IRB-HSR PROTOCOL

Investigator Agreement

BY SIGNING THIS DOCUMENT, THE INVESTIGATOR CONFIRMS:

1. I am not currently debarred by the US FDA from involvement in clinical research studies.
2. I am not involved in any regulatory or misconduct litigation or investigation by the FDA.
3. That if this study involves any funding or resources from an outside source, or if you will be sharing data outside of UVA prior to publication that you will contact the Dean's office regarding the need for a contract and letter of indemnification. If it is determined that either a contract or letter of indemnification is needed, subjects cannot be enrolled until these documents are complete.
4. The proposed research project will be conducted by me or under my close supervision. It will be conducted in accordance with the protocol submitted to and approved by the IRB including any modifications, amendments or addendums submitted and approved by the IRB throughout the life of the protocol.
5. That no personnel will be allowed to work on this protocol until they have completed the IRB-HSR On-line training and the IRB-HSR has been notified.
6. That all personnel working on this protocol will follow all IRB-HSR Policies and Procedures as stated on the IRB-HSR Website http://www.virginia.edu/vprgs/irb/ and on the School of Medicine Clinical Trials Office Website: http://knowledgelink.healthsystem.virginia.edu/intranet/hes/cto/sops/sop_index.cfm
7. I will ensure that all those delegated tasks relating to this study, whether explicitly or implicitly, are capable through expertise, training, and experience or credentialing to undertake those tasks.
8. I confirm that the implications of the study have been discussed with all Departments that might be affected by it and have obtained their agreement for the study to take place.
9. That no subjects will be recruited or entered under the protocol until the Investigator has received the signed IRB-HSR Approval form stating the protocol is open to enrollment
10. That any materials used to recruit subjects will be approved by the IRB-HSR prior to use.
11. That all subjects will sign a copy of the most current consent form that has a non-expired IRB-HSR approval stamp.
12. That any modifications of the protocol or consent form will not be initiated without prior written approval from the IRB-HSR, except when necessary to eliminate immediate hazards to the subjects.
13. Any significant findings that become known in the course of the research that might affect the willingness of subjects to enroll or to continue to take part, will be promptly reported to the IRB.
14. I will report immediately to the IRB any unanticipated problems involving risk to subjects or to others including adverse reactions to biologics, drugs or medical devices.
15. That any serious deviation from the protocol will be reported promptly to the Board in writing.

16. That any data breach will be reported to the IRB, the UVa Corporate Compliance and Privacy Office , UVa Police as applicable.
17. That the continuation status report for this protocol will be completed and returned within the time limit stated on the form.
18. That the IRB-HSR office will be notified within 30 days of a change in the Principal Investigator or of the closure of this study.
19. That a new PI will be assigned if the current PI will not be at UVA for an extended period of time. If the current PI is leaving UVa permanently, a new PI will be assigned PRIOR to the departure of the current PI.
20. All study team members will have access to the current protocol and other applicable documents such as the IRB-HSR Application, consent forms and Investigator Brochures.
21. Signed consent forms and other research records will be retained in a confidential manner. Records will be kept at least 6 years after completion of the study.
22. No data/specimens may be taken from UVa without a signed Material Transfer Agreement between OSP/SOM Grants and Contracts Office and the new institution. Original study files are considered institutional records and may not be transferred to another institution. I will notify my department administration regarding where the originals will be kept at UVa. The material transfer agreement will delineate what copies of data, health information and/or specimens may be taken outside of UVa. It will also approve which HIPAA identifiers may be taken outside of UVa with the health information or specimens.
23. If any member of study team leaves UVa, they are STRONGLY ENCOURAGED to use Exit Checklist found on IRB-HSR website at http://www.virginia.edu/provost/facultyexit.pdf.

The IRB reserves the right to terminate this study at any time if, in its opinion, (1) the risks of further experimentation are prohibitive, or (2) the above agreement is breached.

Investigators Experience

Dr. McCartney (Principal Investigator) has 18 years of experience performing clinical research in the field of reproductive neuroendocrinology. He has extensive experience with frequent sampling protocols, procedures of the Clinical Research Unit, and LH pulse analysis—both in adolescent girls and adult women.

