

# **Effect of Perimenstrual Ovarian Steroid Supplementation on Perimenstrual Suicidality**

*(NIMH Grant Name: “Ovarian Hormone Withdrawal and Suicide  
Risk: An Experimental Approach”; K99MH109667; UNC IRB  
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**Protocol Identifying Number: IRB # 16-2078**

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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 <u>U</u> rgency, lack of <u>P</u> remeditation, lack of <u>P</u> erseverance, <u>S</u> ensation seeking, and <u>P</u> ositive 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:** 45 female outpatients with suicidal ideation but minimal<sup>1,2</sup> 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 two conditions (order randomized across two menstrual cycle phases): (1) natural **perimenstrual E2/P4 withdrawal (placebo)**, and (2) **exogenous stabilization of physiologic premenstrual (luteal phase) E2/P4 to prevent withdrawal**. Analyses will compare

symptoms and suicidality under placebo to symptoms and suicidality under exogenous stabilization of E2 and P4.

**Objectives:**

**The central aim of this mechanistic study is to experimentally test a pathophysiologic mediational model** in which E2/P4 withdrawal increases perimenstrual suicidal desire (via increased hopelessness and negative social appraisals) and suicidal action capacity (via reduced inhibitory control and increased threat sensitivity). We predict that natural perimenstrual E2/P4 withdrawal (compared to stabilization) will be associated with greater perimenstrual 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).

**Endpoint**

**Primary:**

- (1) Mean Within-Person Condition Difference (7 final placebo days – 7 final stabilization days) of SITB Suicidal Ideation subscale score (operationalization of suicidal *desire*)
- (2) Mean Within-Person Condition Difference (7 final placebo days – 7 final stabilization days) of SITB Suicidal Planning subscale score (operationalization of suicidal *action capacity*)

**Secondary:**

- (1) Mean Within-Person Condition Difference (7 final placebo days – 7 final stabilization days) of BHI Hopelessness Score
- (2) Mean Within-Person Condition Difference (7 final placebo days – 7 final stabilization days) of CES-D Depression Total Score
- (3) Mean Within-Person Condition Difference (7 final placebo days – 7 final stabilization days) of UPPS-P Lack of Premeditation Impulsivity subscale
- (4) Mean Within-Person Condition Difference (7 final placebo days – 7 final stabilization days) of PROMIS Anxiety Total Score
- (5) Mean Within-Person Condition Difference (7 final placebo days – 7 final stabilization days) of SSES Social Evaluation/Rejection Sensitivity subscale

**Population:**

45 female outpatients with suicidal ideation but minimal<sup>1,2</sup> imminent risk for attempt

**Phase:**

N/A – Mechanistic Experiment

<b>Number of Sites enrolling participants:</b>	1
<b>Description of Study Agent :</b>	Transdermal Estradiol .1mg/24hr weekly for two weeks, and Oral Micronized Progesterone 200mg (100mg each morning, 100mg each evening) for two weeks
<b>Study Duration:</b>	20 months
<b>Participant Duration:</b>	~3 menstrual cycles (1 menstrual cycle lasts, on average, ~28 days). Therefore, average participation will last roughly 3.5 months.



## SCHEMATIC OF STUDY DESIGN

### Screening and Enrollment

Perform initial telephone screen of potential subjects by inclusion and exclusion criteria, schedule enrollments as appropriate. At Enrollment, obtain informed consent if eligible and interested. Administer SCID, SITBI. Train participants in ovulation testing.

### Randomized Subject to a *Condition Order*

**Condition Order 1:** Placebo during visits 1-3,  
Experimental during visits 4-6 (n=15)

**Condition Order 2:** Experimental during visits 1-3,  
Placebo during visits 4-6 (n=15)

### Visits 1-3

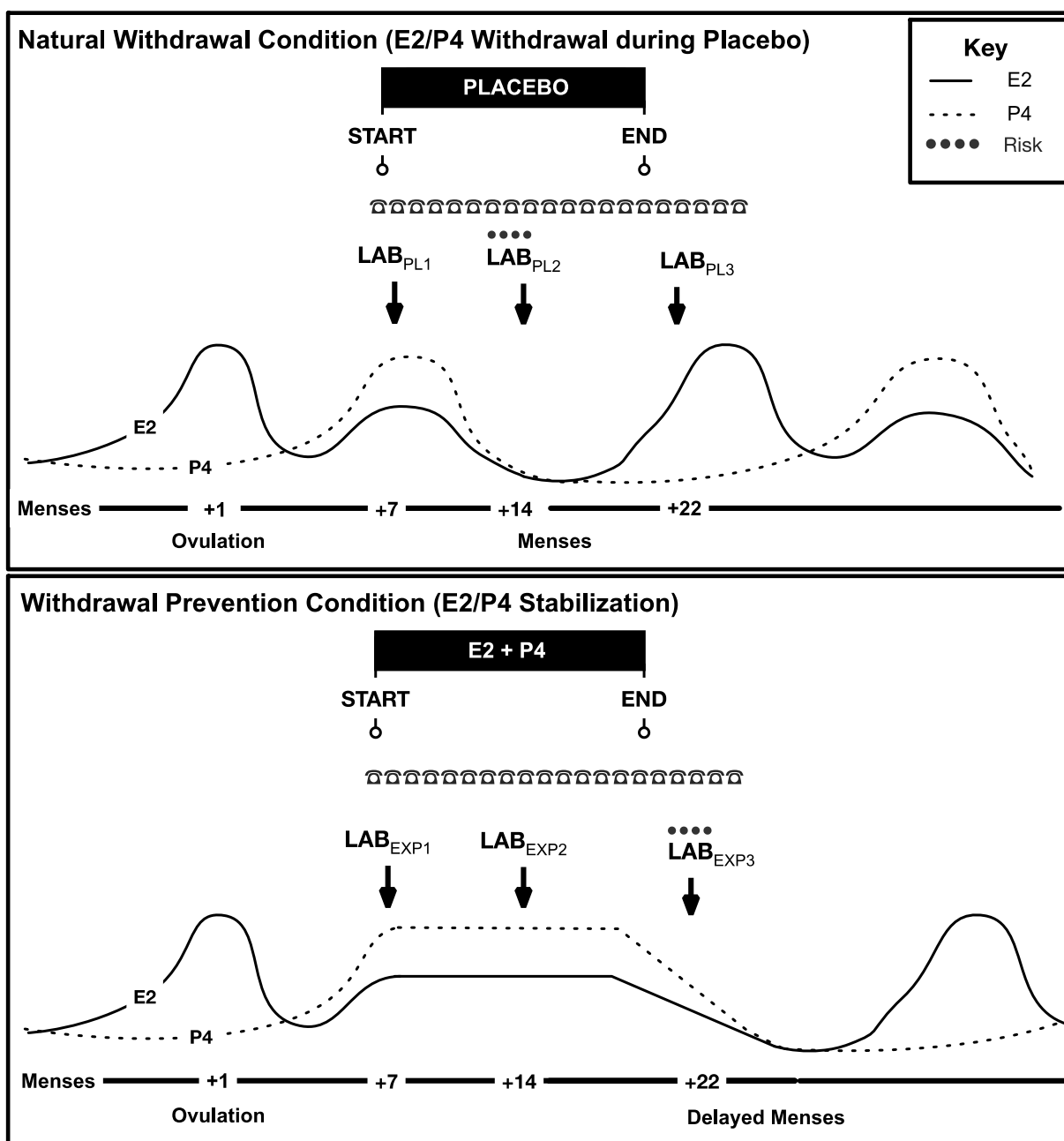
Days 7, 14, and 22 Following 1<sup>st</sup> positive Ovulation test:  
Administer Condition 1 Intervention (Placebo or Experimental).  
Complete Interview Assessment of suicidality (SITBI).  
Complete Computerized Tasks Measuring Inhibitory Control, Hopelessness, Rejection Sensitivity, and Threat Sensitivity. Assess side effects.

