

PROTOCOL

Background

1. Provide the scientific background, rationale and relevance of this project.

PCOS Background

Polycystic ovary syndrome (PCOS) is among the most prevalent endocrine disorders, afflicting 7–15% of reproductive-aged women worldwide.^{1–5} PCOS is characterized by hyperandrogenism (clinical and/or biochemical), ovulatory dysfunction, and polycystic ovarian morphology. PCOS is the most common etiology of anovulatory infertility.⁶ Additionally, it is associated with risks in pregnancy (e.g., 3-fold increased miscarriage rate⁷) and numerous metabolic disorders (e.g., type 2 diabetes, obesity).⁸ Although many advances have been made in understanding PCOS, its underlying pathophysiological mechanisms remain incompletely grasped, and relatively little is known about the causes of ovulatory dysfunction in PCOS. Additional mechanistic clarity would inform improved therapies for PCOS, especially for anovulation and infertility.

Normal Regulation of the Menstrual Cycle and Pre-Ovulatory Gonadotropin Surge

In women with normal ovulatory menstrual cycles (Figure 1), hypothalamic gonadotropin-releasing hormone (GnRH) stimulates pulsatile pituitary secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Higher GnRH frequencies favor LH secretion, while slower frequencies favor FSH secretion.⁹ During most of the normal menstrual cycle, estradiol (E2) and progesterone (P4) regulate GnRH and gonadotropin secretion via negative feedback mechanisms.¹⁰ For example, P4 is the primary inhibitor of GnRH pulse frequency.¹¹ This function appears to require the presence of E2, likely due to E2-induced expression of hypothalamic P4 receptors, as has been demonstrated in the rodent and ewe models.^{12,13} In the luteal phase, when P4 and E2 levels (secreted by the corpus luteum) are very high, GnRH pulse frequency is suppressed; this slow frequency enhances FSH production (although pituitary release of FSH remains restrained by high luteal levels of inhibin A and E2).¹¹ During the transition from the luteal to follicular phase, P4, E2, and inhibin A levels fall. Released from these hormones' negative feedback, GnRH pulse frequency rises and stimulates a pituitary replete with FSH; this selective rise in FSH promotes ovarian follicular development. In the early follicular phase, a dominant follicle emerges (in response to FSH stimulation) and produces steadily increasing amounts of E2. At the end of the follicular phase, ovulation is triggered by the gonadotropin surge—largely in response to the marked increase in E2 secretion by the

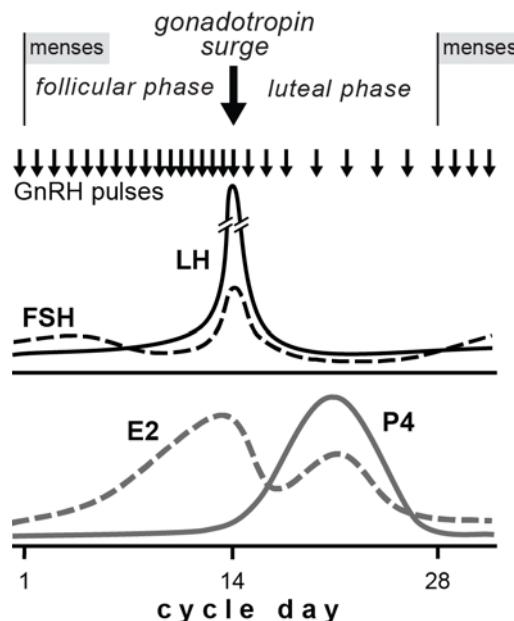


Figure 1. Normal menstrual cycle.

dominant ovarian follicle (i.e., E2 exerts a positive feedback effect on the pituitary).¹⁴ (Note: although a corresponding surge of GnRH occurs in animal models, including primates,¹⁵ gonadotropin surges occur even in GnRH-deficient women treated with exogenous GnRH at a constant dose and frequency.¹⁶) During the normal surge, LH levels rise 10-fold over 2 to 3 days while FSH levels rise 4-fold.¹¹

The gonadotropin surge can be triggered by treatment with exogenous E2 alone.¹⁴ P4 also increases significantly approximately 12 h prior to the gonadotropin surge. When P4 is given in the absence of E2 priming, it cannot independently induce a surge. Nonetheless, P4 has an important role in gonadotropin surge generation.^{14,17-20} In the setting of E2 priming, P4 increases pituitary gonadotropin release in response to GnRH.¹⁸ When P4 receptors are blocked with mifepristone, gonadotropin surge initiation is delayed by up to 3 days.²⁰ Chang and Jaffe demonstrated that, in E2-primed women, administration of GnRH augments the release of LH and FSH more robustly in women treated with P4 compared to those receiving only E2.¹⁹ Liu and Yen used incrementally increasing doses of infused E2 and evaluated the gonadotropin surge with and without a superimposed P4 infusion (begun 48 h into E2 infusion); P4 significantly prolonged the mean duration of the gonadotropin surge.¹⁴ March et al. found that when P4 administration followed incrementally increasing doses of E2, the LH surge occurred earlier (as compared to when E2 was given alone) and was accompanied by an FSH surge (which did not occur in the absence of P4 administration).¹⁷ Taken together, these studies suggest that P4 is necessary for full expression of the gonadotropin surge in terms of timing, magnitude, and duration.

We have previously studied the acute effects of P4 administration in 8 normally-cycling women (late follicular phase) after 3 days of E2 pretreatment (0.2 mg/d transdermally).²¹ This placebo-controlled crossover study was designed to assess the effect of P4 (administered at 1800 h) on LH pulse frequency. Although P4 did not acutely influence LH pulse frequency, we found that mean LH, LH pulse amplitude, and mean FSH increased markedly within 4 h of P4 administration but not following placebo. In a recent placebo-controlled crossover study, we again observed an acute P4-mediated increase in mean LH and FSH in E2-pretreated women.²² Importantly, this experimental model allows evaluation of acute P4 positive feedback on gonadotropin release.

Evidence for Defective Progesterone Negative Feedback in PCOS

In adult women with PCOS, the above-described processes are dysfunctional at multiple levels. Throughout the cycle, LH concentrations are higher than those seen in normal women,²³ a result of LH pulses rising to greater amplitudes and occurring at higher frequencies.²⁴ These alterations in LH pulsatility are largely a consequence of increased frequency of GnRH pulses. As described above, higher GnRH frequencies lead to greater secretion of LH relative to FSH. This shifted ratio of LH to FSH is seen in most women with PCOS,²⁵ and is a factor that contributes greatly to ovulatory dysfunction and increased ovarian androgen production.

Prior studies provide evidence for a relative GnRH pulse generator resistance to P4 negative feedback. Berga and Daniels reported that, with use of oral contraceptives, LH pulse frequency suppression was impaired in PCOS as compared to eumenorrheic women.²⁶ Similarly, Pastor et al. evaluated LH pulse frequency (and by inference GnRH pulse frequency) in women with PCOS

and normal-cycling controls, both before and after 7 days of treatment with E2 (0.2 mg/d transdermally) and P4 (50 mg vaginally every 8 h) to achieve mid-luteal E2 and P4 concentrations.²⁷ LH pulse frequency was suppressed by 60% in normal controls but by only 25% in PCOS. That is, higher plasma levels of P4 were needed to suppress the GnRH pulse generator in PCOS. Of special interest, a subsequent study by our group demonstrated that GnRH pulse generator sensitivity to P4 negative feedback is restored to normal after androgen-receptor blockade with flutamide.²⁸ Reduced GnRH pulse generator sensitivity to P4 negative feedback has also been reported in rodent and sheep models of PCOS.²⁹⁻³¹ Thus, hyperandrogenemia is implicated in the impairment of P4's central negative feedback; this effect may reflect androgen-mediated down-regulation of hypothalamic P4 receptors, as has been demonstrated in rodent models.³⁰ Impairment of P4's negative feedback action on the GnRH pulse generator leads to persistently rapid GnRH pulse frequency with derangement of gonadotropin secretion, as described above.

Evidence for Defective Progesterone Positive Feedback in Regulation of the Surge

We have characterized defective sex steroid negative feedback on the GnRH pulse generator in PCOS, but the potential for dysfunction in sex steroid positive feedback in PCOS remains largely unexplored, particularly in relation to gonadotropin surge generation. Our preliminary data suggest that P4 positive feedback on LH and FSH release may be impaired in women with PCOS. We studied 8 women with PCOS (≥ 7 days after start of last menses) and 11 normally-cycling women (in late follicular phase, cycle days 7-10). (Figure 2.) All subjects were pretreated with transdermal (TD) E2, 0.2 mg/d for a total of 4 days. On day 3 of TD E2, subjects were admitted for frequent blood draws to characterize gonadotropin secretion. On day 4 at 0600 h, subjects received oral P4 100 mg. For this analysis, we calculated mean LH and FSH values in the 4 h preceding P4 administration (0200-0600 h; baseline levels) and 4-8 h after P4 administration (1000-1400 h)—the latter captures peak gonadotropin levels in both normal controls and subjects with PCOS. P4 positive feedback (i.e., P4-induced augmentation of gonadotropin release) was quantified as the ratio of (a) mean LH or FSH before P4 administration to (b) mean LH or FSH after P4 administration—hereafter called the “augmentation index (AI).” (For example, if mean LH from 0200 to 0600 h was 2.0 and mean LH from 1000 to 1400 h was 6.0, the LH AI would be 3.) A representative example of P4-induced augmentation of LH release is in Figure 3.