Signatures

Principal Investigator

_____	_____	_____
Principal Investigator Signature	Principal Investigator Name Printed	Date

The Principal Investigator signature is ONLY required if this is a new protocol, a 5 year update or a modification changing the Principal Investigator.

Department Chair

BY SIGNING THIS DOCUMENT THE DEPARTMENT CHAIR AGREES:

1. To work with the investigator and with the board as needed, to maintain compliance with this agreement.
2. That the Principal Investigator is qualified to perform this study.
3. That the protocol is scientifically relevant and sound.

_____	_____	_____
Department Chair or Designee Signature	Department Chair or Designee Name Printed	Date

The person signing as the Department Chair cannot be the Principal Investigator or a sub-investigator on this protocol.
The Department Chair or Designee signature is ONLY required if this is a new protocol or a modification changing the Principal Investigator.

Brief Summary/Abstract

The rapidity with which progesterone suppresses daytime LH (and by inference GnRH) pulse frequency is unknown. We propose to assess this further using a randomized, cross-over, placebo-controlled study. Normally-cycling women and women with PCOS (both pretreated with estradiol) will undergo a 24-h sampling study. After 10 h of sampling, either oral micronized progesterone (100 mg p.o.) suspension or placebo suspension will be administered (according to randomization). During a subsequent menstrual cycle, subjects will undergo another study identical to the first (including pretreatment with estradiol) except that oral progesterone will be exchanged for placebo or *vice versa* in accordance with the crossover design. We will assess the acute effects of progesterone on LH frequency, with secondary endpoints being mean LH, LH pulse amplitude, and mean FSH. We hypothesize that (1) administration of progesterone (at 0600 h) to adult women during the follicular phase will result in a demonstrable suppression of daytime LH (and by inference GnRH) pulse frequency within 12 hours; and (2) administration of progesterone (at 0600 h) to women with PCOS (during the follicular phase) will result in less suppression of daytime LH pulse frequency than in controls.

Background

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

It remains unknown how rapidly progesterone (P) affects gonadotropin releasing hormone (GnRH) pulse frequency slowing in normal women or in women with PCOS. Through this study, we are pursuing this question to confirm an important assumption in our overall hypothesis regarding how cyclic ovulation is established during puberty.

GnRH stimulates LH and FSH synthesis and secretion from the same gonadotrope cell; nonetheless, LH and FSH levels change differentially throughout ovulatory cycles, with FSH predominance in the early follicular phase and LH predominance in the late follicular phase (1-7). These variations in gonadotropin secretion result in part from different patterns of pulsatile GnRH stimulation, with fast GnRH pulse frequencies favoring LH and slow GnRH pulse frequencies favoring FSH synthesis and secretion (8-12).

In ovulatory women, LH (and by inference GnRH) pulse frequency slows from one pulse every 60-90 min in the follicular phase to approximately one pulse every 4-6 h during the luteal phase. Luteal slowing of GnRH pulse frequency increases FSH synthesis, but high luteal levels of estradiol (E2) and inhibin prevent FSH release from the gonadotrope (13, 14); when E2 and inhibin concentrations fall during the luteal-follicular transition (i.e., when inhibition of FSH secretion is removed), an increasing GnRH pulse frequency leads to the early follicular monotropic rise of FSH. Thus, the ability to slow GnRH pulse frequency during the luteal phase appears to be important for the early follicular rise in plasma FSH and, therefore, follicular development.

Progesterone appears to be the primary effector of GnRH pulse frequency slowing. GnRH pulse frequency slows coincident with P increases in the luteal phase, and administration of P to women during the follicular phase also slows GnRH pulse frequency (15), although the rapidity with which this occurs is unknown. Progesterone's ability to slow GnRH pulse frequency appears to require the permissive presence of E2 (16, 17), possibly reflecting E2's ability to induce hypothalamic P receptors (18-21).