### Visits 4-6

Days 7, 14, and 22 Following 2<sup>nd</sup> positive Ovulation test:  
Administer Condition 2 Intervention (Placebo or Experimental).  
Complete Interview Assessment of suicidality (SITBI).  
Complete Computerized Tasks Measuring Inhibitory Control, Hopelessness, Rejection Sensitivity, and Threat Sensitivity. Assess side effects.

**Final Visit to Evaluate Suicide Risk  
and Facilitate Appropriate  
Referrals as Needed**

## Design Figure. Overview of the Two Within-Person Crossover Study Conditions



## 1 KEY ROLES

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## 2.1 BACKGROUND INFORMATION

**Suicide is the second leading cause of death among women of reproductive age, yet we cannot reliably predict when a woman will attempt suicide.** Suicide accounts for 13% of all deaths among reproductive-age American females<sup>69</sup>. 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 or other factors will translate into acute risk for a suicide attempt**<sup>5,6,70</sup>. By directly studying suicidal individuals<sup>3</sup> and focusing on biologic triggers and cognitive/behavioral mediators of acute changes in suicide risk, the present research responds to public calls from the U.S. President, Congress, Surgeon General, and the NIMH-co-sponsored Suicide Research Prioritization Agenda<sup>5</sup>, to identify strong predictors of acute suicide risk<sup>6</sup>. Such work is imperative in developing targeted interventions for stabilizing and reducing suicidality, 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 withdrawal from E2 and P4, is associated with more hospitalization for suicide attempts<sup>54-56,71</sup>, more lethal attempts<sup>55</sup>, and suicide deaths<sup>57</sup>. Despite these associations, no research has examined a causal role of perimenstrual ovarian steroid withdrawal in suicidality. The rationale for studying hormone withdrawal as a predictor of suicidality comes from work showing that withdrawal from normal levels of E2/P4, rather than differing absolute levels, precipitates affective symptoms and impulsivity in susceptible individuals<sup>39,72</sup>.

**The experiment** will test a model in which **perimenstrual E2/P4 withdrawal (vs. experimental luteal stabilization via transdermal E2 and oral micronized P4; STUDY AGENT) increases acute risk for suicidality** by negatively modulating mediating cognitive behavioral constructs (hopelessness, social appraisals, inhibitory control, threat sensitivity) and parallel clinical symptoms (depression, impulsivity, rejection sensitivity, anxiety). To maximize both safety and generalizability, we will study women with suicidal ideation but minimal<sup>1,2</sup> imminent risk for suicide attempt.

**Theoretical Underpinning for Mediating Constructs.** Selection of mediating constructs through which ovarian steroid withdrawal is most likely<sup>73,74</sup> to influence suicide risk is informed by the Interpersonal Theory of Suicide<sup>61,75</sup>, 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)<sup>61,76</sup>. *Suicidal action capacity* is predicted by poor inhibitory control<sup>77</sup> and threat sensitivity—a tendency toward detection and reactions to threats (fostered by extreme stress exposure)<sup>63,77</sup>. *Because this is an integrative theory, the constructs measured here will allow testing of a variety of theories* (e.g., Neurocognitive Theory<sup>60</sup>).

### **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<sup>78</sup> and outpatients<sup>59</sup>. Furthermore, hopelessness<sup>58</sup> and suicide<sup>60,62,79</sup> 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<sup>75</sup> and serotonergic<sup>76,77</sup> mechanisms, whereas E2 specifically is known to exert antidepressant effects.<sup>78</sup>

2. **Negative social appraisals predict suicidal desire**. Relationships are central to our ability to survive and thrive<sup>80</sup>. Perceptions of social rejection are experienced as painful<sup>81,82</sup> and increase suicidal desire<sup>61</sup>—particularly in reproductive-age women<sup>83,84</sup>. Additionally, suicidality is associated with altered function of brain regions associated with experiences of *social exclusion* and *rejection*<sup>60</sup>.

**Ovarian steroid withdrawal may increase negative social appraisals** (and associated symptoms of rejection sensitivity). In animals, E2 and P4 facilitate social motivation and behavior<sup>85-87</sup>, and ovarian steroid withdrawal precipitates social withdrawal<sup>67</sup>. In women, elevated luteal P4 correlates with social motivation<sup>88</sup> and attention

to social-affiliative opportunities<sup>89</sup>. The PI's longitudinal work indicates that ovarian steroid withdrawal predicts increased daily appraisals of social rejection in women at elevated suicide risk<sup>8,40</sup>.

**3. Poor inhibitory control** enables suicidal desire to escalate into suicidal action<sup>63,90</sup>. Suicidal ideation alone is a poor predictor of suicidal behavior<sup>6</sup>; however, the addition of poor inhibitory control robustly predicts suicide attempts, suggesting its relevance for suicidal action capacity<sup>64,91,92</sup>. Suicide attempters and victims show deficiencies in the functioning of inhibitory circuits<sup>63,93-97</sup>.

**Ovarian steroid withdrawal may reduce inhibitory control** (and increase associated symptoms of impulsivity). Cyclical E2/P4 withdrawal predicts poorer inhibitory control, greater impulsivity,<sup>8,98-100</sup> and decreased frontal cortex inhibitory activity<sup>101</sup>, while high, stable ovarian steroids increase frontal cortex inhibitory activation<sup>102,103</sup>. Cyclical reductions in inhibition are particularly marked in women with low prefrontal dopamine, which corresponds to the greater impulsivity found in suicide victims<sup>60,104</sup>. In animals, baseline impulsive behavior is a risk factor for greater sensitivity to ovarian steroid withdrawal<sup>105,106</sup>.

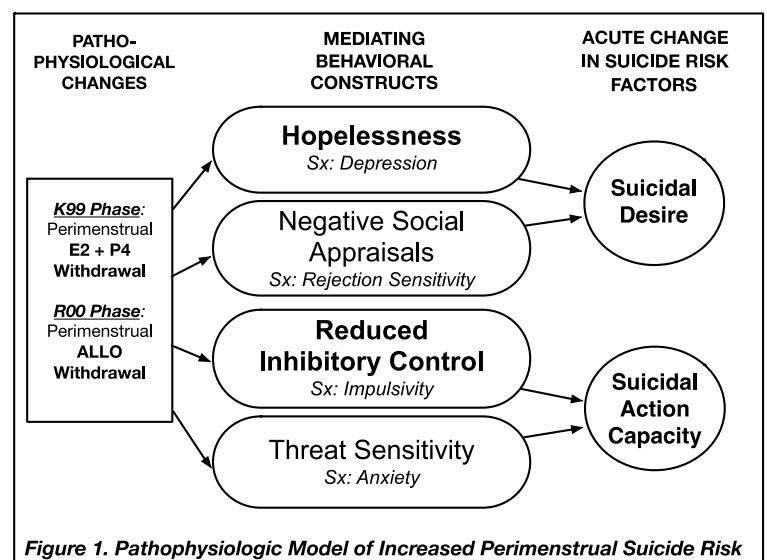
**4. Threat sensitivity**, (defined as increased attention and reflexive responses to threats), predicts suicidal behavior<sup>63,84,107</sup>. Repeated engagement of physiological stress systems also leads to HPA axis dysregulation, which prospectively predicts suicide attempts among chronically suicidal individuals<sup>108</sup>. Other research in suicide victims indicate that generalized threat sensitivity and reactivity to stimuli in daily life are acutely elevated prior to fatal suicidal behavior<sup>107</sup>.

