 PRODUCT STORAGE AND STABILITY

Both Climara and Prometrium can be stored at room temperature for the duration of an individual's study participation. Participants will be instructed to avoid exposing study drugs to extreme temperatures or humidity. More specific information about storage and stability can also be found in the attached inserts for Climara and Prometrium.

6.1.4 PREPARATION

All preparation will be handled by UIC Investigational Drug Services (IDS), who will blind and mask the study drugs. No further preparation will be required by study staff or participants.

6.1.5 DOSING AND ADMINISTRATION

Dosages of E2 and P4 will be fixed. Dosages will not be tied to meals, but will be 12 hours apart in the morning and evening.

6.1.6 ROUTE OF ADMINISTRATION

E2 will be administered via transdermal patch. Micronized P4 will be administered orally in pill form.

6.1.7 STARTING DOSE AND DOSE ESCALATION SCHEDULE

In the experimental conditions of the study, the dosages will be fixed and not changed.

6.1.8 DOSE ADJUSTMENTS/MODIFICATIONS/DELAYS

If the PI determines that a participant must be discontinued from the protocol (see Section 5.4.2), The PI and Dr. Wagner will be unblinded via IDS. If the participant was on active E2/P4, Dr. Wagner will supervise the subject's discontinuation of E2/P4. Regardless of the condition the participant was in, the PI and Dr. Wagner will continue to follow the participant until resolution via appropriate clinical care (e.g., hospitalization for suicidality).

6.1.9 DURATION OF THERAPY

The planned duration of active treatment is 14 days. All days in which participants use patches and pills will be utilized in multilevel modeling analyses, which accommodate unbalanced/missing data.

6.1.10 TRACKING OF DOSE

For patches (E2): E2 patches will be applied (day +7 following ovulation) and changed (day +14 following ovulation) in the laboratory; therefore, no compliance issues are anticipated for E2. One extra patch will be given to each participant in the case that the patch falls off. All used patches will be brought to the lab so that use of patches can be monitored.

For pills (P4): at the first lab visit, study staff will work with participants to develop individualized methods for reminding the participant to take twice-daily study pills, including but not limited to email reminders, phone call reminders, text reminders, app-based reminders (with data stored locally only), pill charts, calendar reminders, and stimulus control (e.g., putting study medication on bedside table. Participants will bring in pill bottles to each visit so compliance can be monitored.

6.1.11 DEVICE SPECIFIC CONSIDERATIONS

Not applicable.

6.2 STUDY AGENT ACCOUNTABILITY PROCEDURES

Research staff pick up agents from IDS and distribute to subjects as outlined in the study schedule below. All unused pills and patches will be returned to study staff, who will return them to IDS. IDS will dispose of unused materials according to regulations.

7 STUDY PROCEDURES AND SCHEDULE

7.1 STUDY PROCEDURES/EVALUATIONS

7.1.1 STUDY SPECIFIC PROCEDURES

PHONE SCREENING

Variable	Interview Measure	Self-Report Measure	Task Measure
Inclusion/Exclusion Criteria	x	-	-
Psychiatric Diagnosis	Self-reported Previous Diagnosis	-	-
Suicidality	Abbreviated SITBI	-	-

ENROLLMENT VISIT – 1 VISIT @ 4 HRS

- **Timing:** Can occur at any time provided that it allows for the participant to complete the protocol in the term of IRB approval.
- **Assessments Administered (see section 4.1 for Abbreviations):**

Variable	Interview Measure	Self-Report Measure	Task Measure
Vitals	-	Height/Weight	-
Inclusion/Exclusion Criteria	x		
Psychiatric Diagnosis	SCID	-	-
Suicidality	Full SITBI	-	-
Depression	-	CESD	-
Hopelessness	-	BHI	-
Inhibitory Control	-	UPPS-P	-
Threat Sensitivity	-	STAI	-
Negative Social Appraisals	-	SSES	-

LABORATORY TESTING VISITS – 9 VISITS @ 1.5-2 HRS

- **Timing:** The nine laboratory visits for each condition will occur on days +7, +14, and +22 following positive ovulation test (day 0) in each condition. The

acceptable windows will be +/- 2 days in either direction for each visit.

- **Assessments Administered (see section 4.1 for Abbreviations):**

Variable	Interview Measure	Self-Report Measure	Task Measure
Urine Pregnancy Screen (1st lab visit of each condition)	-	-	x
Urine Drug Testing	X	X	x
Vitals (1st lab visit of each condition)	-	-	Height/Weight
Blood Sample (48 ml)	-	-	E2/P4/ALLO/Inflammatory Gene Expression
Suicidality	Full SITBI	-	-
Depression	-	CESD	-
Hopelessness	-	BHI	FT-IRAP
Inhibitory Control	-	UPPS-P	SST
Threat Sensitivity	-	STAI	DPT
Negative Social Appraisals	-	SSES	SAR-IAT
Side Effects and Compliance Monitoring	X	-	-
Parasympathetic Activity	-	-	Respiratory Sinus Arrhythmia

DEBRIEFING VISIT – 1 HOUR

- **Timing:** Debriefing will occur within a 15 day window following completion of the second condition.
- Individual results will not be reported to the patient.
- Referral to original care provider will be facilitated as necessary.
- **Assessments Administered (see section 4.1 for Abbreviations):**

Variable	Interview Measure	Self-Report Measure	Task Measure
Final Suicide Risk Assessment	Full SITBI	-	-

7.1.2 STANDARD OF CARE STUDY PROCEDURES

Although the LRAP/UWRAP dictates the provision of basic supportive conversation and other simple interventions to reduce imminent risk in the context of increased suicidality, participants are expected to utilize their mental health provider for any **treatment** needs that arise during the course of the study.