Changes of GnRH pulse frequency are also seen during puberty. During childhood, the hypothalamic-pituitary-gonadal axis is quiescent with low plasma LH and FSH levels, low LH pulse amplitude, and slow LH (GnRH) pulse frequency (i.e., detectable pulses approximately every 3-6 h), and high FSH:LH ratio (22-26). Reactivation of this axis at puberty is heralded by sleep-entrained

amplification of LH (GnRH) pulsatility, with nocturnal increases in both LH pulse amplitude and frequency (23, 24, 27-34). The cause(s) of these diurnal changes in GnRH pulsatility is(are) unclear, but diurnal changes of sex-steroids, which also occur during this time, likely play a major role. Overnight increases of testosterone (T) (35-38) and E2 (25, 39, 40) occur in pubertal boys and girls, respectively. E2 infusion in peripubertal girls (41), in addition to infusions of either T (42, 43) or E2 (33) in peripubertal boys, mitigates nocturnal increases in LH pulsatility. Moreover, in contrast to diurnal changes of LH and E2 in normal pubertal girls, age-matched girls with gonadal dysgenesis exhibit elevated LH pulse frequencies at all time periods (34, 44). Thus, diurnal changes in sex-steroids appear to direct diurnal changes in GnRH pulse frequency.

Since P is the primary effector of GnRH pulse frequency slowing in adult women, and with data that suggests that P increases 2.3-fold overnight in early pubertal girls (45), *we believe that P may be the major effector of diurnal GnRH pulse frequency slowing in peripubertal girls*.

It is unclear what functional significance underlies these diurnal variations in GnRH pulsatility during puberty; however, by analogy to adult women, we suggest that diurnal slowing during puberty may be important for enhancement of FSH synthesis and corresponding follicular development.

Original hypothesis regarding the development of ovulatory cycles in peripubertal girls: We initially proposed that in normal pubertal adolescent girls, sleep-entrained increases of LH pulsatility drive production of sex steroids (P and E2), which in turn suppress pulsatile GnRH (LH) secretion during the subsequent day. (Progesterone production may also be of adrenal origin under diurnal ACTH control.) These diurnal decreases in GnRH pulse frequency enhance FSH production and are therefore important for follicular development. Increasing FSH production results in escalating waves of follicular development and corresponding increases in E2 production. Estradiol concentrations eventually become adequate to trigger an LH surge, and the (ovulatory) follicular remnant is transformed into a corpus luteum that secretes great quantities of P and E2; thus occurs the first luteal phase. Later anovulatory cycles are associated with small increases in P, which appear to be adequate to reduce GnRH pulse frequency in normal women (46).

These processes have major implications for normal pubertal development and for aberrations of this process that appear to occur in adolescents destined to develop PCOS. Hyperandrogenism during adolescence, felt to represent a forerunner of PCOS, is associated with disordered LH secretion. Results from our group (IRB-HSR# 8588, JCM010) suggest that the GnRH pulse generator is relatively resistant to the feedback actions of P and E2 in some hyperandrogenemic adolescents (47), similar to that described in women with PCOS (48, 49). If this is indeed the case, then the aforementioned sequence of events at puberty would be quite different in hyperandrogenemic adolescents. As puberty begins, nocturnal increases of LH (and possibly ACTH) pulsatility would drive production of P and E2. However, these relatively low sex steroid concentrations would be insufficient to overcome GnRH pulse generator resistance, and a relatively rapid GnRH pulse frequency would persist throughout the following day. Persistently rapid GnRH pulses would be expected to result in both LH excess, which would stimulate excessive androgen production, and relative FSH deficiency, which would hinder normal follicular development. These hypotheses are consistent with available data.