Women with PCOS exhibited 44% higher baseline (pre-P4) LH levels compared to controls. P4-induced LH augmentation (Figure 4) was 32% lower in PCOS subjects ($AI 4.9 \pm 1.8$ vs. 3.3 ± 1.0 [expressed as mean \pm SEM]; $p = 0.0388$ by exact Wilcoxon two-sample testing). The

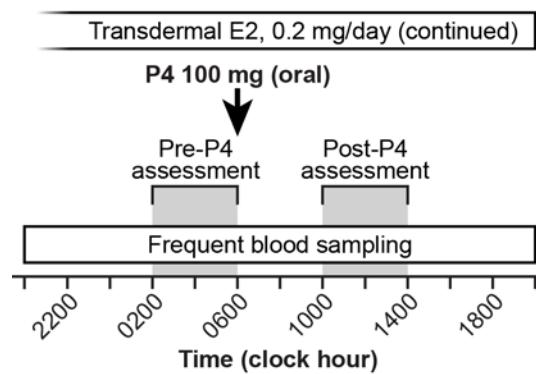


Figure 2. Schematic of study protocol.

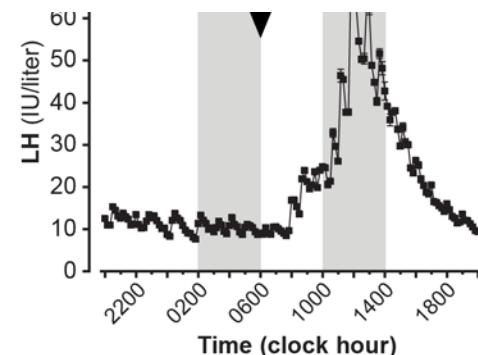


Figure 3. Example, pre- and post-P4 LH.

mean baseline (pre-P4) FSH levels were similar in subjects with PCOS and controls (4.0 ± 1.6 vs. 3.9 ± 1.4 IU/liter; $p = >0.5$). Acute P4-induced augmentation of FSH release (Figure 4, next page) was reduced by 32% in PCOS subjects when compared to controls ($AI 2.3 \pm 0.5$ vs. 1.6 ± 0.3 ; $p = 0.0036$ by exact Wilcoxon two-sample test).

As expected, subjects with PCOS had 3-fold higher testosterone (T) concentrations. Additionally, and not unexpectedly, BMI was higher in the PCOS cohort (31.7 ± 2.6 vs. 22.5 ± 1.0 , $p = 0.0121$). Since circulating gonadotropin levels are inversely related to BMI,²⁵ BMI may have been a confounder in our analysis. However, P4-induced augmentation of FSH and LH release were reduced in PCOS even after adjusting for differences in BMI ($p = 0.0005$ and $p = 0.0281$, respectively). As another potential confounder, achieved E2 levels in PCOS were slightly higher than those in controls (139 ± 24 vs. 103 ± 19 pg/ml). Although this was not statistically significant ($p = 0.23$), one may speculate that higher E2 could have augmented P4-mediated LH release while limiting P4-mediated FSH release in the PCOS group.

Evidence for Defective Estradiol Positive Feedback in Regulation of the Surge

Our preliminary data imply a defect in P4 positive feedback in E2-primed women with PCOS compared to E2-primed controls. However, while P4 positive feedback is believed to be important for the full expression of the gonadotropin surge, E2 positive feedback is of primary importance in generation of the surge. Of interest, prenatally-androgenized (PNA) animals (i.e., animal-models of PCOS) provide evidence for a defect in surge-related E2 positive feedback in PCOS. For example, high-dose E2 fails to initiate the gonadotropin surge in PNA rats, mice, and sheep.^{30,32,33}

The efficacy of E2 positive feedback in women with PCOS has not been thoroughly investigated. Baird *et al.* induced a gonadotropin surge in 12 women with PCOS and 6 normal women with 3 days of ethinyl E2 200 mcg given orally.³⁴ Plasma samples for LH and FSH were collected daily for 4 days before, 3 days during, and 3 days after E2 administration. In this study, the LH peak occurred 24 h earlier in PCOS than in controls, but the LH peak magnitude was similar in both groups. However, we believe that this study had important limitations. Firstly, this protocol did not appear to generate robust surges: LH levels increased only 2 to 3-fold (from mean basal LH 13.6 ± 1.2 mU/mL to peak LH 34.2 ± 6.9 mU/mL), while FSH levels did not reach a statistically significant increase from baseline. Secondly, the control subjects were studied in the early follicular phase (rather than late follicular phase): we speculate that the full effects of E2 positive feedback may not be invoked so soon after the luteal phase – if correct, this may have inappropriately impaired surge generation in controls. Lastly, daily blood samples are a highly imprecise way to quantify the gonadotropin surge.

Defective Sex Steroid Positive Feedback in PCOS: Potential Relevance

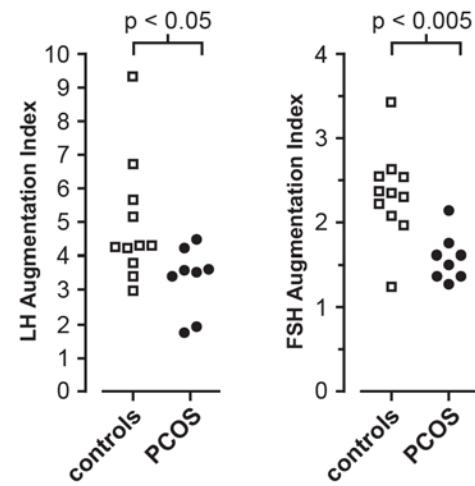


Figure 4. LH and FSH AIs, PCOS vs. controls.

The Endocrine Society PCOS guidelines (2013) recommend short-term clomiphene citrate—and possibly aromatase inhibitors such as letrozole—as preferred pharmacological therapy for ovulation induction in PCOS.³⁵ Clomiphene is a selective estrogen-receptor modulator that antagonizes E2 action at the hypothalamus/pituitary³⁶; letrozole similarly decreases central negative feedback by inhibiting E2 formation.³⁷ Removal from negative feedback results in increased gonadotropin release, which promotes follicular development, selection of a dominant follicle, an increase in E2 production, gonadotropin surge generation, and ovulation. (Note that the latter events occur after drug discontinuation.) However, a significant number of women with PCOS fail to respond to these treatments. In comparison of clomiphene to metformin for ovulation induction, Legro et al. reported that 24.9% of women had no documented ovulations while using clomiphene, and that the live-birth rate was only 22% with use of clomiphene alone.³⁸ In Legro et al.'s subsequent study comparing letrozole to clomiphene, the anovulation rate was 23.4% on clomiphene and 11.5% on letrozole; letrozole resulted in a live-birth rate of 27.5% compared to 19.1% on clomiphene.³⁶ *The failure of many women with PCOS to ovulate with these methods suggests a mechanistic gap in the current standard approach to PCOS infertility management. We propose that impairment in sex-steroid positive feedback and gonadotropin surge generation may provide a functional explanation for this therapeutic deficit.*

Hypothesis to be Tested

We hypothesize that compared to normal controls, PCOS subjects will demonstrate a blunted gonadotropin response to estrogen positive feedback (i.e., a less prominent increase in 24-h average urinary gonadotropin concentration with E2 administration).

Study Design: Biomedical

1. Will controls be used? Yes.

► IF YES, explain the kind of controls to be used.

Controls will be healthy, normally-cycling women without hyperandrogenism.

2. What is the study design?

This is a prospective interventional cohort study. Women with PCOS and controls will receive identical treatment (transdermal estradiol) and will be monitored using the same surveillance protocol. The study does not involve randomization, and it is not blinded.

3. Does the study involve a placebo?

No.

Human Participants

Ages: 18-30

Sex: Female

Race: All races will be recruited and enrolled.

Subjects- see below

1. Provide target # of subjects (at all sites) needed to complete protocol. 28

2. Describe expected rate of screen failure/ dropouts/withdrawals from all sites.

We anticipate a screen failure/dropout/withdrawal rate of up to 33%.