7.2 LABORATORY PROCEDURES/EVALUATIONS

7.2.1 CLINICAL LABORATORY EVALUATIONS

A point-of-care urine **pregnancy test** will be completed in our laboratory on site at the first visit of all three conditions. A positive pregnancy test will result in termination of participation and will be managed by Dr. Wagner.

At the beginning of each experimental condition, we will also collect a urine sample and perform rapid urine toxicology screens for cocaine, opioids, cannabis, and methamphetamine on-site through the CRC nursing staff using Ten Panel Integrated EZ Split Key Drug Test Cup with temperature-sensitive strips. A positive result for cocaine, opioids, or methamphetamine will result in exclusion. THC use will be allowed as long as the participant does not meet criteria for a substance use disorder on the SCID-I.

At all 9 laboratory assessments during active treatment with placebo or E2/P4, participants will provide 48ml of blood to be assayed for E2/P4, ALLO, and inflammatory gene expression, to evaluate the success of the hormone in achieving stable luteal phase levels of E2/P4/ALLO, as well as more stable inflammatory markers. Storage onsite will be handled by the CTSA CRC staff.

7.2.2 OTHER ASSAY PROCEDURES

Gene Expression Measurement and Coding. Total RNA will be extracted using a Qiacube automated nucleic acid preparation system (Qiagen, Valencia, CA). Resulting RNA samples will be tested for suitable mass (Nanodrop ND1000) and integrity (Agilent Bioanalyzer), and converted to fluorescent cRNA for hybridization to human HT-12 BeadChips (Illumina, San Diego, CA). Expression values for all human gene transcripts will be determined, with individual samples subject to quantile normalization and log2-transformation to stabilize variance for linear model-based analyses of differential gene expression and subsequent interpretive bioinformatics.

Genomic data will be included in analyses using composite measures of *a priori*-defined gene sets involved in inflammation, Type I interferon-related antiviral responses, and antibody production. From a specific set of 53 genes, we will form composites of 19 pro-inflammatory genes, 31 Type I interferon-related genes, and 3 antibody-related genes. Composites will be formed by averaging standardized values of each (log2-transformed, quantile normalized) gene transcript. Cronbach's alpha reliability coefficients for such transcript composites are typically in excess of .80, and we expect similarly reliable composites in this study. In addition, we will complete a "bottom-up" analysis of all sets of genes that are systemically up- or down-regulated across the perimenstrual weeks as a result of our experimental conditions (E2, P4) relative to placebo.

7.2.3 SPECIMEN PREPARATION, HANDLING, AND STORAGE

48 ml of blood will be collected using: (1) one 20-ml red-top tube (no additive), (2) one 20-ml lavender-top tube (EDTA additive), and (3) one 8-ml PaxGene RNA tube. 20ml of blood (red-top tube) will be centrifuged and serum will be aliquoted into storage tubes and stored in a -80F freezer in the Clinical Research Center. These will be transported to the core laboratory in batches for estrogen and progesterone assay. Following assay, any additional serum will be stored by the CRC. The other 20 ml tube of blood (lavender-top tube) will be transferred to Dr. Graziano Pinna's laboratory within 48 hours of the draw, and will be centrifuged and separated into plasma aliquots (for measurement of GABAergic neurosteroids using GC-MS) and

lymphocyte aliquots (for use in gene expression assays related to neurosteroid synthesis and function), then stored in a -80 F freezer in the Dr. Pinna's Laboratory. Standard freezer-proof labels will indicate participant ID and study session. A PaxGene RNA blood tube will be utilized to collect an additional 8ml of blood for use in gene expression assays related to inflammation. Following analysis, the PaxGene RNA blood samples will also be destroyed.

7.2.4 SPECIMEN SHIPMENT

Serum transport will be handled by Clinical Research Center staff. Serum samples will analyzed in batches when there are enough aliquots to support purchase of RIA kits. Lavender top tubes will be transported to Dr.Pinna's lab by CLEAR Lab staff members. PaxGene tubes will be stored until assay at the conclusion of the experiment, and will be analyzed in a single batch.

7.3 STUDY SCHEDULE

7.3.1 SCREENING

The screening procedures described below must take place prior to the enrollment visit; because all information about eligibility obtained during the phone screen is confirmed at the enrollment visit, there is no expiration of screening procedures after which they must be repeated.

Following verbal consent, verbal phone screening will include verbal assessment of all inclusion/exclusion criteria described above, including assessment of recent suicidality using the shortened SITBI.

The study will be conducted without accessing protected health information (PHI). We will NOT seek access to any medical records.

7.3.2 ENROLLMENT/BASELINE

Following recruitment screening via telephone, interested individuals will schedule and attend an enrollment visit at which they will provide informed consent and be further assessed for eligibility, which includes assessment of self-reported inclusion/exclusion criteria (including appropriate level of suicide risk on the SITBI-- see information in section 5.1). If ineligible, they will be referred back to their mental health provider and receive payment for the enrollment visit. If eligibility is confirmed, they will be further characterized using the SCID-I and SCID-II and complete various psychosocial questionnaires. In addition, they will be trained in the protocol for urine ovulation testing, daily telephone assessments, and the patch/pill administration schedule. Participants will be randomized to one of six possible condition orders at that time.