**Ovarian steroid withdrawal may increase threat sensitivity** (and associated symptoms of anxiety). In animals, E2/P4 withdrawal increases threat sensitivity and reactivity<sup>65</sup> and causes threat-sensitizing alterations in associated brain circuitry, including the amygdala<sup>109-111</sup>, the bed nucleus of the stria terminalis<sup>111,112</sup>, and the periaqueductal grey<sup>113,114</sup>, mediated by increased expression of GABA<sub>A</sub> receptor subunits that elicit anxiogenic effects of P4-derived neurosteroids<sup>109,115-121</sup>. In women, correlates of suicidality including social stress<sup>122</sup>, social isolation<sup>123</sup>, lifetime abuse<sup>40</sup>, impulsivity<sup>105</sup>, and PTSD<sup>124,125</sup>, 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<sup>73,126,127</sup>.

Despite robust animal and correlational human evidence that E2/P4 withdrawal negatively modulates key mediators of suicide risk as outlined above, **the present research will be the first to experimentally confirm (or refute) a pathophysiologic role for steroid hormone withdrawal on suicide risk factors in humans, generally, and in at risk women, specifically.** The study delineates the within-person effects of ovarian steroid withdrawal on suicidal desire (via mediators hopelessness and social appraisals), and suicidal action capacity (via mediators inhibitory control and threat sensitivity).

## 2.2 RATIONALE

**The objective of this experiment is to experimentally determine the pathophysiologic role of perimenstrual ovarian steroid hormone withdrawal on acute suicide risk.** Suicidal behavior arises from two proximal risk factors: **Suicidal desire**, defined as the wish to die, and **suicidal action capacity**, defined as a readiness to act on suicidal urges. **Hopelessness**<sup>58-60</sup> and **Negative social appraisals**<sup>60-62</sup> are constructs reflecting dysregulation in central reward and social processing networks, and, in turn, strongly predict **suicidal desire**. **Poor inhibitory control**<sup>60,63</sup> and **threat sensitivity**<sup>60,63,64</sup> (cognitive bias to interpret stimuli in a threat-related manner) are constructs reflecting dysregulation in central cognitive control and threat avoidance networks, and, in turn, are strong predictors of **suicidal action capacity** (right side of Figure at right).



The **RATIONALE for examining ovarian steroid withdrawal in suicide risk** stems from evidence that withdrawal from estradiol (E2) and progesterone (P4), such as that occurring naturally during the perimenstrual timeframe, modulate each of the mediating constructs and associated clinical symptoms<sup>8</sup> (Figure above) toward risk, exerting robust anxiogenic, depressogenic, and impulsogenic effects in animals<sup>65-67</sup> and increasing depression, anxiety, rejection sensitivity, and impulsivity in susceptible women (preliminary work by PI)<sup>8,40</sup>. **However, no experimental work has directly evaluated whether perimenstrual E2/P4 withdrawal influences suicidality.** The experiment will determine the effects of perimenstrual E2/P4 withdrawal on suicidal desire and action capacity in at-risk women, testing mediation by risk-related behavioral constructs.

**Design Overview:** 30 female outpatients with suicidal ideation but minimal<sup>1,2</sup> imminent risk for attempt will complete behavioral tasks measuring *hopelessness, social appraisals, inhibitory control, and threat sensitivity* as well as clinical interviews in each of two conditions (order randomized across two menstrual cycle phases): (1) natural **perimenstrual E2/P4 withdrawal (placebo)**, and (2) **exogenous stabilization of physiologic premenstrual (luteal phase) E2/P4 to prevent withdrawal**. Daily calls will also assess symptoms and suicidality.

**CENTRAL HYPOTHESIS: Experimentally test a pathophysiologic mediational model** in which late luteal (perimenstrual) E2/P4 withdrawal (vs. experimental luteal E2/P4 stabilization) 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 natural E2/P4 withdrawal (compared to stabilization) 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).

## **JUSTIFICATION OF E2/P4 DOSING AND SCHEDULE**

In collaboration with Drs. Girdler, Young, and Rubinow, the PI has chosen formulations that are bioidentical to human E2 and P4 and will mimic luteal phase concentrations of E2 and P4.

**General 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 roughly to normative luteal E2 levels<sup>4</sup>. 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 our hypotheses. Thus, oral prometrium will be dosed b.i.d. to achieve luteal P4 levels<sup>4</sup> to create more **steady-state levels** and minimize the variability that can occur with the oral route.

**Rationale for Timing and Duration of E2/P4.** Administration for 14 days, starting 7 days after a positive urine test for ovulation, will span the range of time in which natural perimenstrual withdrawal would occur and prevent natural withdrawal, thereby allowing us to determine the effects of natural hormone withdrawal vs. experimentally stabilized hormones across the same perimenstrual time period of the menstrual cycle.

**Compliance checks:** Participants will: **1)** be educated on the importance of replacing their patches every 7 days, with individualized strategies to enhance compliance; **2)** be asked about patch adherence and pill compliance on each daily call; **3)** record date of patch application on calendars, reviewed at lab visits; **4)** bring used and unused patches to visits; **5)** be given extra patches for immediate replacement; and **6) Serum Levels of E2 and P4** will be collected at each session to both confirm compliance and that exogenous hormones replicate physiologic<sup>4</sup> luteal phase levels.

**Specific Rationale for 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<sup>169-171</sup>. Transdermal E2 also has a superior safety profile than oral estrogen for thromboembolic and metabolic risk<sup>172-174</sup>. The use of a 7 day transdermal system provides significantly more



stable concentrations and fewer patch adherence problems than twice weekly patches<sup>175</sup>. 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 at the .1mg/24hrs dosage correspond roughly to normative luteal levels of E2<sup>4</sup>. Climara achieves peak levels of E2 within 24 hours.

**Specific Rationale for 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, 200mg oral prometrium will be dosed daily (100mg each morning, 100mg each evening) to achieve luteal levels P4<sup>4</sup> 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.

**Other possible hormone manipulations** were considered: a) mid-luteal administration of human chorionic gonadotropin to extend the luteal phase by preserving the corpus luteum, which would not isolate E2/P4 withdrawal from other hormones; b) mid-luteal GnRH antagonist administration to precipitously drop hormones, which would have a menopausal side effect profile and a precipitous decline in E2/P4 that would not resemble perimenstrual E2/P4 decline; c) use of continuous oral contraceptives to eliminate cyclical hormone flux, which would stabilize E2/P4 in follicular phase ranges and not allow modeling of luteal phase levels.

## 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 women who report these issues<sup>3,164</sup>. 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 women<sup>39,50</sup>, 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 hormone manipulation mimics (i.e., stabilizes) the luteal hormone levels that women naturally experience<sup>4</sup>, the study is not expected to pose any greater risk to participants than what they experience in daily life.** Furthermore, although withdrawal from exogenous E2/P4 (which will mimic normal late luteal phase hormone withdrawal given that exogenous levels will mirror normal luteal physiologic ranges) 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 women that is simultaneously: (1) experiencing recent suicidal ideation, yet (2) at acceptably low<sup>1,2,149</sup> current risk for

**suicidal crisis/attempt** based on an assessment conducted during a screening visit (see **Experimental Eligibility Table in later section**). Because ovarian hormone changes are implicated in psychiatric symptoms in some women<sup>39,50</sup>, 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 hormone manipulation mimics (i.e., stabilizes) the normal physiologic range of luteal hormone levels<sup>4</sup> that women naturally experience, the study is not expected to expose participants to greater risk for suicidality than what they experience in daily life.**

### **3) Hormone Side Effects**

We do not expect any adverse side effects associated with the hormonal manipulation 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<sup>176-179</sup>. 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 women with mood disorders, and no severe psychological adverse events were reported.

#### **SIDE EFFECTS OF ESTRADIOL**

##### **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

E2 is widely prescribed; given the modest dose and duration administered here, severe adverse events are unlikely. Given our plans to administer transdermal E2 to women: (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 cancer and venous thromboembolism, the **risk of serious adverse events is exceedingly low.**

#### **SIDE EFFECTS OF PROGESTERONE**

##### **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

Progesterone is widely prescribed, with indications including dysfunctional uterine bleeding, endometriosis, mastodynia, galactorrhea, and precocious puberty<sup>180</sup>. In our recent study, a dose of 300 mg of oral micronized progesterone (higher than that given in the present study) was given to a sample of women with premenstrual mood disturbance and was well tolerated by this sample<sup>146</sup>. Given the modest dose and duration used in the present trial, severe adverse events are unlikely.