3. How many subjects will be enrolled at all sites? 37

4. How many subjects will sign a consent form under this UVa protocol? 37

5. Provide an estimated time line for the study.

Year 1: 33.3% enrolled.

Year 2: 33.3% enrolled.

Year 3: 33.3% enrolled.

Year 4: Completion of data analysis.

Inclusion/Exclusion Criteria

1. List the criteria for inclusion

- All included subjects will be adult women ages 18-30 years. We will use age 30 as a cutoff because early manifestations of reproductive senescence (e.g., evidence for reduced ovarian reserve) are more common in women older than age 30 years. Additionally, the risk of development of venous clots on estrogen treatment appears to increase beyond age 35 years, so by limiting our population to women age 18-30, risk associated with estrogen treatment will be limited in a healthy population.
- Adult women with PCOS and normally-cycling, non-hyperandrogenic controls
- General good health (excluding, for example, overweight, obesity, hyperandrogenism, PCOS, and adequately-treated hypothyroidism)
- Capable of and willing to provide informed consent
- Willing to strictly avoid pregnancy with use of non-hormonal methods during the study period

2. List the criteria for exclusion

- Incapacity to provide informed consent including due to cognitive impairment
- Males will be excluded as E2-induced gonadotropin surges and PCOS are relevant to females only
- Children (age <18 years) will be excluded
- Prisoners will be excluded due to the high frequency of required visits
- Evidence for etiologies of anovulation/hyperandrogenism due to conditions other than PCOS
- Positive pregnancy test or current lactation
- Menstrual cycle frequency <26 days: Cycles <26 days suggest the possibility of relatively short follicular phases (e.g., < 12 days). If a subject with a follicular phase shorter than 12 days participates in our protocol, they could experience an endogenous gonadotropin surge under surveillance. Since we wish to capture only experimentally-induced surges, we will exclude such subjects.
- History or physical exam evidence suggesting Cushing's syndrome or adrenal insufficiency
- Evidence of virilization (e.g., rapidly progressive hirsutism, deepening of the voice, clitoromegaly)
- Total testosterone >150 ng/dL, which suggests the possibility of virilizing neoplasm

- DHEA-S greater than the upper limit of reference range for controls; mild elevations may be seen in PCOS, and elevations <1.5 times the upper limit of normal will be accepted in these groups.
- Early morning follicular phase 17-hydroxyprogesterone greater than the follicular phase reference range, which suggests the possibility of congenital adrenal hyperplasia (if elevated during the luteal phase, the 17-hydroxyprogesterone will be repeated during the follicular phase). *NOTE:* If a 17-hydroxyprogesterone level greater than the follicular phase reference range is confirmed on repeat testing, an ACTH stimulated 17-hydroxyprogesterone < 1000 ng/dL will be required for study participation.
- Previous diagnosis of diabetes, fasting glucose ≥ 126 mg/dL, or a hemoglobin A1c $\geq 6.5\%$
- Abnormal thyroid stimulating hormone (TSH). Note that subjects with stable and adequately treated primary hypothyroidism, reflected by normal TSH values, will not be excluded.
- Hyperprolactinemia. Any degree of hyperprolactinemia (confirmed on repeat) will be grounds for exclusion for controls. Mild prolactin elevations may be seen in women with PCOS; elevations <50% higher than the upper limit of the reference range will be accepted in this group.
- Persistent hematocrit <36% and hemoglobin <12 g/dL.
- Severe thrombocytopenia (platelets <50,000 cells/microL) or leukopenia (total white blood count <4,000 cells/microL)
- Persistent liver laboratory study abnormalities, with these exceptions: Mild bilirubin elevations will be accepted in the setting of known Gilbert's syndrome; mild transaminase elevations may be seen in PCOS, therefore elevations < 1.5 times the upper limit of normal will be accepted in this group
- Significant history of cardiac or pulmonary dysfunction (e.g., known or suspected congestive heart failure; asthma requiring intermittent systemic corticosteroid; etc.)
- Decreased renal function evidenced by GFR <60 mL/min/1.73m²
- No medications known to affect the reproductive system can be taken in the 3 months prior the study. Such medications include hormonal contraceptives, spironolactone, metformin, progestins, glucocorticoids, antipsychotic psychotropic drugs, etc.
- Personal history of any disorders that may potentially be complicated or exacerbated by high-dose estradiol administration, such as hypertension (persistent, appropriately-measured blood pressure $>140/90$ mmHg), severe hypertriglyceridemia (fasting triglycerides >500 mg/dL), venous thromboembolism (deep venous thrombosis, pulmonary embolism), proven or suspected hypercoagulability, arterial thrombosis, stroke, coronary artery disease, myocardial infarction, migraine with aura, and breast, ovarian, or endometrial cancer
- History of any other cancer diagnosis and/or treatment (with the exception of basal cell or squamous cell skin carcinoma) unless they have remained clinically disease free (based on appropriate surveillance) for five years
- History of allergy or intolerance to transdermal estradiol
- BMI less than 18 kg/m² or greater than or equal to 40 kg/m²
- Subjects with body weight <110 lbs. will be excluded from the study

NOTE 1: *All potentially exclusionary laboratory studies will be confirmed by repeat testing to exclude laboratory error.*

NOTE 2: *Subjects with mild anemia (e.g., hematocrit 36%) will be offered the option to take iron in the form of ferrous gluconate (325 mg twice daily) for up to 60 days, after which they can return to the CRU to have their hemoglobin or hematocrit rechecked. Such subjects will be allowed to proceed with study*

if hemoglobin or hematocrit is ≥ 12 g/dL or $\geq 36\%$, respectively. Iron will not be offered to subjects with a history of any disorders that may potentially be complicated by long-term iron supplementation, such as hemochromatosis and polycythemia vera.

3. List any restrictions on use of other drugs or treatments.

No medications known to affect the reproductive system can be taken in the 3 months prior the study. Such medications include hormonal contraceptives, spironolactone, metformin, progestins, glucocorticoids, antipsychotic psychotropic drugs, etc.

Statistical Considerations

1. Is stratification/randomization involved?

No.

2. What are the statistical considerations for the protocol?

This is a prospective interventional cohort study. Women with PCOS and controls will receive identical treatment (transdermal estradiol) and will be monitored using the same surveillance protocol. The study does not involve randomization, and it is not blinded.

This study is informed by our finding that progesterone-mediated augmentation of serum LH and FSH levels are abnormal (reduced) in PCOS compared to normal controls. Available data are inadequate to address whether E2-mediated augmentation of serum LH and FSH levels are abnormal (reduced) in PCOS.

The primary endpoint in this study will be E2-induced fold-increase in 24-h average urinary gonadotropin concentration, defined as the peak 24-h mean value after E2 administration divided by the nadir 24-h mean value (prior to or during E2 administration).

3. Provide a justification for the sample size used in this protocol.

The power analysis is based a comparison of progesterone-mediated augmentation of serum LH and FSH levels (i.e., LH and FSH Augmentation Indices) between healthy normal and PCOS study populations (described above).

Tables 1 and 2 show, as a function of sample size, the minimum detectable differences in mean LH Augmentation Indices that would lead at least 80% of the time to rejecting the null hypothesis that the true underlying mean LH AI are the same for healthy normal and PCOS study populations. Estimates in Table 1 are based on differences in progesterone-mediated LH Augmentation Indices between healthy normal and PCOS study populations; while estimates in Table 2 are based on differences in progesterone-mediated FSH Augmentation Indices between healthy normal and PCOS study populations.

Table 1. Data based on differences in progesterone-mediated LH Augmentation Indices between healthy normal and PCOS study populations.

Number of Healthy Controls	Number of PCOS	Minimum Detectable Difference in the Mean LH AI
10	10	2.19

11	11	2.09
12	12	2.00
13	13	1.92
14	14	1.85
15	15	1.79
16	16	1.73

Table 2. Differences in progesterone-mediated FSH Augmentation Indices between healthy normal and PCOS study populations.

Number of Healthy Controls	Number of PCOS	Minimum Detectable Difference in the Mean LH AI
10	10	0.73
11	11	0.69
12	12	0.66
13	13	0.64
14	14	0.61
15	15	0.59
16	16	0.57

Since progesterone appears to be especially important for FSH augmentation (previous study), and estradiol is most important for LH augmentation (current study), we believe that the data in Table 2 may possibly be more relevant for our study. However, we wish to power our study according to the preliminary LH data—a more conservative approach to help ensure adequate statistical power for our study.

If 14 women with PCOS and 14 controls complete this study, we expect at least 80% power to detect relevant differences in E2-induced augmentation of urinary LH and urinary FSH concentrations.