The study agent will not be administered until Lab assessment 1 (i.e., not at this enrollment visit).

7.3.3 FOLLOW-UP

Following the baseline/enrollment visit, participants will remain in contact with study staff and will begin ovulation testing as instructed. When ovulation testing is positive (day 0), the participant will contact the study staff to schedule the first three laboratory assessment visits (visits for condition 1) on days +7, +14, and +22. All laboratory assessment visits are identical in structure and content, with the singular exception that the first laboratory visit of each condition will include: (1) a pregnancy test that must be negative prior to dispensing study medication, (2) a urine drug screen, and (3) assessment of height and weight (BMI). Between conditions, participants will again remain in contact with study staff to schedule ovulation testing in the next menstrual cycle. Once again, when ovulation is confirmed (day 0), the final 3 laboratory assessments (for condition 2) will be scheduled on days +7, +14, and +22. These visits may be scheduled up to +/-2 days in either direction.

- **Follow-Up Assessments Administered (see section 4.1 for Abbreviations):**

Variable	Interview Measure	Self-Report Measure	Task Measure
Vitals (first lab of each condition)	-	-	Height/Weight
Urine Pregnancy Screen (first lab of each condition)	-	-	x
Urine Drug Screen (first lab of each condition)	x	x	x
Blood Sample (48 ml)	-	-	E2/P4/ALLO/Inflammatory Gene Expression
Suicidality	Full SITBI	-	-
Depression	-	CESD	-
Hopelessness	-	BHI	FT-IRAP
Inhibitory Control	-	UPPS-P	SST
Threat Sensitivity	-	STAI	DPT
Negative Social Appraisals	-	SSES	SAR-IAT
Side Effects, AEs, and Compliance Monitoring	X	-	-
Parasympathetic Activity	-	-	Respiratory Sinus Arrhythmia

Follow-Up Timeline:

Condition 1 (Follow-Up Visits 1-3):

- Ovulation Testing
- Positive Ovulation Test #1= Day 0
- Study Followup Visits 1-3 Scheduled for days +7, +14, +22
- Study Followup Visit 1 (Day +7): Assessments, pregnancy test, drug test, vitals, administration of study agent patch A and dispensation of pills **(Condition 1)**
- Study Followup Visit 2 (Day +14): Assessments, replacement of study agent patch A with patch B **(Condition 1)**
- Removal of study patch and end of pills on day +21
- Study Followup Visit 3 (Day +22): Assessments

Condition 2 (Follow-Up Visits 4-6):

- Ovulation Testing
- Positive Ovulation Test #2= Day 0
- Study Followup Visits 4-6 Scheduled for days +7, +14, +22
- Study Followup Visit 4 (Day +7): Assessments, pregnancy test, drug test, vitals, administration of study agent patch A and dispensation of pills **(Condition 2)**
- Study Followup Visit 5 (Day +14): Assessments, replacement of study agent patch A with patch B **(Condition 2)**
- Removal of study patch and end of pills on day +21
- Study Followup Visit 6 (Day +22): Assessments

Condition 3 (Follow-Up Visits 7-9):

- Ovulation Testing
- Positive Ovulation Test #2= Day 0
- Study Followup Visits 4-6 Scheduled for days +7, +14, +22
- Study Followup Visit 4 (Day +7): Assessments, pregnancy test, drug test, vitals, administration of study agent patch A and dispensation of pills **(Condition 3)**
- Study Followup Visit 5 (Day +14): Assessments, replacement of study agent patch A with patch B **(Condition 3)**
- Removal of study patch and end of pills on day +21
- Study Followup Visit 9 (Day +22): Assessments, Schedule Debriefing

7.3.4 FINAL STUDY VISIT

Debriefing visits will be scheduled within 15 days of study completion. In this final visit, a final risk assessment will be conducted and the participant will be referred as appropriate for additional clinical care. This final risk evaluation visit is included in order to reduce the likelihood of emergent risk following withdrawal from exogenous hormones in participants who had the placebo-experimental condition order. Participants will not be provided with individualized study results.

7.3.5 EARLY TERMINATION VISIT

Should early termination be deemed necessary (following procedures detailed above), and the situation does not involve a need for immediate hospitalization of the participant, then the participant will be invited to an early termination visit in which the full typical debriefing interview (i.e., risk assessment and referral as appropriate) will be conducted.

7.3.6 UNSCHEDULED VISIT

There will be no unscheduled visits. Should a participant present at the laboratory at the wrong time, a member of the study staff will reschedule the visit if needed or refer her to her mental health provider as appropriate.