#### **4) Confidentiality**

A breach of confidentiality could indicate to others a participants' history of psychiatric symptoms.

#### **5) Venipuncture**

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

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### **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. There is no direct benefit to the participants 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) E2/P4 withdrawal (vs. experimental luteal E2/P4 stabilization via exogenous transdermal E2 and oral micronized P4) 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 natural E2/P4 withdrawal (compared to stabilization) 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).

## **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.

**Participants:** 30 female outpatients with recent suicidal ideation but **low<sup>1,2</sup> imminent risk for suicide attempt** (see **Human Subjects** section for details) receiving treatment as usual from a mental health provider at UNC will be recruited. Women must: be 18 to 45 years of age, have normal menstrual cycles (25-35 days), not pregnant (verified with urine screens), breastfeeding, or trying to get pregnant, not taking hormones, no history of serious medical illness, normal weight (BMI 18-28 kg/m<sup>2</sup>), and low risk for thrombotic events (factor V Leiden mutation based on family history). Use of psychotropic medications are expected in the majority of participants. *Most outpatients with suicidality take psychotropic medications; therefore, to increase feasibility and generalizability, medication use will be carefully measured daily and covaried in analyses if common enough.*

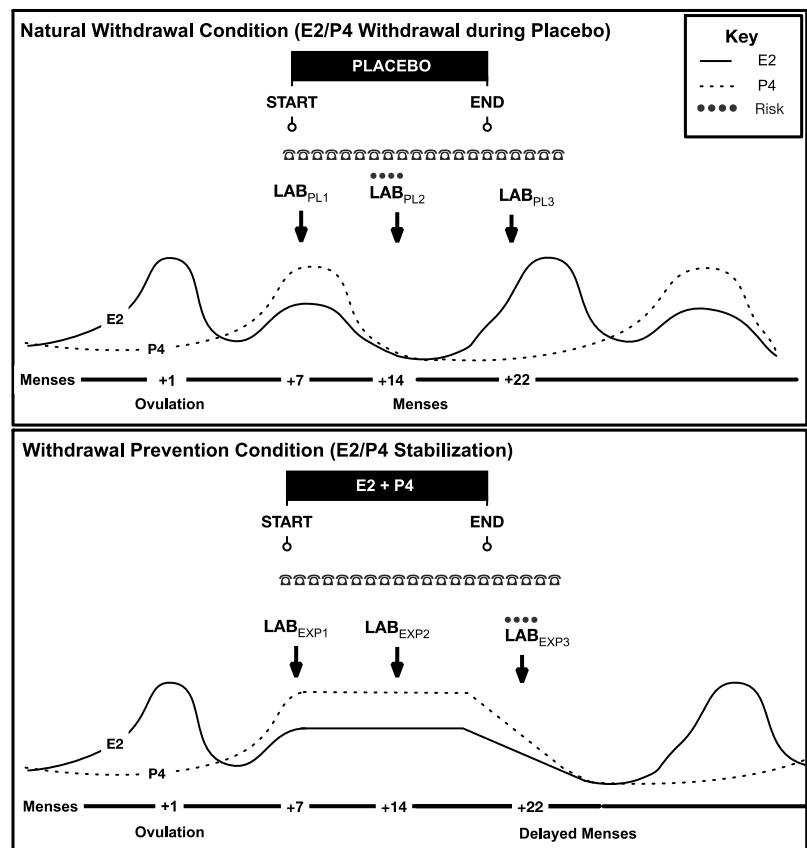
**Psychiatric Diagnoses.** Women will undergo initial SCID-I and SCID-II interviews. *History of manic episode, psychosis (except dissociation), or substance abuse disorder will be exclusionary due to increased risk of suicidal crises<sup>1,149</sup>.* Additionally, a self-report history of treatment for postpartum depression or a current diagnosis of premenstrual dysphoric disorder will be exclusionary, since these reproductive mood disorders could be characterized by a different pathophysiological pattern of sensitivity to ovarian steroid flux<sup>150</sup>. On the basis of work by Dr. Prinstein<sup>108</sup>, we expect that 50 - 75% of our sample will meet criteria for major or persistent depressive disorder, ≥ 50% will meet criteria for BPD, and ≥ 25% will meet criteria for an anxiety disorder.

**Study Design** (see figure at right; top = naturally occurring cyclicity of E2/P4 under placebo, bottom = E2/P4 withdrawal prevention using exogenous E2/P4). THIS IS NOT A CLINICAL EFFICACY TRIAL. However,

a within-person crossover RCT design with transdermal 17 $\beta$ -E2 and oral micronized P4 (or placebo) will investigate the pathophysiologic role of perimenstrual steroid withdrawal in suicidal desire and action capacity. Consenting participants will be trained in the protocol, including urine ovulation testing (LH surge preceding ovulation 24-36 hours), the daily phone assessment, and the patch/pill schedule. Using a double-blind, **within-subject cross-over design**, participants will be randomized as to the order in which they receive (1) active 17 $\beta$ -E2 + P4 or (2) placebo patch/pills. The UNC Investigational Drug Services will manage the randomization and dispensing of medications. During the E2/P4 stabilization condition, for two weeks, participants will wear a weekly patch (Climara®) delivering 0.10 mg E2/24 hrs—a dose tolerated by > 94% of the 200+ women in Dr. Girdler's recent study (NIMHR01087619)—

and will take oral progesterone (Prometrium®) at a dose of 200 mg (100mg each morning, 100mg each evening). A higher dose (300mg/day) was well tolerated by 100% of premenopausal women in Dr. Girdler's prior research (NIMHR01051246). These doses will achieve (stabilize) luteal E2/P4 levels<sup>4</sup>.

**Timing of Hormone Administration.** Based on home urine ovulation testing, participants will apply the first seven-day transdermal E2 patch (or placebo patch) and begin daily oral P4 (or oral placebo) seven days after urine-confirmed ovulation (cycle 1, +7) to coincide with a time in the natural cycle when E2/P4 are elevated. The goal of the active E2+P4 manipulation is to **extend and maintain the mid-luteal phase hormone profile** beyond the point in the cycle that would normally be associated with natural E2/P4 withdrawal. In the active E2/P4 condition, the luteal phase hormone profile will be maintained for 14 days by applying a new patch on day 8 (extended luteal phase spanning +7 to +21 in lower panel), preventing natural withdrawal and stabilizing E2/P4 in physiologic<sup>4</sup> luteal range. The placebo condition uses the same patch/pill schedule.



**Assessment of Outcomes.** Participants will complete phone measures of symptoms and suicidality starting on the first day of patches/pills and ending 5 days following the last patch/pill (phone symbols in figure above). Three lab sessions will measure mediating constructs using behavioral tasks. In each condition, lab visits will occur on days +7, +14, and +22 following ovulation.

**Primary Windows of Comparison for Hypothesis Testing.** To test whether prevention of E2/P4 withdrawal is associated with mediating behavioral constructs and suicide risk, we will compare tasks and interviews in **LAB<sub>PL2</sub>** with **LAB<sub>EXP2</sub>**.

**Blinding of Participants and Staff.** *All study staff performing assessments will remain blinded to condition. The natural within-woman variation in menstrual cycle length from one cycle to another will help maintain the participant blind.* Few women have regular 28 day cycles, and between-cycle variation up to 8 days is normal<sup>4</sup>. Demand characteristics regarding the impact of hormonal manipulation will be minimized by informing participants that women vary a great deal in their responses to hormones, and our current evidence does not allow us to predict for certain whether hormones have a positive, negative, or neutral effect on women's symptoms (of note, this is scientifically accurate<sup>39</sup>). Finally, although some women may become unblinded later in the extended hormone phase, work by the mentoring team suggests that pharmacologic delay of menses does not influence the course of perimenstrual mood symptoms<sup>144</sup>.