Calculation Assumptions: For these calculations, we assumed that E2-induced changes in urinary gonadotropins—including within-group variability and between-group differences—will approximate those observed for P4-induced changes in serum gonadotropins. The power calculations also assumed that the LH AI will be normally distributed in the two study populations and that the standard deviations of the two underlying distributions will not exceed 1.75 units. Furthermore, the power calculations assume that the two-sided type I error rate of the statistical test will not exceed 0.05.

4. What is your plan for primary variable analysis?

The primary endpoint in this study will be the E2-induced fold-increase in 24-h average urinary gonadotropin concentration (a.k.a., the LH Augmentation Index), defined as the peak 24-h mean value after E2 administration divided by the nadir 24-h mean value (prior to or during E2 administration).

A comparison of the mean LH Augmentation Index (LH AI) values between the healthy normal and the PCOS study populations will be conducted by way of analysis of covariance (ANCOVA). Determining whether the component of variability in the LH AI attributed to “Study Group” (i.e. healthy normal versus PCOS) is a significant component of the overall variability in the LH AI will be the central hypothesis that will be tested. Variability in LH AI attributed to baseline disparities in mean LH will be accounted for in testing this central

hypothesis by treating subject-specific baseline mean LH as a covariate in the ANCOVA model. A $p \leq 0.05$ decision rule will be utilized as the null hypothesis rejection criterion for testing the central null hypothesis that the mean LH AI is the same for healthy normal and the PCOS subjects who have common baseline LH.

5. What is your plan for secondary variable analysis?

As a secondary analysis, the potential role of obesity will be explored by adding subject-specific BMI as a covariate in the ANCOVA model.

As another secondary analysis, comparison of the mean FSH Augmentation Index values between the healthy normal and the PCOS study populations will be conducted by way of analysis of covariance (ANCOVA), with procedures identical to those described for LH Augmentation Index (above).

6. Have you been working with a statistician in designing this protocol?

Yes.

IF YES, what is their name?

James Patrie (Department of Public Health Sciences)

7. Will data from multiple sites be combined during analysis?

No.

Biomedical Research

1. What will be done in this protocol?

All procedures performed in this protocol are being done solely to answer a research question and generate generalizable knowledge.

Outpatient Consent and Screening

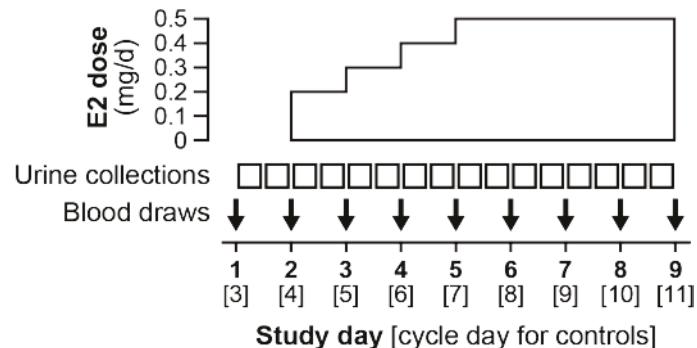
After a potential subject is identified, we will arrange for her to come to the CRU to obtain informed consent and to perform outpatient screening procedures. The goals and procedures of the study will be explained to the potential subject, and she will be given the opportunity to ask any questions. If the potential subject wishes to proceed with the study, she will be asked to sign the consent form. A study physician then will record a medical history and perform a physical examination. Blood will be drawn for screening laboratory tests. Prior to the blood draw, the subject will need to fast for a minimum of 8 hours. Blood obtained will be for screening of: Complete blood count, comprehensive metabolic panel, hemoglobin A1c, insulin, thyroid stimulating hormone, prolactin, LH, FSH, estradiol, progesterone, total testosterone, sex hormone binding globulin, 17-hydroxyprogesterone, DHEA-S, and beta-human chorionic gonadotropin. If any of these screening tests suggest exclusionary results, they may be repeated to exclude laboratory error. Since adiposity could be a potential confounder in this study, measures of adiposity will be recorded. This will include measurement of waist circumference (using standard technique) to provide an estimate of abdominal adiposity. Hip circumference will also be measured. Additionally, BOD POD® will be used to measure total fat mass, fat free mass, and percent body fat.

Study Day 1: Baseline (Pre-Transdermal Estradiol) Assessment

- For control subjects with regular menstrual cycles, Day 1 of the study (Study Day 1) will occur on day 3 of the menstrual cycle. For PCOS subjects (who will have oligo-/amenorrhea), Study Day 1 will occur no earlier than menstrual cycle day 3.
- At 8:00 a.m. on Study Day 1, subjects will present to the CRU to obtain urine collection supplies (sufficient for Study Days 1 and 2) to take home, and to have a pregnancy test (urine or serum hCG) and blood drawn for measurement of LH, FSH, estradiol (E2), progesterone, testosterone, SHBG, inhibin A, inhibin B, and antimullerian hormone (AMH).
- NOTE:** For each study day in this protocol, we will obtain blood samples for measurement of LH, FSH, E2, progesterone, testosterone, SHBG, inhibin A, inhibin B, and AMH. To reduce variability in results, blood and urine samples will be held until the completion of each subject's study, and then assays will be performed in the CRR's Ligand Assay Core. These results from the Ligand Assay Core will be used for formal analyses. In addition, for each study day, we will also obtain blood samples for E2, LH, and progesterone to be assayed immediately in the UVA Clinical Laboratory (to ensure rapid turnaround). These particular tests will be used for (a) real-time monitoring of serum E2 levels (to ensure achievement of target levels), (b) real-time monitoring for an LH surge (so that the protocol can be stopped once an LH surge occurs), and (c) real-time monitoring for an early luteal phase (so that the protocol can be stopped if this occurs). Details of such surveillance are provided below.
- Subjects will use provided urine collection supplies to obtain complete urine collections in 12-hour blocks for a total of 48 hours. Subjects will be instructed to keep these urine collections refrigerated or in an iced cooler.
 - Note regarding urine collections:** Subjects will collect *all* urine produced in these 12-hour blocks. Urine will be stored for later assay of LH, FSH, and creatinine in the Center for Research in Reproduction's Ligand Assay Core. (All LH and FSH concentrations will be normalized to creatinine concentrations.)
- The pregnancy test will be done in the CRU (if done via urine testing) or in the UVAHS Clinical Laboratory (if done via blood testing). If the pregnancy test is positive and/or if the Study Day 1 serum progesterone is >1.5 ng/mL, the subject will be notified of this information and they will not proceed with the study at that time (but plans to begin at a later date will be discussed).

Study Day 2: Start Transdermal Estradiol

- At approximately 8:00 am on Study Day 2, subjects will present to the CRU to return the refrigerated Study Day 1 urine samples and to have blood drawn for LH, FSH, E2, progesterone, testosterone, SHBG, inhibin A, inhibin B, and AMH.
- NOTE:** As above, E2, LH, and progesterone will be assayed in both
 - (a) the UVA Clinical Laboratory (to ensure rapid turnaround) and (b) the CRR's Ligand Assay



Core. And on study day 2 only, we will also obtain blood to be sent for total testosterone by LC-MS-MS (send out to Mayo Clinic Laboratory).

- After blood collection, two transdermal estradiol patches (each patch delivering 0.1 mg/day for a total dose 0.2 mg/day) will be applied by CRU staff at 8:15-8:30 a.m. Throughout this protocol, transdermal estradiol patches will be preferentially placed on the abdomen.
 - NOTE: In our previous studies^{21,22}, transdermal estradiol doses of 0.2 mg/day produce mean serum E2 level approximating 100-120 pg/ml.*
- Subjects will be provided with urine collection supplies in a sufficient quantity to last through Study Day 9.
- Subjects will also be provided with 5 extra transdermal 0.1 mg/day estradiol patches to take home. These extra patches will be used for replacement purposes in the event of patch loss (e.g., patch[es] falling off). [NOTE: Extra transdermal patches will be subsequently provided as needed to maintain a reserve of at least 5 patches.]

Study Day 3: Monitor + Increase Transdermal Estradiol Dose to 0.3 mg/day

- At approximately 8:00 a.m. on Study Day 3, the subject will present to the CRU to return the refrigerated Study Day 2 urine samples and to have blood drawn for LH, FSH, E2, progesterone, testosterone, SHBG, inhibin A, inhibin B, and AMH. As above, E2, LH, and progesterone will be assayed in both (a) the CRR's Ligand Assay Core and (b) the UVA Clinical Laboratory.
- The presence and condition of the prior patches will be verified and recorded; the patches will then be removed.
- Three transdermal estradiol patches (each patch delivering 0.1 mg/day for a total dose 0.3 mg/day) will be applied by CRU staff.