7.3.7 SCHEDULE OF EVENTS TABLE

Procedures	Screening	Enrollment/Baseline	IDS RANDOMIZATION	Follow-Up (Condition 1, Visit 1)	Follow-Up (Condition 1, Visit 2)	Follow-Up (Condition 1, Visit 3)	Follow-Up (Condition 2, Visit 4)*	Follow-Up (Condition 2, Visit 5)*	Follow-Up (Condition 2, Visit 6)*	Final Visit/ Debriefing Visit
Informed Consent	Verbal x	Written x								
Demographics	x	x								
Medical History/Inclusion/Exclusion Criteria	x	x								
SCID – Diagnostic Interview		x								
SITBI – Suicidality Interview		x	x	x	x	x	x	x	x	x
Randomization			x							
Administer Study Agent E2 (or placebo) Patch				x	x		x	x		
Dispense Study Agent P4 (or placebo) Pills				x	x		x	x		
Height/Weight				x			x			
Pregnancy and Drug Tests				x			x			
E2/P4/ALLO/Inflammatory Gene Expression Blood Measure				x	x	x	x	x	x	
CESD - Depression Self-Report				x	x	x	x	x	x	
BHI – Hopelessness Self-Report				x	x	x	x	x	x	
STAI – Anxiety/Threat Sensitivity Self-Report				x	x	x	x	x	x	
SSES – Negative Social Appraisals Self-Report				x	x	x	x	x	x	
UPPS-P – Impulsivity/Inhibitory Control Self-Report				x	x	x	x	x	x	
FT-IRAP – Hopelessness Task				x	x	x	x	x	x	
SAR-IAT – Negative Social Appraisal Task				x	x	x	x	x	x	
SST – Inhibitory Control Task				x	x	x	x	x	x	
DPT – Threat Sensitivity Task				x	x	x	x	x	x	
Adverse Event/Side Effect Evaluation				x	x	x	x	x	x	
Parasympathetic Activity – Respiratory Sinus Arrythmia				x	x	x	x	x	x	

*Condition 3, Visits 7-9 are identical to Condition 2, Visits 4-6.

7.4 JUSTIFICATION FOR SENSITIVE PROCEDURES

Placebo control is required to demonstrate unique effects (vs. expectancy effects) of hormones on indices of suicidality.

Ongoing Assessment of Suicidality

The PI (Dr. Eisenlohr-Moul) is a clinical psychologist with nine years of experience in risk assessment and empirically supported treatment of chronic suicidality (e.g., Dialectical Behavior Therapy); Dr. Wagner, whose office is adjacent to Dr. Eisenlohr-Moul's, is an experienced clinical psychiatrist with clear expertise in suicide risk assessment and management. These clinical qualifications will allow the PI and Dr. Wagner, the prescribing physician for the study, to carefully assess and manage any emergent changes in imminent suicide risk.

The available literature[3] indicates that ***participants in research studies do not generally become acutely upset in response to the psychological assessments conducted in the present study.*** If participants do become upset during an assessment, they will be reminded of their right to discontinue participation, and mood changes or suicidality will be assessed and managed using the University of Washington Risk Assessment and Management Procedure (**UWRAP**), the suicide risk management protocol recommended by NIH in the context of RCTs for mood disorders.

7.5 CONCOMITANT MEDICATIONS, TREATMENTS, AND PROCEDURES

All concomitant prescription medications taken during study participation will be recorded. For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported are concomitant prescription medications, over-the-counter medications and non-prescription medications.

7.5.1 PRECAUTIONARY MEDICATIONS, TREATMENTS, AND PROCEDURES

There are no medications that are prohibited for concomitant use with the study agents, with the exception of any hormonal preparations (that are exclusionary).

7.6 PROHIBITED MEDICATIONS, TREATMENTS, AND PROCEDURES

There are no prohibited medications or procedures except hormonal preparations, which are exclusionary. However, inclusion/exclusion criteria exclude those reporting ongoing treatment for a chronic (non-psychiatric) medical condition, as well as excluding those with a positive enrollment drug screen for opioids, methamphetamines, or cocaine, since recreational use of these drugs may indicate heightened risk of a suicide attempt.

7.7 PROPHYLACTIC MEDICATIONS, TREATMENTS, AND PROCEDURES

Not applicable.

7.8 RESCUE MEDICATIONS, TREATMENTS, AND PROCEDURES

Not Applicable.

Hospitalization will serve as a “rescue treatment” should imminent suicidal risk emerge.

7.9 PARTICIPANT ACCESS TO STUDY AGENT AT STUDY CLOSURE

Participants will not be provided access to study medications following the termination of this study; as noted above, this is a mechanistic experimental study and not a treatment study.

8 ASSESSMENT OF SAFETY

The safety of the study and its participants (as described in 2.3.1) will be monitored via (1) Supervising physician Melissa Wagner, M.D./Ph.D. (hormone-related AEs) and Tory Eisenlohr-Moul, Ph.D. (mood and suicide-related AEs) as well as (2) a Data and Safety Monitoring Board (DSMB).

AEs will be assessed daily during the telephone contacts in the two administration conditions, and will be assessed weekly at laboratory visits. All AEs will be reported immediately to the PI and Dr. Wagner, who will determine severity and next steps.

A DSMB of three experts outside the PI's department will evaluate AEs that are submitted during scheduled reviews (every 6 months). The DSMB will be composed of MDs and PhDs from relevant disciplines, including clinical psychology, gynecology, and endocrinology.

Although not expected given the doses and duration of hormone exposure, the DSMB will, during scheduled reviews, specifically evaluate the frequency of AEs related to the hormonal RCT. The DSMB will specify the tables and data it wishes to have presented to it at all meetings, including but not limited to, moderate to severe side effects and all serious adverse events. **DSMB review of this study will occur every six months**. At these meetings the DSMB will also review data from participants who have been discontinued by study staff or who have withdrawn or dropped out of the study, and the reasons for discontinuation. The DSMB will also identify if any study procedures should be altered or stopped in the event of an indication of harm to participants attributable to the study interventions. The DSMB will evaluate issues of participant safety as well as the adequacy and integrity of accumulating data, review of enrollment data, and making recommendations regarding safety.