**Lab Construct Measures.** *Each computerized task is scored using within-task comparisons, controlling for experience- or training-related changes in response time.* **Hopelessness** will be measured via the Future Thinking Implicit Relational Assessment Procedure (FT-IRAP)<sup>151</sup>, in which 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<sup>151</sup>. **Validity:** Performance predicts clinical hopelessness and depression<sup>151</sup>. **Negative Social Appraisals** will be measured using the Social Acceptance-Rejection Implicit Association Test (SAR-IAT)<sup>152</sup>, 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. **Validity:** Performance predicts explicit social appraisals and heightened negative affect in social interactions per clinical interviews<sup>152</sup>. **Inhibitory Control** will be measured using performance on the Stop-Signal Task (SST)<sup>153</sup>, an index of the ability to withhold a prepotent response. **Validity:** Performance predicts prefrontal cortex function and lower impulsive clinical outcomes<sup>153</sup>. **Threat Sensitivity** will be measured as attentional bias for threat pictures on a Dot Probe Task (DPT)<sup>154</sup>. **Validity:** Attentional bias to threat on this task is linked to anxiety disorders<sup>155</sup> and multimodal indices of threat sensitivity in daily life<sup>154,156</sup>.

**Daily Telephone Symptom Measures (PRIMARY AND SECONDARY ENDPOINTS): Depressive Symptoms and Hopelessness** using the 10-item short form of the CES-D<sup>161</sup> and the Beck Hopelessness Scale<sup>162</sup>, **Anxiety Symptoms** (reflecting Threat Sensitivity) using the 6-item short form of the Patient-Reported Outcome Measurement Information System (PROMIS) Anxiety scale<sup>163</sup>; **Impulsive Symptoms** using a five item version of the UPPS-P Lack of Premeditation (i.e., classic impulsivity) subscale<sup>158</sup>; and **Rejection Sensitivity Symptoms** (reflecting social appraisals) using two items with high item-total correlations from the Social Evaluation subscale of the State Self-Esteem Scale<sup>157</sup>. *Each of these inventories has been extensively validated for the measurement of the target construct.* **Suicidal desire and action capacity** will be measured daily using a shortened version of the SITBI<sup>160</sup>.

**Participant Safety:** Emergent changes in suicidality will be managed using the structured University of Washington Risk Assessment and Management Protocol (UWRAP)<sup>3,164</sup>, a safety protocol recommended by NIMH for RCTs since it allows for the safe study of individuals with suicidality<sup>3</sup>. If imminent risk for suicide emerges and cannot be satisfactorily managed by the UWRAP protocol (as determined by Dr. Prinstein), the protocol for discontinuation will be followed. **However, because the manipulation mimics luteal E2/P4 levels that**

women **naturally** experience, the hormonal manipulation is not expected to pose greater risk than a normal<sup>4</sup> menstrual cycle—the risk will simply be shifted in the stabilization condition.

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#### 4.2.1 PRIMARY ENDPOINT

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

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

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#### 4.2.2 SECONDARY ENDPOINTS

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

- (6) Mean Within-Person Condition Difference (7 final placebo days – 7 final stabilization days) of BHI Hopelessness Score
- (7) Mean Within-Person Condition Difference (7 final placebo days – 7 final stabilization days) of CES-D Depression Total Score
- (8) Mean Within-Person Condition Difference (7 final placebo days – 7 final stabilization days) of UPPS-P Lack of Premeditation Impulsivity subscale
- (9) Mean Within-Person Condition Difference (7 final placebo days – 7 final stabilization days) of PROMIS Anxiety Total Score
- (10) Mean Within-Person Condition Difference (7 final placebo days – 7 final stabilization days) of SSES Social Evaluation/ Rejection Sensitivity subscale

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#### 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, an individual must also meet all of these study-specific following criteria.

1. Must be biologically **female** and between the ages of **18-45 years**.
  - **Justification:** Women 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 25 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.
  - **Justification:** Because we intend to recruit women 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 women may introduce error variance into the experiment.
5. Must be of **normal weight** (BMI between 18-29 kg/m<sup>2</sup>); 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 women.
6. 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 hormone withdrawal/stabilization of E2/P4) on within-person changes in suicide risk. The present sampling strategy **ensures generalizability**<sup>3</sup> 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).
7. Must be categorized as having **acceptably low imminent risk for suicidal crisis/attempt** according to established clinical and research guidelines<sup>149</sup> (see Eligibility Table), which have been adapted for the current study in collaboration with the co-mentor, Dr. Prinstein. As co-mentor Dr. Prinstein notes in his 2014 methodological paper on research-based suicide risk assessment<sup>2</sup>, 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<sup>2,149</sup> 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 hormone protocol simply prolongs the physiologic luteal hormone profile, they are **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 women with elevated imminent risk for suicidal crisis/attempt** (determined by structured interview and established guidelines<sup>2,149</sup>). Individuals with histories of suicidality often have ongoing, habitual thoughts about death or suicide that are highly distinguishable from **imminent risk for suicide attempt**<sup>1,149,166</sup>. 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<sup>166</sup>, yet less than .01% die by suicide each year<sup>149,166</sup>.

## **SPECIFICS OF SUICIDE RISK-RELATED ELIGIBILITY CRITERIA**

The study will **include only women 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 women at highest risk for attempt, as noted below). In order to minimize the risk of suicidal crises during the study, we will **exclude women who meet evidence-based criteria**<sup>2,149</sup> **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, women 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<sup>1,149</sup>.
  - 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<sup>1,149</sup>.
  - 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<sup>1</sup>.
  - 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<sup>1,149</sup>.
  - 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<sup>149</sup>.
2. Second, women 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 Eligibility Table.
  - a. Eligibility criteria in 1a-1e above will again be re-assessed and applied.
  - b. Using a clinical interview based on the SITBI interview<sup>160</sup> and the Scale for Suicidal Ideation interview<sup>168</sup>, the PI will evaluate each woman on a variety of risk factors for suicidal crisis and evaluate whether a woman has acceptably low<sup>1,2,149</sup> imminent risk of suicidal crisis/attempt using the decision rules described in the leftmost column in Eligibility Table and summarized below:
    - ***The evidence-based determination of acceptably low risk<sup>1,2,149</sup> of suicidal crisis/attempt will be based on the count of positive findings in each Row of Eligibility Table (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. Finally, Dr. Eisenlohr-Moul (the PI) will review the results of each eligibility visit interview with Dr. Prinstein at weekly meetings (see Training Plan and Co-Mentor Letter) to verify that the individual demonstrates acceptably low<sup>1,2,149</sup> imminent risk of suicidal crisis/attempt before approving participation in the experiment. Should Drs. Eisenlohr-Moul and Prinstein determine the patient is at imminent risk of suicidal crisis/attempt despite meeting the criteria described previously, the patient will not continue with participation. However, should Dr. Prinstein agree that the patient indeed demonstrates acceptably low<sup>1,2,149</sup> risk of suicidal crisis/attempt as outlined in Eligibility Table, the participant will continue with randomization and participation in the study.
4. *Note that ongoing participant suicide risk monitoring and management is described in later sections.*

## EXPERIMENTAL ELIGIBILITY TABLE

The goal of the suicide-related exclusion/inclusion criteria (outlined below) for the study 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 the co-mentor, Dr. Prinstein<sup>1,2,149</sup>. History of mania, psychosis, or substance abuse disorder will also be exclusionary. Table notes: Recent = In the past month. MHP = Mental Health Provider. <sup>1</sup>SITBI items 3a, 3b, 4a, 4b, 4c. <sup>2</sup>SITBI items 1a, 1b, 1c, 1f, 2c. <sup>3</sup>SITBI items 2a, 2b.