Study Day 4: Monitor + Increase Transdermal Estradiol Dose to 0.4 mg/day

- At approximately 8:00 a.m. on Study Day 4, the subject will present to the CRU to return the refrigerated Study Day 3 urine samples and to have blood drawn for LH, FSH, E2, progesterone, testosterone, SHBG, inhibin A, inhibin B, and AMH.
- As above, E2, LH, and progesterone will be assayed in both (a) the CRR's Ligand Assay Core and (b) the UVA Clinical Laboratory. These particular tests will be run through the UVA Clinical Laboratory so that results will be available rapidly. These results will be used as follows:
 - We will perform ***real-time monitoring of serum E2 levels (i.e., real-time monitoring of transdermal estradiol dosing) to ensure achievement of appropriate target levels.*** That is, we want to ensure that serum E2 levels are high enough to trigger a gonadotropin surge (~250-300 pg/ml), but we wish to avoid prolonged exposure to excessive serum E2 levels (especially levels exceeding 400 pg/ml). Accordingly:
 - If a subject's serum E2 level was < 250 pg/ml on the preceding Study Day, the transdermal estradiol dose will be increased by 0.1 mg/day per protocol.
 - If a subject's serum E2 was 250-350 pg/ml on the preceding Study Day, that same transdermal estradiol dose will be maintained for the next 24 hours ^{footnote 1}. (NOTE:

¹ For example, if a subject's serum E2 level was 275 pg/ml on Study Day 4 — when the subject was receiving 0.3 mg/day transdermal estradiol — the transdermal estradiol dose would be maintained at 0.3 mg/day on Study Day 5.

Since a given day's serum E2 levels are unlikely to be available before the subject leaves the CRU, and since the protocol may dictate an increase in the transdermal estradiol dose during the CRU visit, continuation of the same dose could require that the subject pull a single estradiol patch off her skin shortly after the CRU visit.)

- If a subject's serum E2 level is > 350 pg/ml on a given dose of transdermal estradiol – which we think will be unlikely in this protocol – the transdermal estradiol dose will be decreased to the previous dose that had been associated with a serum E2 value approximating 250-350 pg/mL. If there is uncertainty regarding the appropriate dose reduction, we will proportionally reduce the dose according to measured serum E2 levels, aiming to achieve serum E2 levels of 250-350 pg/ml ^{footnote 2}.
- We will perform ***real-time monitoring for an LH surge*** (so that the protocol can be stopped once an LH surge occurs). If a subject demonstrates an LH surge, further monitoring will not be required. Therefore, if the subject's serum LH peaks to >5 -fold (500%) her Study Day 1 or 2 serum values and then subsequently falls to within 2-fold (200%) of the Study Day 1 or 2 serum values, the subject's study will be stopped on the following day (e.g., after the next morning's blood sample is drawn).
- We will perform ***real-time monitoring for entry into the luteal phase***. Specifically, a serum progesterone level of 2.5 ng/ml or higher would provide compelling evidence of an early luteal phase, which would strongly suggest previous gonadotropin surge generation and ovulation. Accordingly, if serum progesterone ever equals or exceeds 2.5 ng/ml, we will stop the protocol on the following day (e.g., after the next morning's blood sample is drawn). (We do not expect this to occur in this protocol.)
- The presence and condition of the prior patches will be verified and recorded; the patches will then be removed.
- New transdermal E2 patches will then be applied by CRU staff:
 - If the subject's serum E2 level was < 250 pg/ml on Study Day 3 (most likely scenario), then the transdermal estradiol dose will be increased to 0.4 mg/day.
 - If the subject's serum E2 was 250-350 pg/ml on Study Day 3 (possible but unlikely scenario), that same transdermal estradiol dose will be continued.
 - If the subject's serum E2 level was > 350 pg/ml on Study Day 3 (we believe that this will be a highly unlikely scenario), then the transdermal estradiol dose would have been reduced (see above), and the reduced dose will be continued.

Study Day 5 – Study Day 8: Monitor + Adjust Transdermal Estradiol Dose According to Serum E2 Levels

- At approximately 8:00 a.m. on Study Days 5 - 8, the subject will present to the CRU to:
 1. return the refrigerated prior Study Day's urine samples;

² For example, if serum E2 levels are 400 pg/ml on 0.5 mg/d transdermal estradiol, each 0.1 mg/d dose reduction would be expected to lower serum E2 levels by ~ 80 pg/ml (400 divided by 5), so a dose reduction to 0.4 mg/d would be expected to achieve an acceptable serum E2 level of ~ 320 pg/ml.

2. have blood drawn for LH, FSH, E2, progesterone, testosterone, SHBG, inhibin A, inhibin B, and AMH (with E2, LH, and progesterone also immediately assayed in the UVA Clinical Laboratory for real-time monitoring as described above);
3. have the presence and condition of the prior patches verified and recorded, and subsequently removed; and
4. have new transdermal estradiol patches applied by CRU staff:
 - If the subject's serum E2 level was < 250 pg/ml on the preceding Study Day, then the transdermal estradiol dose will be increased by 0.1 mg/day (maximum dose = 0.6 mg/day).
 - If the subject's serum E2 was 250-350 pg/ml on the preceding Study Day, that same transdermal estradiol dose will be continued.
 - If the subject's serum E2 level was > 350 pg/ml on the preceding Study Day, then the transdermal estradiol dose would have been reduced (see above), and the reduced dose will be continued.

Optional ovarian ultrasound on Study Day 6 or 7: To evaluate for a dominant ovarian follicle, subjects may elect to undergo transvaginal ovarian ultrasound in the CRU or in the Endocrinology Clinic (Fontaine Research Park) on study day 6 or 7. This ultrasound will be optional (i.e., subjects may opt out with no negative consequences). Dominant follicle size and endometrial stripe thickness will be recorded.

Study Day 9: Monitor + Discontinue Transdermal Estradiol

- At approximately 8:00 a.m. on Study Day 9, the subject will present to the CRU to:
 1. return the refrigerated prior Study Day's urine samples;
 2. have blood drawn for LH, FSH, E2, progesterone, testosterone, SHBG, inhibin A, inhibin B, and AMH;
 3. have the presence and condition of the prior patches verified and recorded, and subsequently removed.

Final Study Day (7-9 days after estradiol patch discontinuation)

- Subject will present to the CRU to have blood drawn for LH, FSH, E2, progesterone, testosterone, SHBG, inhibin A, inhibin B, and AMH.

Data and Safety Monitoring Plan

1. Definition:

1.1 How will you define adverse events (AE) for this study?

 X An adverse event will be considered any undesirable sign, symptom or medical or psychological condition **even if the event is not considered to be related** to the investigational drug/device/intervention. Medical condition/diseases present before starting the investigational drug/intervention will be considered adverse events only if they worsen after starting study treatment/intervention. An adverse event is also any undesirable and unintended effect of

research occurring in human subjects as a result of the collection of identifiable private information under the research. Adverse events also include any problems associated with the use of an investigational device that adversely affects the rights, safety or welfare of subjects.

Will use definitions provided in the non IRB Protocol (Sponsor's, Investigator-Initiated, CTEP etc.)

1.2 How will you define serious adverse events?

A serious adverse event will be considered any undesirable sign, symptom, or medical condition which is fatal, is life-threatening, requires or prolongs inpatient hospitalization, results in persistent or significant disability/incapacity, constitutes a congenital anomaly or birth defect, is medically significant and which the investigator regards as serious based on appropriate medical judgment. An important medical event is any AE that may not result in death, be life-threatening, or require hospitalization but may be considered an SAE when, based upon appropriate medical judgment, it may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in the definitions of SAEs.

1.3 What is the definition of an unanticipated problem?

An unanticipated problem is any event, experience that meets ALL 3 criteria below:

- Is unexpected in terms of nature, severity or frequency given the research procedures that are described in the protocol-related documents AND in the characteristics of the subject population being studied
- Related or possibly related to participation in research. This means that there is a reasonable possibility that the incident may have been caused by the procedures involved in the research study.
- The incident suggests that the research placed the subject or others at greater risk of harm than was previously known or recognized OR results in actual harm to the subject or others

1.4 What are the definitions of a protocol violation and/or noncompliance?

A **protocol violation** is defined as any change, deviation, or departure from the study design or procedures of research project that is NOT approved by the IRB-HSR prior to its initiation or implementation. Protocol violations may be major or minor violations.

Noncompliance can be a protocol violation OR deviation from standard operating procedures, Good Clinical Practices (GCPs), federal, state or local regulations. Noncompliance may be serious or continuing.

Additional Information: see the IRB-HSR website at

http://www.virginia.edu/vpr/irb/HSR_docs/Forms/Protocol_Violations_%20Enrollment_Exceptions_Instructions.doc

1.5 If pregnancy occurs how will this information be managed?

Adverse Event- will follow adverse event recording and reporting procedures outlined in section 3.

Unanticipated Problems- will follow Unanticipated Problem recording and reporting procedures outlined in section 3.

Note that pregnancy could not be deemed to be related/possibly related to this study, as none of the procedures increases likelihood of pregnancy, and subjects are specifically required to avoid pregnancy.