8.1 SPECIFICATION OF SAFETY PARAMETERS

8.1.1 DEFINITION OF ADVERSE EVENTS (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention inhumans, whether or not considered intervention-related (21 CFR 312.32 (a)).

8.1.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

Serious adverse event or serious suspected adverse reaction. An AE or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, or a persistent or significant incapacity. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical intervention to prevent one of the outcomes listed in this definition.

****Notable examples of serious adverse events in the present study include development of a venous thromboembolism or suicide attempt.***

8.1.3 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

OHRP considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

This study will use the OHRP definition of UP.

8.2 CLASSIFICATION OF AN ADVERSE EVENT

8.2.1 SEVERITY OF EVENT

For AEs not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating.

8.2.2 RELATIONSHIP TO STUDY AGENT

For all AEs, Dr. Eisenlohr-Moul will urgently collaborate with Dr. Wagner to determine the AE's causality based on temporal relationship and clinical judgment. The degree of certainty about causality will be graded using the categories below.

Definitely Related – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event occurs in a plausible time relationship to drug administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.

Probably Related – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event occurs within a reasonable time after administration of the drug, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and

follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.

Possibly Related – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or"definitely related," as appropriate.

Unlikely to be related – A clinical event whose temporal relationship to drug administration makes a causal relationship improbable (e.g., theevent did not occur within a reasonable time after administration of the trial medication) and inwhich other drugs or chemicals or underlying disease provides plausible explanations (e.g., theparticipant's clinical condition, other concomitant treatments).

Not Related – The AE is completely independent of study drug administration, and/or evidenceexists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by Dr. Wagner or Dr. Eisenlohr-Moul.

8.2.3 EXPECTEDNESS

Dr. Eisenlohr-Moul will collaborate with Dr. Wagner to make determinations about whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study agent.

8.3 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews of a study participant. All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate documentation. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Pre-existing medical conditions are exclusionary for participation in the trial, with the exception of psychiatric conditions. Any psychiatric condition or symptom that is present at the time that the participant is screened, including mood symptoms and suicidal *ideation*, will be considered as baseline and not reported as an AE. However, if the study participant's condition or symptom deteriorates significantly with regard to the severity or impact of the symptom on the participants functioning or safety (as determined by Drs. Eisenlohr-Moul and Wagner) at any time during the study, it will be recorded as an AE. UPs will be recorded in the data collection system throughout the study.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The PI will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each phone call and study visit, study staff will inquire about the occurrence of AE/SAEs since the last visit. All AEs will be immediately reported to the PI, who will respond (psychiatric AEs) or consult with Dr. Wagner as soon as possible (for medical AEs). Events will be followed for outcome information until resolution or stabilization.

8.4 REPORTING PROCEDURES

8.4.1 ADVERSE EVENT REPORTING

Study staff will screen for all AEs at each telephone call and study visit. Dr. Eisenlohr-Moul will be on call for all visits and telephone calls to ensure that emergent mood deterioration or suicide risk can be immediately handled by a licensed clinical psychologist with experience in suicide risk assessment (Eisenlohr-Moul) using the LRAP/UWRAP protocol (the tool recommended by NIH for clinical trials with suicidal subjects). Medical/physical AEs will be discussed as soon as possible with Dr. Wagner.

The PI Dr. Eisenlohr-Moul (in consultation with Dr. Wagner) will review all protocol data at monthly meetings, including enrollment and retention statistics and aggregate reports of side effects/AEs. As the contact PI, Dr. Eisenlohr-Moul will be the one responsible for reporting any severe AEs to the IRB and DSMB within 1 week. Since we are employing a marketed pharmaceutical product (i.e., a non-IND study), unexpected Serious AEs will be also be reported to the FDA Medwatch Program. The NIMH program officer will be notified of any study modifications or suspension imposed by the DSMB or local IRB in response to an AE.

8.4.2 SERIOUS ADVERSE EVENT REPORTING

The PI (Dr. Eisenlohr-Moul) will complete a SAE Form within the following timelines:

- All deaths and immediately life-threatening events, whether related or unrelated, will be recorded on the SAE Form and submitted to the DCC/study sponsor within 24 hours of site awareness. See **Section 1, Key Roles** for contact information.

All SAEs will be followed until satisfactory resolution or until the PI deems the event to be chronic or the adherence to be stable. Other supporting documentation of the event may be requested by the DSMB or study sponsor and will be provided as soon as possible. The study sponsor will be responsible for notifying FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information.

8.4.3 UNANTICIPATED PROBLEM REPORTING

Incidents or events that meet the OHRP criteria for UPs require the creation and completion of an UP report form. It is the site investigator's responsibility to report UPs to their IRB and to the study sponsor. The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB projectnumber;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcomerepresents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- All UPs will be reported to the IRB, the DSMB, and to the study sponsor within 1 week of the investigator becoming aware of the event.

8.4.4 EVENTS OF SPECIAL INTEREST

Not applicable.

8.4.5 REPORTING OF PREGNANCY

Pregnancy will result in immediate withdrawal from the study and a breaking of the blind to Dr. Wagner; Dr. Wagner will oversee appropriate clinical care, including supervised hormonal discontinuation.