ROW	Suicide Attempts <sup>1</sup>		Suicidal Thoughts <sup>2</sup>				Recency of Last Suicidal Planning <sup>3</sup>	Current Access to Means	Recent Stressors/ Loss	Current Frequency of MHP Contact	Current Social Support
	Number	Recency	Recency	Recent Frequency	Recent Severity	Recent Intent					
<b>1</b>	Never	Never	In past year	Less than monthly	1f=1	2c=1	Never	No Access to Means	None	>1x/ month	Multiple Strong Supporters
<b>2</b> <i>No more than 3 total positive findings allowed in Rows 2,3, +4</i>	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
<b>3</b> <i>No more than 2 total positive findings allowed in Rows 3 + 4</i>	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
<b>4</b> <i>No more than 1 positive finding allowed in Row 4</i>	1 attempt	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
<b>5</b> <i>No positive findings allowed in Row 5</i>	>1 attempt	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 condition. Women 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.
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.
  - **Justification:** The use of additional exogenous hormones poses safety risks and would undermine experimental control of hormones.
  - **Women 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.**
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 women 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* women.
9. Must not currently smoke **cigarettes**.
  - **Justification:** Administration of ovarian hormones to women who smoke may increase the risk of thromboembolic events.
10. 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<sup>39</sup> from that which we expect in the more general clinical population of women with suicidality<sup>8</sup>. Because these distinctions are not yet clear, women with a self-reported history of clinical diagnosis with these disorders will be excluded.
11. 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<sup>1,149</sup>.

### 5.3 STRATEGIES FOR RECRUITMENT AND RETENTION

#### ***Recruitment and Informed Consent***

To ensure adequate enrollment of an adequate number of participants, we will employ a number of recruitment strategies. First, Participants will be recruited from the University of North Carolina at Chapel Hill Outpatient Psychiatry Clinic, where the PI currently follows psychotherapy patients and therefore have access to recruitment pathways. Second, we will advertise to the Raleigh, Durham, Chapel Hill (i.e., the “Triangle”) community using email and listserv advertisements (sent to the UNC-Chapel Hill community), flyers in local businesses and buses, and radio/newspaper/website ads.

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<sup>160</sup> and the Scale for Suicidal Ideation Interviews<sup>168</sup> 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 women. 45 women will be included in the study.

The UNC Center for Women’s Mood Disorders has a strong commitment to the enrollment of minority women in research projects, and this commitment will be reflected in this study. As shown below, the ethnic and racial composition of the population from which subjects will be recruited, including Orange, Durham, and Wake counties, is similar to the population of the United States as a whole.

Race/Ethnicity	Wake	Durham	Orange	Adjusted Average
White	70%	54%	78%	69%
Black or African American	21%	39%	12%	24%
American Indian/Alaska Native	1%	1%	1%	1%
Asian	6%	5%	7%	6%
Hispanic or Latino Origin	10%	14%	8%	11%

**The PI plans to recruit similar proportions of ethnic and racial minorities for the study. We have conservatively estimated our ability to recruit a sample that is 76% White, 20% Black or African American, 4% Asian, and with 8% identifying as Hispanic or Latina.**

Recruitment Plan for Minorities : We realize, however, that specialized efforts will be needed to meet these goals. Because minorities underutilize mental health care, the PI will also advertise in the community to recruit subjects who are currently experiencing emotional symptoms. Dr. Girdler, primary mentor, has a long-standing track record of successful recruitment of African Americans (our largest minority population) into clinical research studies. In her most recent ethnic differences research (RO1-DA13705), she was successful at recruiting over 125 African American men and women (50% of the entire sample) and in each of her current studies (RO1s MH087619 and MH099076), 30% of the women enrolled identify as African American. We will employ the strategies that have proven successful in her prior studies. The single most effective strategy involves the use of minority research staff in the recruitment and retention process. Other strategies involve the use of culturally sensitive language in advertisements, including assurances that the study involves no experimental drugs or devices. We will also advertise in periodicals targeting African American populations and work with community-based minority programs. Statistics will be kept throughout the study documenting recruitment strategy and enrollment rates, allowing the PIS to adjust initiatives accordingly. Should additional initiatives be necessary, we will work directly with leaders from the African American community.

## **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/P4) on within-person changes in suicide risk. The present sampling strategy **ensures generalizability**<sup>3</sup> of our findings to clinical populations of interest (i.e., not simply depressed women, 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 women with elevated imminent risk for suicidal crisis/attempt** (determined by structured interview and established guidelines<sup>2,149</sup>). Individuals with histories of suicidality often have ongoing, habitual thoughts about death or suicide that are highly distinguishable from **imminent risk for suicide attempt**<sup>1,149,166</sup>. 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<sup>166</sup>, yet less than .01% die by suicide each year<sup>149,166</sup>.

## 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<sup>108</sup> women with suicidal ideation but low<sup>2,149</sup> imminent risk of attempt. Furthermore, the majority of individuals with suicidal ideation do not evidence imminent risk for suicide attempt<sup>2,166</sup>, supporting the feasibility of recruiting from this population. With regard to retention, the PI has demonstrated the capacity to recruit and retain women 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. The PI has also worked on recruitment and retention (using the same psychiatric exclusion criteria) in her new clinical fellow role with Dr. Prinstein, who has great success in the recruitment, phone engagement, and retention of suicidal outpatients<sup>108</sup>. Empirically-supported strategies<sup>167</sup> for retention will include study branding, frequent contact, monetary rewards, and graduated compensation schedules.

### Study subjects will receive the following monetary incentives:

\$100 pay for one enrollment visit

\$50 pay for each laboratory testing session x 6 laboratory sessions = \$300 possible testing visit pay

\$200 pay for daily phone calls (prorated at \$5 per day if subject withdraws)

= For a total possible compensation of \$600

## 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 as determined by the DSMB or Dr. Eisenlohr-Moul in collaboration with Dr. Prinstein

(severe mood deterioration, severe changes in suicidality), Dr. Rubinow (hormonal side effects). Dr. Rubinow may consult Dr. Young with regard to gynecologic issues as necessary.

- 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 UWRAP protocol for assessing and managing suicide risk will be closely followed, supervised by Dr. Eisenlohr-Moul and Dr. Prinstein (see Appendix for UWRAP). However, if a participant is judged to be at imminent risk of suicide and therefore in need of immediate hospitalization, the participant will be immediately discontinued from participation and hospitalized. Our offices are in close proximity to UNC emergency department, and participants requiring immediate emergency care can easily be transferred there. An immediate follow-up phone call to the participant's mental health provider alerting them to the change in symptoms (see information below regarding consent to provider-researcher communication) will occur immediately. If the participant is taking study medication currently, Dr. Rubinow will immediately be contacted and he will break the blind. If it is revealed that the participant is currently in the placebo condition, he will take no further action. However, if it is revealed that the participant was currently in an active hormone stabilization condition, he will oversee the subject's discontinuation of the exogenous hormone according to his best clinical judgment.

#### 5.5 PREMATURE TERMINATION OR SUSPENSION OF STUDY

At six month intervals, the study biostatistician (Dr. Xia) will test whether there are significant differences in the occurrence of moderate, severe, and serious AEs in the placebo vs. the experimental condition with alpha set at .05. These results will be provided to the Data Safety and Monitoring Board (DSMB) along with the summary report of enrollment.

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. **Dr. Xia will again perform an unblinded statistical analysis to further determine whether active study medication (E2/P4) is significantly related to greater risk of AEs relative to placebo**. 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.

The DSMB will consider stopping the entire study based on the results of the statistical tests provided by Dr. Xia: specifically, if the experimental hormone stabilization condition is associated with greater risk of severe or serious AEs than placebo, and there is evidence (as determined by David Rubinow) that such AEs are causally related to the study drug, the study

will be terminated.