The rapidity (over hours to days) with which P slows LH pulse frequency in normal women remains unknown. This is a critical point: for the above hypothesis regarding the control of diurnal changes in GnRH pulsatility during puberty to be viable, these sex steroids would have acute actions, effecting GnRH pulse frequency changes over hours rather than days. An earlier study by this group (48) chronicled the time course of LH pulse frequency suppression in response to P and E2 administration during the follicular phase in normal women. Maximal suppression (to approximately 80% of baseline) occurred on day 10 ($P < 0.05$ *vs*. day 1). Although LH pulse frequency decreased by a mean of 50% by

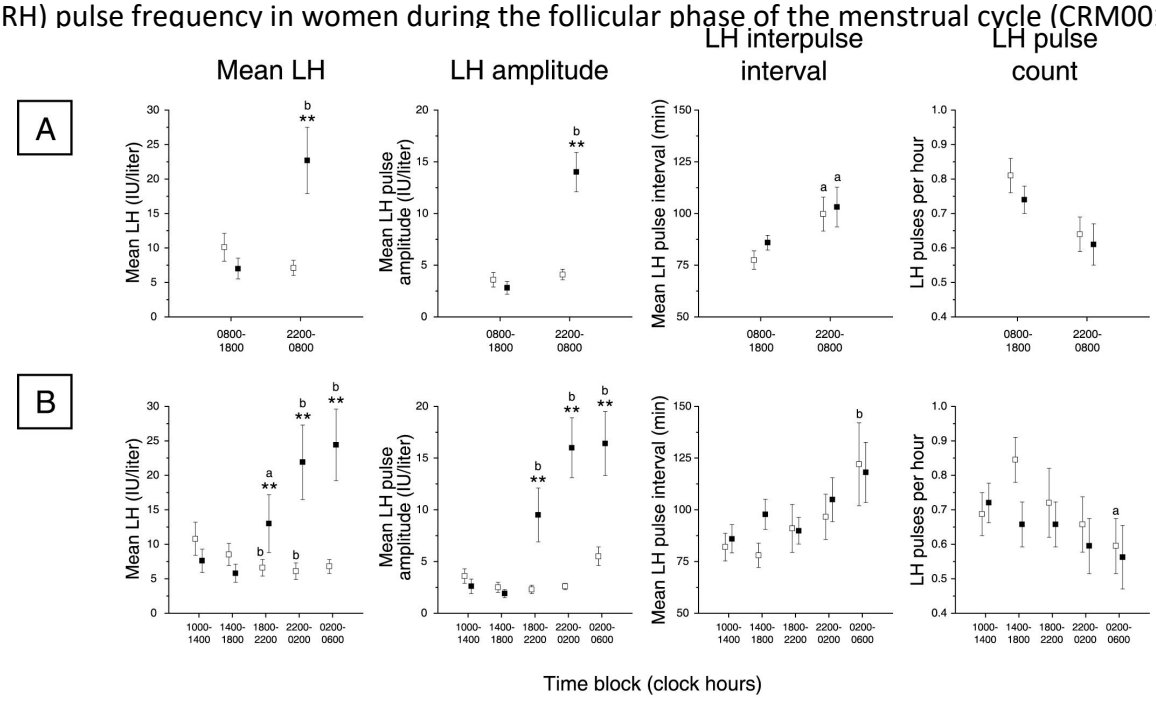

Figure 1. LH pulse characteristics over 10-hour (A) and 4-hour (B) time blocks. Data points for the placebo and progesterone admissions are shown as open and closed squares, respectively. P and PBO are given at 1800 h. ** = P < 0.01 change after P vs. change after PBO; a = P < 0.05 vs. baseline; b = P < 0.01 vs. baseline.

day 5, this did not reach statistical significance, likely a reflection of the small number (n=5) of subjects studied. Subsequent review of the raw data revealed that 4 of 5 women had clear suppression of LH pulse frequency (67% reduction on average) and a characteristically luteal LH pulse pattern by day 5 (the earliest observation after initiation of P); LH pulse frequency slowed from 6 to 1 pulses/8 h by day 3 in one such subject. These data strongly suggest that P reduces GnRH pulse frequency within 3-5 d. Further data in a man revealed that 100 mg oral P suspension (which increased plasma P from < 1 to 10 ng/ml over 2 h) reduced LH by 40% within 3 h and T by 50% over 6 h.