8.5 STUDY HALTING RULES

Administration of study agent will be halted when **three serious AEs* determined to be "probably related" (i.e., with new onset during study patches/pills) are observed**. The PI will immediately notify the DSMB when this occurs. The DSMB will notify the study sponsor and investigators immediately when the third severe event is reported and enrollment screens will stop accepting new study participants. **The DSMB will request the statistical analyses and tables that they wish to review, and these will be prepared by an unblinded biostatistician associated with the study.** All requested documentation will be provided to the DSMB. The DSMB will convene an ad hoc meeting by teleconference or in writing as soon as possible. The DSMB will provide recommendations for proceeding with the study to the PI and the NIH. The study sponsor will inform all appropriate regulatory agencies of the temporary halt and the disposition of the study.

Statistically, the most likely examples of serious adverse events in the present study include development of a venous thromboembolism or a suicide attempt during an experimental phase of the study.

8.6 SAFETY OVERSIGHT

Safety oversight will be under the direction of a DSMB composed of individuals with the appropriate expertise as determined by their regulatory guidelines. The DSMB will meet every 6 months to assess safety and efficacy data in each condition of the study. The DMSB will operate under the rules of an approved charter that will be written and reviewed at the organizational meeting of the DSMB. At this time, each data element that the DSMB needs to assess will be clearly defined. The DSMB will provide its input as required to NIMH.

9 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of human subjects are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with GCP, and with applicable regulatory requirement(s).

- Madeline Divine, the project manager, will be responsible for the adequate clinical documentation of all events as described in the current protocol, including data verification and backup.
- Evaluation of the safety of the study will take place via the DSMB every six months and as needed when AEs emerge (see sections above).

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL AND ANALYTICAL PLANS

There will be no formal SAP. The PI will conduct all analyses at the end of the trial.

10.2 STATISTICAL HYPOTHESES

Hypotheses. *For suicidal desire and suicidal action capacity—our primary endpoints)—we predict that perimenstrual increases in suicidal desire and action capacity (Lab 2 – Lab 1 within each condition) will be larger in the placebo (natural E2 + P4 withdrawal) condition and experimental E2 stabilization (P4 withdrawal) condition than in the experimental P4 stabilization (E2 withdrawal) condition. The associated null hypothesis is that there will be no difference in the perimenstrual increase in suicidality across conditions. For our secondary endpoints, including hopelessness, negative social appraisals, reduced inhibitory control, and threat sensitivity, we predict the same pattern of results.*

There are no “efficacy” endpoints in the present trial since it is mechanistic and not a treatment study.

10.3 ANALYSIS DATASETS

An intent-to-treat dataset will be utilized. The study statistician will use a carry-forward approach to evaluate the within-person condition effect using this intent-to-treat sample.

10.4 DESCRIPTION OF STATISTICAL METHODS

10.4.1 GENERAL APPROACH

The design of this study is a three-period crossover design, where each of three study periods is a perimenstrual frame of the menstrual cycle (days +7 to +22 following ovulation where positive ovulation test day=0). Each participant will complete all three conditions in a randomized order.

Primary hypothesis tests will be evaluated using a alpha level of .05, with a two-tailed test.

All outcomes will be checked for normality; given the low likelihood that suicidality will follow a normal distribution even in this clinical sample, we expect that we will need to utilize nonparametric procedures.

10.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

Primary and secondary endpoints are operationalized above (i.e., mean within-person differences between conditions) for the daily SITB suicidal ideation and planning subscales (primary endpoints) as well as the daily UPPS-P impulsivity, PROMIS anxiety, CESD depression, BHI hopelessness, and SSES rejection sensitivity subscales (secondary endpoints).

Analyses will proceed identically for all outcomes listed above. First, we will calculate each subject's mean daily rating (via phone) on the outcome measure *during* each condition.

Second, we will utilize repeated measures ANCOVA to examine the within-person condition effects. Covariates (medicines, BMI, age) will be examined and included. These analyses will be conducted on the intent-to-treat dataset.

If necessary due to violations of model assumptions, we will utilize similar nonparametric tests.

10.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

See above.

10.4.4 SAFETY ANALYSES

All adverse events will be measured daily and recorded at the end of each condition for each subject, calculated dichotomously based on its emergence during the treatment timeframe. These outcomes will be presented in tables for review by the DSMB, and such outcomes will be accompanied by descriptive and inferential statistics (both mean

levels and change from baseline in each condition) to determine whether AEs and changes in AEs are more likely to occur in the experimental conditions.

Below, we list the adverse events that are most likely to occur in the course of the study and will therefore be monitored most closely (at every daily and weekly participant contact during administration of study agents). However, participants will have the opportunity to report any other adverse event in addition to those listed below.

GENERAL ADVERSE EVENTS

- Significant deterioration in mood
- Significant deterioration in suicidality
- Any adverse event related to venipuncture
- Any adverse event related to emotional or behavioral reactions to interviews, questionnaires, or tasks

ESTRADIOL PHYSICAL SIDE EFFECT ADVERSE EVENTS

The most frequent side effects associated with estradiol use include:
- breast tenderness (occurs in 29% of patients)
- abdominal cramps (occurs in 16% of patients)
- headache (occurs in 13% of patients)
- edema (swelling) (occurs in 10% of patients)
- nausea (occurs in 6% of patients)
- acne (occurs in 3 – 12% of patients)
- skin rash or irritation may also occur at site where the patch is placed (occurs in 3 - 12% of patients)

Rare side effects (<1%) include:
- jaundice (yellowing of skin)
- increased blood pressure
- worsening of migraines or asthma
- enlargement of uterine fibroids
- intolerance to contact lenses
- dizziness
- changes in appetite and weight