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

## 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 UNC Investigational Drug Services from their respective manufacturers.

IDS will order climara + prometrium, placebo patches have been shipped, donated from Wisner.

#### 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 UNC IDS) are formulated and designed to be identical in appearance to these agents; all packaging and blinding will be accomplished via UNC 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 UNC 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 arm, the dosages will be fixed and not changed.

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#### 6.1.8 DOSE ADJUSTMENTS/MODIFICATIONS/DELAYS

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

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#### 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.

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#### 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. Women will bring in pill bottles to each visit so compliance can be monitored.

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#### 6.1.11 DEVICE SPECIFIC CONSIDERATIONS

Not applicable.

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### 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.

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## 7 STUDY PROCEDURES AND SCHEDULE

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### 7.1 STUDY PROCEDURES/EVALUATIONS

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#### 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 @ 1.5 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
Urine Pregnancy Screen	-	-	x
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 – 6 VISITS @ 1.5 HRS

- **Timing:** The three laboratory visits for each condition will occur on days +7, +14, and +22 following positive ovulation test (day 0). 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 (3 <sup>rd</sup> visit only)	-	-	x
Blood Sample (10 ml tube)	-	-	Serum E2/P4
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	-	-

## DEBRIEFING VISIT – 30 MINUTES

- **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. Therefore, participation will require that the participant consents to phone contact between the PI and their current mental health provider in two circumstances: (1) one standard phone contact occurring prior to engagement in the first condition in which the PI describes the experiment and explains the protocol for daily risk assessment and discontinuation of participation should it be necessary; and (2) as-needed phone contact in the event that the participant must be discontinued to alert the provider to the severe change in mood symptoms or suicidality.

## 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 on two occasions: prior to randomization at the enrollment visit, and at the third study visit of the first condition.

At all 6 laboratory assessments during active treatment with placebo or E2/P4, women will provide 10ml **blood samples** to be assayed for E2/P4 to evaluate the success of the hormone in achieving stable luteal phase levels of E2/P4. Cheryl Walker's laboratory at UNC will conduct assays. Storage onsite will be handled by medical technician Joe Hodges in Dr. Susan Girdler's laboratory.

### 7.2.3 SPECIMEN PREPARATION, HANDLING, AND STORAGE

Blood will be centrifuged and serum will be aliquotted into storage tubes and stored in a -80 F freezer in Dr. Girdler's lab. Standard freezer-proof labels will indicate participant

ID and study session. These will be transported under dry ice conditions to Dr. Cheryl Walker's laboratory in batches. Following assay, any additional serum will be stored in Dr. Girdler's laboratory.

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#### 7.2.4 SPECIMEN SHIPMENT

Serum transport will be handled by Joe Hodges to Cheryl Walker's Bioendocrine lab under dry ice conditions. Samples will be sent in batches when there are enough aliquots to support purchase of RIA kits.

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### 7.3 STUDY SCHEDULE

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#### 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.

Women will be recruited primarily through chart review at the UNC outpatient psychiatry clinic; only those women agreeing to be recontacted for research procedures can be contacted. Dr. Eisenlohr-Moul will identify women via chart review who may be eligible for the study, and contact them to explain the study, and conduct phone screening in interested individuals.

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.

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#### 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; assessment for eligibility includes assessment of height and weight (BMI), urine HcG pregnancy test, consent to contact their mental health provider at appropriate intervals for study participation, and other 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 two possible condition orders at that time.

Pregnancy test must be negative in order for the participant to continue with the study. The study agent will not be administered until Lab assessment 1 (i.e., not at baseline/enrollment).

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#### 7.3.3 FOLLOW-UP

Following the baseline/enrollment visit, women 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 third laboratory visit in the first condition will include a pregnancy test that must be negative prior to starting the second condition. When the first condition is completed and the second pregnancy test is negative at the third laboratory visit, women 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	-	-	Weight
Urine Pregnancy Screen (3 <sup>rd</sup> follow-up only)	-	-	x
Blood Sample (10 ml tube)	-	-	Serum E2/P4
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	-	-

**Follow-Up Timeline:**

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

- Negative Pregnancy Test Confirmed at Enrollment
- 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, 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, Pregnancy Test

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

- Negative Pregnancy Test Confirmed at Visit 3
- 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, 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, Schedule Debriefing

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#### 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 women who had the placebo-experimental condition order. Participants will not be provided with individualized study results.

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#### 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.

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#### 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

	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
<b>Procedures</b>										
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								
E2/P4 Serum 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	

### 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 seven years of experience in risk assessment and empirically supported treatment of chronic suicidality (e.g., Dialectical Behavior Therapy); Dr. Prinstein, the consulting clinical psychologist, has over 20 years of experience in risk assessment and treatment of chronic suicidality. The PI and her mentors have also conducted several studies assessing depressive symptoms in women with borderline personality disorder or chronic suicidality, most of which included structured interviews for the assessment of suicidality and other psychiatric symptoms. The available literature<sup>3</sup> 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.

## 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 clinicians David Rubinow, M.D. (hormone-related AEs) and Mitch Prinstein, 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. Girdler, who will determine severity and next steps in consultation with the appropriate clinical supervisor (Rubinow for physical adverse events, Prinstein for psychological and behavioral adverse events).

The DSMB will evaluate AEs that are submitted during scheduled reviews. The North Carolina Translational and Clinical Sciences (NCTraCS) Data and Safety Monitoring Board (NCTraCS DSMB) has agreed to serve in this role. The Board is composed of MDs from a variety of disciplines, an R.N. from the UNC School of Nursing, a Biostatistician, a Regulatory Expert, and for purposes of this study, a psychiatrist or clinical psychologist may serve as an ad hoc member.

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 in humans, 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, Drs. Girdler and Eisenlohr-Moul will urgently collaborate with appropriate clinical supervisors (Rubinow for physical/medical events, Prinstein for behavioral/psychological events) to determine the AE’s causality based on temporal relationship and his/her 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., the event did not occur within a reasonable time after administration of the trial medication) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).

**Not Related** – The AE is completely independent of study drug administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by Dr. Rubinow or Dr. Prinstein.

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### 8.2.3 EXPECTEDNESS

Drs. Girdler and Eisenlohr-Moul will collaborate with appropriate clinical supervisors (Rubinow for physical/medical, Prinstein for psychological/behavioral) 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 participant's functioning or safety (as determined by Drs. Eisenlohr-Moul, Girdler, Rubinow, and Prinstein) 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 (within 2 hours) reported to the PI, who will consult with Drs. Girdler, Rubinow, and Prinstein in the 24 hours that follow. 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 or Dr. Prinstein 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 or Prinstein) 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, and within the following 24 hours, with Drs. Girdler and Rubinow.

The PI Dr. Eisenlohr-Moul (in consultation with Drs. Girdler, Rubinow, and Prinstein) will review all protocol data at weekly 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.

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#### 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 project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents 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.

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#### 8.4.4 EVENTS OF SPECIAL INTEREST

Not applicable.

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#### 8.4.5 REPORTING OF PREGNANCY

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

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### 8.5 STUDY HALTING RULES

At six month intervals, the study biostatistician (Dr. Xia) will test whether there are significant differences in the occurrence of moderate, severe, and serious AEs in the placebo vs. the experimental condition with alpha set at .05. These results will be provided to DSMB along with the summary report of enrollment.

Administration of study agent will be halted when three serious AEs\* determined to be "probably related" 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. Dr. Xia will again perform an unblinded statistical analysis to further determine whether active study medication (E2/P4) is significantly related to greater risk of AEs relative to placebo. 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.

The DSMB will consider stopping the entire study based on the results of the statistical tests provided by Dr. Xia: specifically, if the experimental hormone stabilization condition is associated with greater risk of severe or serious AEs than placebo, and there is evidence (as determined by David Rubinow) that such AEs are causally related to the study drug, the study will be terminated.