1.6 What is the definition of a Protocol Enrollment Exception?

NA- No outside sponsor

1.7 What is the definition of a data breach?

A data breach is defined in the HITECH Act (43 USC 17932) as an unauthorized acquisition, access, or use of protected health information (PHI) that compromises the security or privacy of such information.

Additional Information may be found on the IRB-HSR Website: [Data Breach](#)

2. Identified risks and plans to minimize risk

2.1 What risks are expected due to the intervention in this protocol?

Expected Risks related to study participation.	Frequency
Risk of withholding specific medications that would interfere with protocols	<p>Subjects with PCOS commonly are prescribed medications including hormonal contraceptives, spironolactone, and metformin for management of PCOS, hyperandrogenism, and insulin resistance. Women without PCOS commonly are prescribed hormonal contraceptives for purposes including pregnancy prevention.</p> <p>We believe that elective withholding of such medications for up to 4 months would pose minimal risk to subjects with PCOS.</p> <p>Subjects previously using oral contraceptives for the purpose of reducing risk of pregnancy would be at risk of an unplanned pregnancy if they remain sexually active without use of non-hormonal methods of contraception.</p>
Risks associated with venipuncture	
Discomfort	<p><input checked="" type="checkbox"/> Occurs frequently</p> <p><input type="checkbox"/> Occurs infrequently</p> <p><input type="checkbox"/> Occurs rarely</p> <p><input type="checkbox"/> Frequency unknown</p>
Bruising	<p><input checked="" type="checkbox"/> Occurs frequently</p> <p><input type="checkbox"/> Occurs infrequently</p> <p><input type="checkbox"/> Occurs rarely</p> <p><input type="checkbox"/> Frequency unknown</p>

Infection	<input type="checkbox"/> Occurs frequently <input type="checkbox"/> Occurs infrequently <input checked="" type="checkbox"/> Occurs rarely <input type="checkbox"/> Frequency unknown
Blood clot at the site of intravenous catheter insertion	<input type="checkbox"/> Occurs frequently <input type="checkbox"/> Occurs infrequently <input checked="" type="checkbox"/> Occurs rarely <input type="checkbox"/> Frequency unknown
Risks associated with transdermal estradiol use	
Deep venous thrombosis/Pulmonary embolism	<input type="checkbox"/> Occurs frequently <input type="checkbox"/> Occurs infrequently <input type="checkbox"/> Occurs rarely <input checked="" type="checkbox"/> Frequency unknown. This occurs very rarely with long-term estrogen use (for example, in oral contraceptive pills), so we would expect this to be exceedingly rare with short-term administration as in this protocol. In addition, transdermal estradiol use is associated with lower deep venous thrombosis risk compared to oral estrogen regimens (e.g., oral contraceptives), and we are aiming to achieve preovulatory (i.e., physiological) serum estradiol levels for a relatively short period of time. We will be excluding subjects with history of DVT, pulmonary embolism, or clotting disorders.
Myocardial infarction	<input type="checkbox"/> Occurs frequently <input type="checkbox"/> Occurs infrequently <input type="checkbox"/> Occurs rarely <input checked="" type="checkbox"/> Frequency unknown – This is thought to be extremely rare. The risk of this will be minimized by excluding any subjects with a history of coronary artery disease or myocardial infarction.
Stroke	<input type="checkbox"/> Occurs frequently <input type="checkbox"/> Occurs infrequently <input type="checkbox"/> Occurs rarely <input checked="" type="checkbox"/> Frequency unknown – This is thought to be extremely rare. The risk of this will be minimized by excluding any subjects with a history of stroke or migraine with aura.
Hypertension	<input type="checkbox"/> Occurs frequently <input type="checkbox"/> Occurs infrequently <input checked="" type="checkbox"/> Occurs rarely – Risk of this will be minimized by excluding any subjects with a history of hypertension or sustained blood pressure >140/90 on screening assessment.

	<input type="checkbox"/> Frequency unknown <input type="checkbox"/> Occurs frequently <input type="checkbox"/> Occurs infrequently <input checked="" type="checkbox"/> Occurs rarely – Risk of this will be minimized by excluding any subjects with a history of hypertriglyceridemia. Also, the short-term risks of hypertriglyceridemia are minimal.
Hypertriglyceridemia	<input type="checkbox"/> Frequency unknown <input type="checkbox"/> Occurs frequently <input type="checkbox"/> Occurs infrequently <input checked="" type="checkbox"/> Occurs rarely – Risk of this will be minimized by excluding any subjects with a history of breast, ovarian, or endometrial cancers. Also, no short-term risks are expected in this regard.
Stimulation of growth of breast, ovarian, or endometrial cancers	<input type="checkbox"/> Frequency unknown <input type="checkbox"/> Occurs frequently <input type="checkbox"/> Occurs infrequently <input checked="" type="checkbox"/> Occurs rarely – Risk of this will be minimized by excluding any subjects with a history of breast, ovarian, or endometrial cancers. Also, no short-term risks are expected in this regard.
Rash or skin irritation at site of patch application	<input type="checkbox"/> Frequency unknown <input type="checkbox"/> Occurs frequently <input checked="" type="checkbox"/> Occurs infrequently – 15-20% of subjects. Risk will be minimized by daily rotation of application sites. <input type="checkbox"/> Occurs rarely <input type="checkbox"/> Frequency unknown
Mild nausea	<input type="checkbox"/> Frequency unknown <input type="checkbox"/> Occurs frequently <input checked="" type="checkbox"/> Occurs infrequently <input type="checkbox"/> Occurs rarely
Swelling or breast tenderness	<input type="checkbox"/> Frequency unknown <input type="checkbox"/> Occurs frequently <input checked="" type="checkbox"/> Occurs infrequently <input type="checkbox"/> Occurs rarely
Temporary hot flashes after discontinuation	<input type="checkbox"/> Frequency unknown <input type="checkbox"/> Occurs frequently <input checked="" type="checkbox"/> Occurs infrequently <input type="checkbox"/> Occurs rarely
Risks associated with ferrous gluconate supplementation	
Constipation	<input type="checkbox"/> Frequency unknown <input checked="" type="checkbox"/> Occurs frequently <input type="checkbox"/> Occurs infrequently <input type="checkbox"/> Occurs rarely
Darkening of stools	<input checked="" type="checkbox"/> Occurs frequently <input type="checkbox"/> Occurs infrequently <input type="checkbox"/> Occurs rarely <input type="checkbox"/> Frequency unknown
Nausea	<input type="checkbox"/> Occurs frequently

	<input checked="" type="checkbox"/> Occurs infrequently <input type="checkbox"/> Occurs rarely <input type="checkbox"/> Frequency unknown
Reproductive Risks	Minimized due to the requirements of this protocol.
Violation of subject's privacy and confidentiality	Minimized due to the requirements of the privacy plan in this protocol

2.2 List by bullet format a summary of safety tests/procedures/observations to be performed that will minimize risks to participants:

- Risk of venipuncture will be reduced by use of sterile technique.
- Risks of transdermal estradiol will be reduced by screening for and exclusion of any subjects who may be at increased risk of developing complications while using E2, including: those with history of hypertension (persistent, appropriately-measured blood pressure $>140/90$ mmHg), severe hypertriglyceridemia (fasting triglycerides >500 mg/dL), venous thromboembolism (deep venous thrombosis, pulmonary embolism), proven or suspected hypercoagulability, arterial thrombosis, stroke, coronary artery disease, myocardial infarction, migraine with aura, and breast, ovarian, or endometrial cancer. Additionally, if a history of allergy or intolerance to estradiol is reported, the subject will be excluded from the study. Also, daily serum E2 levels will be monitored in order to detect and correct excessively high estradiol exposure (described in detail above).
- Risk of skin irritation from use of estradiol patch will be reduced by rotation of sites of E2 patches to avoid skin irritation and/or rash.
- Risk of fetal exposure to the study drug will be reduced by having a pregnancy test assessed prior to initiation of transdermal estradiol. If the volunteer is pregnant, the study will be discontinued.
- Risk from iron supplementation (when used) will be reduced by instructing subjects to take their iron tablets with food and a full glass of water. They will be told to include whole grains and fruits and vegetables in their diet for fiber. Also, iron supplementation will not be offered to subjects with a history of any disorders that may potentially be complicated by long-term iron supplementation, such as hemochromatosis and polycythemia vera.
- We will exclude subjects with abnormal screening labs with confirmatory testing showing: severe thrombocytopenia (platelets $<50,000$ cells/microL), leukopenia (total white blood count $<4,000$ cells/microL), persistent liver laboratory study abnormalities (with these exceptions: Mild bilirubin elevations will be accepted in the setting of known Gilbert's syndrome; mild transaminase elevations may be seen in PCOS, therefore elevations < 1.5 times the upper limit of normal will be accepted in this group), Decreased renal function evidenced by GFR <60 mL/min/1.73m².
- We will exclude subjects who report on screening history a significant history of cardiac or pulmonary dysfunction (e.g., known or suspected congestive heart failure; asthma requiring intermittent systemic corticosteroid; etc.).