PROGESTERONE PHYSICAL SIDE EFFECT ADVERSE EVENTS

The most common side effects associated with progesterone include:
- breast tenderness (occurs in 16% of patients)
- dizziness (occurs in 24% of patients)
- abdominal cramping (occurs in 20% of patients)
- headache (occurs in 16% of patients)
- viral infection (occurs in 12% of patients)
- joint pain (occurs in 12% of patients)
- diarrhea (occurs in 8% of patients)
- menstrual bleeding, sometimes consistent with a heavy menstrual period (occurs in 20-30% of

patients)
- drowsiness (occurs in 9% of patients)

Rare (<1%) side effects include:
- vaginal discharge
- chest pain
- abdominal bloating

10.4.5 ADHERENCE AND RETENTION ANALYSES

Adherence to hormone administration protocols will be characterized following the close of the study using serum assays for E2 and P4 during the active conditions relative to hormonal profiles during the placebo condition; it will not be monitored in real time.

Participants will be monitored in their compliance daily during each study condition via a phone call with study staff. Any deterioration in adherence will be immediately remedied via increased reminders and phone calls.

10.4.6 BASELINE DESCRIPTIVE STATISTICS

Groups (counterbalanced orders) will be compared on demographic factors such as age, severity of perimenstrual mood change in the placebo condition, and general suicidality. However, inferential statistics are not necessary as our primary outcome modeling procedures will control for any baseline differences that exist.

10.4.7 PLANNED INTERIM ANALYSES

10.4.7.1 SAFETY REVIEW

Administration of study agent will be halted when **three serious AEs* determined to be "probably related" (i.e., with new onset during study patches/pills) are observed**. The PI will immediately notify the DSMB when this occurs. The DSMB will notify the study sponsor and investigators immediately when the third severe event is reported and enrollment screens will stop accepting new study participants. **The DSMB will request the statistical analyses and tables that they wish to review, and these will be prepared by an unblinded biostatistician associated with the study.** All requested documentation will be provided to the DSMB. The DSMB will convene an ad hoc meeting by teleconference or in writing as soon as possible. The DSMB will provide recommendations for proceeding with or terminating the study to the PI and the NIH. The study sponsor will inform all appropriate regulatory agencies of the temporary halt and the disposition of the study (i.e., terminate, continue).

Statistically, the most likely examples of serious adverse events in the present study include development of a venous thromboembolism or a suicide attempt during an experimental phase of the study.

10.4.7.2 EFFICACY REVIEW

There are no efficacy endpoints in the present study because this is not a treatment study and the intervention used is not expected to be an effective treatment for any condition.

10.4.8 ADDITIONAL SUB-GROUP ANALYSES

The study is not powered to conduct sub-group analyses, and therefore they will not be performed.

10.4.9 MULTIPLE COMPARISON/MULTIPLICITY

N/A

10.4.10 TABULATION OF INDIVIDUAL RESPONSE DATA

Individual response data will be tabulated only if requested by the DSMB.

10.4.11 EXPLORATORY ANALYSES

Not applicable.

10.5 SAMPLE SIZE

An unlimited number of potential participants will be screened. 60 females will be enrolled in the study, with the goal of 40 participants completing the study.

Power analyses are described below; alpha is set at .05, power at 80%. The null hypothesis is no link between the study experimental condition and risk for suicidal ideation, intent, and planning; the alternate hypothesis is that hormone stabilization in the experimental condition will prevent flux in these suicide-related outcomes. Because no previous studies have examined the impact of experimental hormones on suicidality, we have conducted power analyses using existing effect sizes on related constructs (e.g., BPD features).

Power Analysis. For primary hypothesis tests, 80% power to detect a conventionally medium-sized effect of condition (f = .25) is achieved with 24 participants[117]. To allow for attrition and improve generalizability and power, we propose to include 40 participants in the final sample.

10.6 MEASURES TO MINIMIZE BIAS

10.6.1 ENROLLMENT/ RANDOMIZATION/ MASKING PROCEDURES

Individuals will be enrolled following phone screen and an enrollment visit as described above. Individuals will be randomized by IDS to one of six condition orders (permutations of A-B-C) following enrollment visit if they meet study criteria and give informed consent. Masking and blinding will be handled by IDS according to standard procedures for double blinding, and will be

fully double-blind. Randomization codes will be kept by IDS until the completion of the study, and will be shared only as needed for unblinding as needed for safety monitoring (described above).

Blinding and Compliance: Pills are dispensed in a double-blind manner. Patches will be placed by CTSA staff at UIC. All study staff will remain blinded. Preliminary analyses from the preceding similar experiment revealed that the blinding was successful; participants were not more likely to report believing they were on active treatment in the active condition relative to the placebo condition (*p* = .58). Participants will: **1)** be educated on the importance of compliance; **2)** be asked about compliance on each daily call; and **3) have *steroid levels*** measured to confirm compliance.

10.6.2 EVALUATION OF SUCCESS OF BLINDING

At the conclusion of the study, each participant will be asked to guess which condition order they had. Blinding will be evaluated by determining whether the accuracy of these reports is greater than 50% (chance).

10.6.3 BREAKING THE STUDY BLIND/PARTICIPANT CODE

Breaking of blind will occur to Dr. Eisenlohr-Moul and Dr. Wagner in the case of severe physical AEs (e.g., VTE) or psychiatric AEs (hospitalization/suicide attempt).