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

## 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 DSMB 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).

- Katherine McCann, 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).

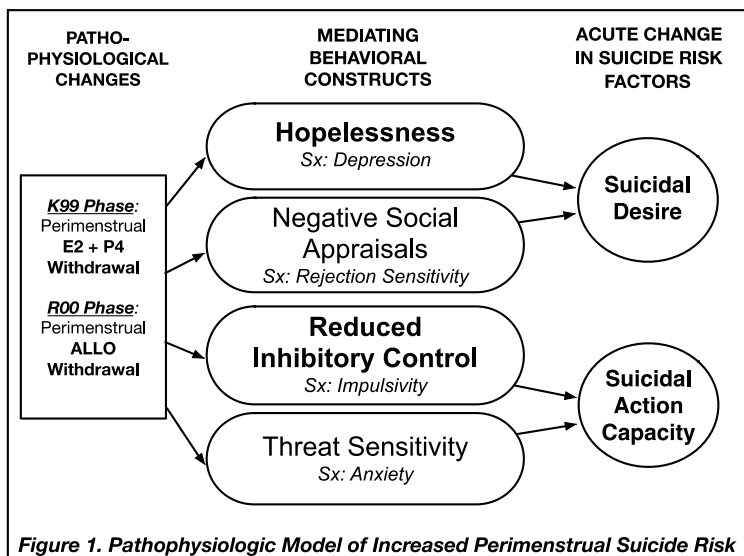
## 10 STATISTICAL CONSIDERATIONS

### 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 (see far right side of figure in Figure at right)—we predict that perimenstrual increases in suicidal desire and action capacity (Lab 2 – Lab 1 within each condition) will be larger in the placebo condition than in the experimental hormone stabilization condition. The associated null hypothesis is that there will be no difference in the perimenstrual increase in suicidality across condition. 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 two-period crossover design, where each of two 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 both conditions in a randomized order.

The primary windows of comparison will be the final seven days of each administration phase, which will allow for a comparison of a woman's mean scores in the placebo condition compared to her mean scores in the experimental condition.

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.

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#### 10.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

Primary and secondary endpoints are operationalized above (i.e., mean within-person differences between placebo and experimental in final seven days of manipulation) 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 *in the final seven days* of both the placebo condition (placebo mean score) and the experimental condition (experimental mean score).

Second, we will utilize paired samples t-tests to examine the within-person condition effect. If proposed covariates (medicines, BMI, age) show sufficient variability, we will instead use a repeated measures ANCOVA to evaluate the condition effect. 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.

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#### 10.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

See above.

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#### 10.4.4 SAFETY ANALYSES

All adverse events will be recorded once per 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 condition.

***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

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#### 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 condition relative to hormonal profiles during the placebo condition; it will not be monitored in real time.

Women 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.

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#### 10.4.6 BASELINE DESCRIPTIVE STATISTICS

Groups (counterbalanced order as placebo-experimental or experimental-placebo) 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.

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#### 10.4.7 PLANNED INTERIM ANALYSES

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##### 10.4.7.1 SAFETY REVIEW

At six month intervals, the study biostatistician (Dr. Xia) will test whether there are significant differences in the occurrence of moderate, severe, and serious AEs in the placebo vs. the experimental condition with alpha set at .05. These results will be provided to DSMB along with the summary report of enrollment.

Administration of study agent will be halted when three serious AEs\* determined to be “probably related” 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. Dr. Xia will again perform an unblinded statistical analysis to further determine whether active study medication (E2/P4) is significantly related to greater risk of AEs relative to placebo. 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.

The DSMB will consider stopping the entire study based on the results of the statistical tests provided by Dr. Xia: specifically, if the experimental hormone stabilization condition is associated with greater risk of severe or serious AEs than placebo, and there is evidence (as determined by follow up with David Rubinow using unblinded data provided by Dr. Xia) that such AEs are causally related to the study drug, the study will be terminated.



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

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#### 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.

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#### 10.4.8 ADDITIONAL SUB-GROUP ANALYSES

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

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#### 10.4.9 MULTIPLE COMPARISON/MULTIPLICITY

N/A

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#### 10.4.10 TABULATION OF INDIVIDUAL RESPONSE DATA

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

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#### 10.4.11 EXPLORATORY ANALYSES

Not applicable.

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### 10.5 SAMPLE SIZE

An unlimited number of women will be screened. 45 women will be enrolled in the study, with the goal of 30 women 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 the contrast testing the primary hypothesis, we will have power to detect a conventionally medium-sized effect of hormone withdrawal ( $r = .30$ ;  $d = .60$ ) at 80% power with just 17 participants<sup>165</sup>. The PI's previous work in women with BPD suggests that attrition could be as high as 25% across two conditions; should attrition be this high, enrollment of 22 participants would be required for adequate power. However, to improve generalizability of our findings and to ensure 80% power to detect small-to-medium effect sizes ( $r = .20$ ;  $d = .40$ ), we aim to include 30 completers (with 45 women randomized). This number is especially reasonable given the

much larger effects found in the candidate's recent studies for the effect of natural ovarian steroid changes (average  $r = .58$ ;  $d = 1.38$ ) on daily symptoms of depression, rejection sensitivity, anxiety, and impulsivity<sup>40</sup>.

## 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 two counterbalanced condition orders (placebo-experimental or experimental-placebo) 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 of Participants and Staff.** *All study staff performing assessments will remain blinded to condition. The natural within-woman variation in menstrual cycle length from one cycle to another will help maintain the participant blind.* Few women have regular 28 day cycles, and between-cycle variation up to 8 days is normal<sup>4</sup>. Demand characteristics regarding the impact of hormonal manipulation will be minimized by informing participants that women vary a great deal in their responses to hormones, and our current evidence does not allow us to predict for certain whether hormones have a positive, negative, or neutral effect on women's symptoms (of note, this is scientifically accurate<sup>39</sup>). Finally, although it is possible that some women may become unblinded later in the extended hormone phase if they have extremely regular cycles, work by the mentoring team suggests that pharmacologic delay of menses does not influence the course of perimenstrual mood symptoms<sup>144</sup>.

### 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. Rubinow in the case of severe physical AEs (e.g., VTE) or hospitalization/suicide attempt, and Dr. Prinstein will also be unblinded if severe AEs are related to suicidality.

## 11 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

All data collected in this study will be maintained on the UNC protected servers and in clinical binders kept under lock and key in Dr. Girdler'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 Katherine McCann. 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 UNC Chapel Hill. 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 and archived on UNC Chapel Hill 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.

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##### 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 suicidality and psychiatric symptoms. No genetic testing will be performed.
- **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 UNC server. Disposition at the completion of the study: All serum samples will be kept under locked access areas at UNC and on secure UNC servers. 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 and stored at Dr. Susan Girdler's laboratory at UNC. After the study is completed, the de-identified, archived data will remain in Dr. Girdler's laboratory, under the supervision of medical technician Joe Hodges, for use by other researchers including those outside of the study. Permission to retain samples in Dr. Girdler's laboratory will be included in the informed consent.

With the participant's approval and as approved by local IRBs, de-identified biological samples will be stored at the Dr. Girdler's laboratory. These samples could be used for research into the causes of suicidality and other psychiatric symptoms, and to improve treatment.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to biosample storage will not be possible after the study is completed.

The data and samples collected in this study 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 UNC 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 will be retained for a minimum of 2 years following the end of the study. These documents will be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

## 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 take specific steps to ensure compliance with NIH implementation of FDAAA.

## 15 STUDY ADMINISTRATION

### 15.1 STUDY LEADERSHIP

The PI and her key mentors (Girdler, Prinstein, Rubinow, Young) will govern the conduct of the study, along with NIMH program officer Mark Chavez. The PI will meet weekly with key mentors weekly as described in the NIMH K99 Career Development 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.

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## APPENDIX

Version	Date	Significant Revisions