2.3 Under what criteria would an INDIVIDUAL SUBJECT'S study treatment or study participation be stopped or modified

At subject, PI or sponsor's request

X Treatment would be stopped if the subject had a serious adverse event deemed related to study

Refer to the non- IRB Protocol (Sponsor's, Investigator-Initiated, CTEP protocol etc.)

2.4 Under what criteria would THE ENTIRE STUDY need to be stopped.

X Per IRB, PI, DSMB, or sponsor discretion

2.5 What are the criteria for breaking the blind/mask?

X NA – Not blinded/masked

2.6 How will subject withdrawals/dropouts be reported to the IRB prior to study completion?

X IRB-HSR continuation status form

3. Adverse Event / Unanticipated Problem Recording and Reporting

3.1 Will all adverse events, as defined in section 1.1, be collected/recorded? No.

► IF NO, what criteria will be used?

X Only adverse events deemed related/possibly related to study

3.2 How will adverse event data be collected/recorded?

X Paper AE forms/source documents

X Spreadsheet: paper or electronic

Database

3.3. How will AEs be classified/graded?

X Mild/Moderate/Severe

X Serious/Not serious Required for all protocols

3.4 What scale will the PI use when evaluating the relatedness of adverse events to the study participation?

X The PI will determine the relationship of adverse events to the study using the following scale:

Related: AE is clearly related to the intervention

Possibly related: AE may be related to the intervention

Unrelated: AE is clearly not related to intervention

3.5 When will recording/reporting of adverse events/unanticipated problems begin?

X After subject begins study drug/ device placement/intervention /study-related procedure/specimen collection

3.6 When will the recording/reporting of adverse events/unanticipated problems end?

Subject completes intervention and follow up period of protocol

3.7 How will Adverse Events, Unanticipated Problems, Protocol Violations and Data Breaches be reported? Complete the table below to answer this question

Type of Event	To whom will it be reported:	Time Frame for Reporting	How reported?
Any internal event resulting in death that is deemed DEFINITELY related to (caused by) study participation <i>An internal event is one that occurs in a subject enrolled in a UVa protocol</i>	IRB-HSR	Within 24 hours	IRB Online and phone call www.irb.virginia.edu/
Internal, Serious, related/possibly related to study, Unexpected adverse event	IRB-HSR	Within 7 calendar days from the time the study team received knowledge of the event. <i>Timeline includes submission of signed hardcopy of AE form.</i>	IRB Online www.irb.virginia.edu/
Unanticipated Problems that are not adverse events or protocol violations This would include a Data Breach.	IRB-HSR	Within 7 calendar days from the time the study team received knowledge of the event.	Unanticipated Problem report form. http://www.virginia.edu/vprgs/irb/HSR_docs/Forms/Reporting_Requirements-Unanticipated_Problems.doc
Protocol Violations/Noncompliance <i>The IRB-HSR only requires that MAJOR violation be reported, unless otherwise required by your sponsor, if applicable.</i> OR Enrollment Exceptions <i>See definition- only allowed if there is a commercial sponsor or a DSMB that has granted the enrollment exception.</i>	IRB-HSR	Within 7 calendar days from the time the study team received knowledge of the event.	Protocol Violation, Noncompliance and Enrollment Exception Reporting Form http://www.virginia.edu/vprgs/irb/hsr_forms.html <i>Go to 3rd bullet from the bottom.</i>

Data Breach	The UVa Corporate Compliance and Privacy Office ITC: if breach involves electronic data Police if breach includes items that are stolen: Stolen on UVA Grounds OR Stolen off UVa Grounds- contact police department of jurisdiction of last known location of PHI	As soon as possible and no later than 24 hours from the time the incident is identified. As soon as possible and no later than 24 hours from the time the incident is identified. IMMEDIATELY.	UVa Corporate Compliance and Privacy Office- Phone 924-9741 ITC: Information Security Incident Reporting procedure, http://www.itc.virginia.edu/security/reporting.html UVa Police-Phone- (434) 924-7166
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4. How will the endpoint data be collected/recorded.

Protocol specific case report forms
 Source documents
 Database: On a secure UVA server.

5. Data and Safety Oversight Responsibility

5.1. Who is responsible for overseeing safety data for this study?

DSMB/ DSMC

5.2. What is the composition of the reviewing body and how is it affiliated with the sponsor?

Other- The DSMB is composed of an endocrinologist sub-specializing in reproductive endocrinology, a pediatric endocrinologist, and a Ph.D. statistician. The board meets at least annually and as needed in the case of any severe AE that may be related to or is related to a study.

5.3. What items will be included in the aggregate review conducted by the PI?

NA- PI is not the overall person overseeing the safety data for this study.

5.4 How often will aggregate review occur?

For additional information on aggregate review

see: www.virginia.edu/vpr/irb/hsr/continuations.html#aggreview

Annually

5.5. How often will a report, regarding the outcome of the review by the DSMB/DSMC, be sent to the UVa PI?

Annually

5.6. How will a report of the information discussed in question 5.4 OR 5.5 be submitted to the IRB?

Part of IRB-HSR continuation status form

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APPENDIX: Drug Information

1. What is the drug name, manufacturer and IND# if available?

Estradiol (Vivelle), transdermal patch, Novartis.

2. If IND application has been submitted to the FDA, who is the Principal Investigator on the IND?

N/A

3. What is the phase or stage of this study?

N/A

APPENDIX: Pharmacy-Investigational Drugs/Biologics

1. What is the name of the investigational drug/biologic?

Estradiol, transdermal patch

2. Where will the subjects be seen for the administration/dispensing of the drug?

Outpatient Unit: Subjects will have the transdermal patch applied in the Clinical Research Unit each morning of the study.

3. What dose will be utilized in this study?

Subjects will receive graded transdermal E2 dosing (e.g., 0.2 mg/d x 1 d, then 0.3 mg/d x 1 d, then 0.4 mg/d x 1 d, then 0.5 mg/d). In addition, we will perform real-time monitoring of serum E2 levels (i.e., real-time monitoring of transdermal estradiol dosing) to ensure achievement of appropriate target levels. That is, we want to ensure that serum E2 levels are high enough to trigger a gonadotropin surge (~250-350 pg/ml), but we wish to avoid prolonged exposure to excessive serum E2 levels (> 400 pg/ml). Accordingly:

- If a subject's serum E2 level was < 250 pg/ml on the preceding Study Day, the transdermal estradiol dose will be increased by 0.1 mg/day per protocol (maximum dose = 0.6 mg/day).

- If a subject's serum E2 was 250-350 pg/ml on the preceding Study Day, that same transdermal estradiol dose will be maintained for the next 24 hours.
- If a subject's serum E2 level is > 350 pg/ml on a given dose of transdermal estradiol – which we think will be unlikely in this protocol – the transdermal estradiol dose will be decreased to the previous dose that had been associated with a serum E2 value approximating 250-350 pg/mL. If there is uncertainty regarding the appropriate dose reduction, we will proportionally reduce the dose according to measured serum E2 levels, aiming to achieve serum E2 levels of 250-350 pg/ml.

Through the above dose-adjustment protocol, we will avoid substantial subject exposure to supraphysiologic serum E2 levels (i.e., serum E2 levels > 400 pg/ml).

4. What will be the frequency of dosing in this study?

Patch application will occur daily through the duration of the study.

5. What will be the duration of dosing in this study?

The maximum duration of dosing would be from the morning of Study Day 2 through the morning of Study Day 9 (i.e., 7 days).

6. What route of administration will be utilized?

Transdermal patches will be rotated (daily) on locations on the abdominal wall.

7. Will drug need to be prepared by the UVa Investigational Drug Service (IDS)?

X YES

NO- Drug will be prepared and/or administered per package insert

► IF YES, complete the following information under 7a-7d.

If you need assistance completing this section contact the Investigational Pharmacists at 982-1048

7a. Concentration

X Standard

Non- Standard- Specify Answer/Response:

7b. Diluents

X Standard

Non- Standard- Specify Answer/Response:

7c. Stability after prepared

X Standard

Non- Standard- Specify Answer/Response:

7d. Special storage requirements

X Standard

Non- Standard- Specify Answer/Response:

8. Are there any special handling instructions mandated by the study (e.g. weighing hazardous materials)?

No.

9. Does the protocol provide provisions for dose titration, dose reductions, and or re-challenged (if drug is stopped), etc.? No.