11 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

All data collected in this study will be maintained on the UIC protected servers and in clinical binders kept under lock and key in Dr. Eisenlohr-Moul's locked laboratory.

12 QUALITY ASSURANCE AND QUALITY CONTROL

QC procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated; this will be carried out by study project manager Madeline Divine. Any missing data or data anomalies will be communicated to the PI for clarification/resolution.

Following written SOPs, the monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

13 ETHICS/PROTECTION OF HUMAN SUBJECTS

13.1 ETHICAL STANDARD

The investigator will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Subjects of Research codified in 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and/or the ICH E6.

13.2 INSTITUTIONAL REVIEW BOARD

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether previously consented participants need to be re-consented.

13.3 INFORMED CONSENT PROCESS

13.3.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study agent, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study product. The following consent materials are submitted with this protocol including the adult informed consent and the consent for storage of specimens.

13.3.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation will be provided to the participants and their families. Consent forms will be IRB-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants will have the opportunity to discuss the study with family or health care providers and think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. The participants may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

13.4 PARTICIPANT AND DATA CONFIDENTIALITY

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by local IRB and Institutional regulations.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored on protected servers at UIC. Identifying information will be linked to ID number only in one password-protected file. Individual participants and their research data will otherwise be identified by a unique study identification number. The study data entry and study management systems used by study staff will be secured and password protected. At the end of the study, all study databases will be de-identified (linking files destroyed) and archived on UIC servers.

Certificate of Confidentiality

To further protect the privacy of study participants, a Certificate of Confidentiality will be obtained from the NIH. This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

13.4.1 RESEARCH USE OF STORED HUMAN SAMPLES,SPECIMENS OR DATA

Study data include blood samples from laboratory visits and all psychosocial data from study participation.

- Intended Use: Samples and data collected under this protocol may be used to study the role of E2, P4, ALLO, and inflammatory gene expression in suicidality and psychiatric symptoms.

- Storage: Access to stored samples will be limited using locked access areas. Samples and data will be stored using codes assigned by the investigators. Data will be kept in password-protected computers. Only investigators will have access to the samples and data.

- Tracking: Data will be tracked using password-protected files on the UIC server. Disposition at the completion of the study: All serum samples will be kept under locked access areas at UIC. Study participants who request destruction of samples will be notified of compliance with such request and all supporting details will be maintained for tracking.

13.5 FUTURE USE OF STORED SPECIMENS

Data collected for this study will be analyzed at UIC within 2 years of the time they are collected. The data and samples collected in this study will not be stored for future use, and will not be shared with other investigators.

14 DATA HANDLING AND RECORD KEEPING

14.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the staff at the site under the supervision of the PI. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL.

Copies of the electronic data will be provided for use as source documents and maintained for recording data for each participant enrolled in the study. Data reported in the electronic data file derived from source documents should be consistent with the source documents or the discrepancies should be explained and captured in a progress note and maintained in the participant's official electronic study record.

Clinical data (including AEs, concomitant medications, and expected adverse reactions data) and laboratory data will be entered into a local database on UIC servers. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

14.2 STUDY RECORDS RETENTION

Study documents including identifying information (e.g., names, phone numbers, addresses, and other identifying information collected as part of data collection from the participant) will be retained until the end of data collection and cleaning (maximum 1 year following the completion of the study), after which all identifying information on study documents will be be destroyed or deleted. Deidentified study documents (documents without identifying information, redacted if necessary) will be retained indefinitely.

14.3 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or MOP requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site to use continuous vigilance to identify and report deviations within 7 working days of identification of the protocol deviation. All significant deviations must be addressed in study source documents, reported to NIMH Program Official and DSMB. Protocol deviations must be sent to the local IRB per their guidelines. The PI/study staff is responsible for knowing and adhering to their IRB requirements.

14.4 PUBLICATION AND DATA SHARING POLICY

This study will comply with the NIH Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a clinical trials registration policy as a condition for publication. The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or concurrent comparison or control groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Medical interventions include drugs, surgical procedures, devices, behavioral treatments, process-of-care changes, and the like. Health outcomes include any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and adverse events. The ICMJE policy, and the Section 801 of the Food and Drug Administration Amendments Act of 2007, requires that all clinical trials be registered in a public trials registry, which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies. For interventional clinical trials performed under NIH IC grants and cooperative agreements, it is the grantee's responsibility to register the trial in an acceptable registry, so the research results may be considered for publication in ICMJE member journals. The ICMJE does not review specific studies to determine whether registration is necessary; instead, the committee recommends that researchers who have questions about the need to register err on the side of registration or consult the editorial office of the journal in which they wish to publish.
FDAAA mandates that a "responsible party" (i.e., the sponsor or designated principal investigator) register and report results of certain "applicable clinical trials":

- Trials of Drugs and Biologics: Controlled, clinical investigations, other than Phase I investigations, of a product subject to FDA regulation;

The PI will ensure compliance with NIH implementation of FDAAA.

15 STUDY ADMINISTRATION

15.1 STUDY LEADERSHIP

The PI (Eisenlohr-Moul) and Dr. Melissa Wagner will govern the conduct of the study, along with NIMH program officer Mark Chavez. The PI will continue to consult as needed with key mentors as described in the NIMH R00 award on which this protocol is based.

16 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the trial. The study leadership in conjunction with the NIMH has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

17 LITERATURE REFERENCES

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APPENDIX

Version	Date	Significant Revisions