10. How will missed doses be handled?

All interruptions of transdermal E2 dosing will be documented in the subject's study record. If transdermal E2 dosing is interrupted for more than 6 consecutive hours, then the subject's involvement in the study will be stopped.

11. Will a comparator (active or placebo) be utilized in the protocol? No.

12. Does this study involve research on a drug, biologic, supplement or food additive? No.

13 Are you using a drug/supplement/ food additive in a manner not approved by the FDA? Yes.

13a. Describe pertinent animal data that is available regarding the toxicity/safety of this drug.

Since human data regarding estradiol safety are abundant, animal data regarding estradiol safety is not felt to be relevant.

13b. Describe pertinent human data that is available regarding the toxicity/safety of this drug.

There are no data regarding extremely short-term use of transdermal estradiol. However, with the exception of local skin reactions, any adverse reactions are anticipated to be exceedingly rare. This is related to (a) the very short-term use of estradiol in this study; (b) the fact that *physiological* estradiol concentrations will be achieved in this study; and (c) the fact that only healthy young subjects are included in this study. The primary clinical use of unopposed estrogen (often in the form of combined equine estrogens, but not uncommonly in the form of estradiol) is long-term (months to years) use in the setting of menopausal symptoms. The largest study of long-term unopposed estrogen (combined equine estrogens taken orally) was the Women's Health Initiative (WHI) in healthy postmenopausal women ages 50 to 79. The WHI unopposed estrogen versus placebo trial in nearly 11,000 women showed no difference in coronary heart disease outcomes over an average follow-up of 6.8 years (note: there was a suggestion—but not a statistically significant one—of a protective effect in the younger women [ages 50 to 59]). This trial showed a slightly increased risk of stroke (HR 1.39, HR 1.1 to 1.77), an absolute excess risk of 12 additional strokes per 10,000 person-years. The WHI also showed an increased rate of deep venous thromboembolism. However, transdermal estrogens, which have little effect on hemostasis, may be associated with a lower VTE risk than oral estrogens. No increased breast cancer risk was observed. An increased risk of biliary tract disease (e.g., cholecystitis) was observed. *Overall, the baseline risk of coronary artery disease, stroke, venous thromboembolism, and biliary tract disease is very low in healthy women aged 18-30 y. Thus, the attributable risk of unopposed estrogen use in such a population would remain exceedingly low.* Reported adverse reactions of estradiol may include the following (NOTE: some are observed with estrogen/progestin combination therapy; and the frequency of these reactions are not clearly defined, but are rare): DVT, edema, hypertension, MI, stroke, venous

thromboembolism, anxiety, dementia, dizziness, epilepsy exacerbation, headache, irritability, mental depression, migraine, mood disturbances, nervousness, angioedema, chloasma, erythema multiforme, erythema nodosum, hemorrhagic eruption, hirsutism, loss of scalp hair, melasma, rash, pruritus, urticaria, breast cancer, breast enlargement, breast pain, breast tenderness, fibrocystic breast changes, galactorrhea, glucose intolerance, hypocalcemia, libido changes, nipple pain, serum triglycerides/phospholipids increased, vaginal discharge, vaginitis, abdominal cramps, abdominal distension, abdominal pain, bloating, cholecystitis, cholelithiasis, diarrhea, flatulence, gallbladder disease, nausea, pancreatitis, vomiting, weight gain/loss, alterations in frequency and flow of menses, cervical secretion changes, dysmenorrhea, endometrial cancer, endometrial hyperplasia, metrorrhagia, ovarian cancer, Pap smear suspicious, urinary tract infection, uterine leiomyomata size increased, uterine pain, vaginal candidiasis, aggravation of porphyria, cholestatic jaundice, hepatic hemangioma enlargement, thrombophlebitis, burning, erythema, irritation, arthralgia, back pain, chorea, leg cramps, muscle cramps, contact lens intolerance, corneal curvature steepening, retinal vascular thrombosis, asthma exacerbation, pulmonary thromboembolism, anaphylactoid/anaphylactic reactions, liver function tests increased, leg pain.

13c. Have there been any human deaths associated with this drug?

We are unaware of any human deaths directly attributable to short-term transdermal estradiol use.

13d. In how many humans has this drug been used previously?

It is unknown exactly how many women have received transdermal estradiol, but the numbers must be substantial, since transdermal estradiol is commonly used for the clinical care of patients with menopause and estrogen deficiency.

13e. If this protocol will be used in children describe any previous use of this drug with children of a similar age range.

N/A

14. Do the following criteria apply?

_____ The investigation is intended to be reported to FDA as a well-controlled study in support of a new indication for use or intended to be used to support any other significant change in the labeling for the drug;

_____ If the drug that is undergoing investigation is lawfully marketed as a prescription drug product, the investigation is intended to support a significant change in the advertising for the product;

_____ The investigation does involve a route of administration or dosage level or use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product.

If Not checked- explain why you believe the risk to subjects is not increased:

An FDA-approved transdermal estradiol preparation will be used in this study with the intention of reproducing the physiologic serum estradiol levels seen in the late follicular (pre-ovulatory) phase of the menstrual cycle. That is, we are using estradiol as a physiological probe.

In the normal menstrual cycle, the late follicular estradiol levels are the **peak** estradiol levels within the cycle. Peak pre-ovulatory estradiol levels usually exceed 200 pg/mL, but commonly approach (and sometimes exceed) 400 pg/mL (Welt CK, McNicholl DJ, Taylor AE, Hall JE. Female reproductive aging is marked by decreased secretion of dimeric inhibin. *J Clin Endocrinol Metab* 1999; 84:105.) In general, preovulatory estradiol levels remain elevated > 200 pg/mL for 3-4 days, and the high estradiol levels provoke (by a positive feedback action) the preovulatory gonadotropin surge. In our study, we aim to achieve serum estradiol levels greater than 250 pg/mL, but less than 350 pg/mL, for 3-4 days to provoke a gonadotropin surge. That is, we aim to reproduce estradiol levels that normal women experience during each menstrual cycle.

In clinical practice, when transdermal estradiol is used for full hormone replacement therapy (for example, in young women who have undergone menopause prematurely), a typical transdermal estradiol dose would be 0.1-0.2 mg/day. In these clinical scenarios, the goal is to achieve serum estradiol levels of about 70-100 pg/mL, because this value approximates that average estradiol level observed *across the cycle* for a menstruating female. (Note that lower doses [e.g., 0.05 mg/day] may be sufficient when the primary goal is to mitigate menopausal symptoms such as hot flashes.)

Because this study's intention is to produce a gonadotropin surge, which is contingent upon achieving physiologic peak estradiol levels (e.g., 250-350 pg/mL), we must use higher doses than are used therapeutically for simple hormone replacement. To our knowledge, the safety profile of short-term use of estradiol \geq 0.4 mg/day transdermally has not been defined; but based on an understanding of normal physiology and the benefits of transdermal delivery (compared to oral delivery), we have no reason to expect excess risk related to these estradiol doses as compared to what a cycling subject would routinely experience during the late follicular (pre-ovulatory) phase of the cycle. To reduce the risk of excessively high (i.e., non-physiological) estradiol exposure, we will monitor estradiol levels by assessing serum estradiol concentrations daily (through the UVA Clinical Laboratory for more rapid result turnaround). If a subject's serum E2 level is at the target level (250-350 pg/ml), the transdermal estradiol dose will not be increased further; and if a subject's serum E2 level is above our target level (i.e., > 350 pg/ml) on a given dose of transdermal estradiol, the transdermal estradiol dose will be decreased (as described in detail above).

Importantly, many of the adverse events related to exogenous estrogen use (e.g., venous thromboembolism) appear to be largely if not exclusively related to (a) oral administration with absorption/entry into the hepatic portal circulation (increased hepatic exposure can alter liver production of various products [e.g., products influencing coagulability]) and/or (b) long-term use of supraphysiological doses (e.g., doses required to suppress follicular development and ovulation). Transdermal estradiol is chemically and biologically identical to the native (ovarian) estradiol, which allows accurate assessment of delivered doses. Moreover, transdermal estradiol delivers estradiol into

the systemic circulation (mimicking ovarian estradiol delivery in this regard), and it effectively avoids first-pass liver effects.

In summary, our use of estradiol in this protocol uses the natural hormone via a near-physiological route, with the amount delivered producing normal concentrations for the late follicular (preovulatory) phase of the menstrual cycle. For these reasons, we do not expect excess risk related to the proposed estradiol doses as compared to what a cycling subject would routinely experience during the late follicular (pre-ovulatory) phase of the cycle.

X The investigation will be conducted in compliance with the requirements for institutional review set part in part 21CFR56 and with the requirements for informed consent set forth in part 21CFR50 ; and This item must be checked.

X The investigation will be conducted in compliance with the requirements of 21CFR312.7 (Promotion and charging for investigational drugs)
This item must be checked.

15. Is this a post-marketing study? No.