In a recent study of 8 normally cycling women (nearly identical to the current study), we administered a single dose of P at 1800 h and monitored changes (before vs. after P) of LH pulse characteristics. Each subject also underwent a GCRC admission where placebo (PBO) was administered at 1800 h. A manuscript describing our findings has been published (50). The 10-hour mean P concentration increased from 0.6 $\pm$ 0.1 ng/ml before P (0800-1800 h) to 3.9 $\pm$ 0.3 ng/ml after P administration (2200-0800 h; P < 0.01). LH pulse frequency (based on both [a] LH pulse counts over 10 hours and [b] ten-hour mean LH interpulse intervals) decreased significantly after both P and placebo administration, with no significant difference between P and placebo (Figure 1, next page). In contrast, mean LH, LH amplitude, and mean FSH increased significantly within 4 hours of P administration, but not after placebo.

We concluded that in E_2-pretreated women in the late follicular phase, (1) *nocturnal* LH pulse frequency is not acutely (within 12 hours) influenced by P administration; (2) an acute increase in P causes pronounced augmentation of gonadotropin pulse amplitude within 4 hours; and (3) LH pulse frequency slows overnight during the second half of the follicular phase.

The absence of P-induced slowing of LH frequency is in contrast to animal studies that suggest a rapid (within 2-6 hours) decrease in LH and GnRH pulse frequency after P administration. For instance, in ovariectomized but E_2-replete ewes, P dramatically suppresses GnRH pulse frequency over 12 h; this

effect appears to begin within 2 h and is blocked by the progesterone-receptor antagonist mifepristone (RU-486) (51). Similarly, in bovine females, changes in LH pulse frequency are observed within 6 h of altered P concentrations (52). Although species differences may account for this discordance, we have considered an alternative explanation.

Nocturnal, sleep-related slowing of LH pulse frequency during the early follicular phase is well described. Our data (above) and several studies by Loucks and colleagues (53-55) suggest that late follicular LH pulse frequency also slows by 20-30% during sleep. Nocturnal slowing of LH frequency in the early follicular phase (and presumably in the late follicular phase) appears to be specifically related to sleep and is likely driven by higher CNS systems.

These nocturnal changes in LH pulse frequency are interesting in light of what is observed during puberty. In early pubertal girls (and boys), nocturnal LH frequency *increases* compared to daytime LH frequency. It is generally believed that the overnight increase of LH pulsatility during puberty is driven by higher CNS systems.

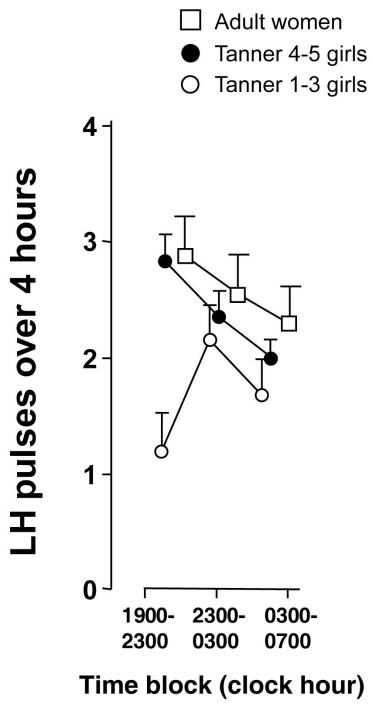

Figure 2. LH pulse count over 4-h time blocks in normal Tanner 1-3 girls (open circles; n = 7), normal Tanner 4-5 girls (closed circles; n = 9), and normal adult women who were studied in the late follicular phase under the JCM016 protocol (squares; n = 8).

Of particular interest, our preliminary data in peripubertal girls suggests that nocturnal LH frequencies in early pubertal girls, late pubertal girls, and the 8 adult women studied under JCM016 are very similar, while late evening LH frequencies (i.e., from 1900-2300 h – a surrogate for daytime frequency) are varied (Figure 2). These data are consistent with the notion that higher CNS systems may in large part determine nocturnal GnRH pulse frequency during puberty and in adults. Accordingly, sleep-associated (and presumably CNS-driven) LH frequency may be similar in pubertal girls and adult women, with daytime frequencies being controlled by other factors.

In addition, two of the aforementioned studies by Loucks and colleagues reported that dietary restriction slows daytime—but not nighttime—LH pulse frequency in the late follicular phase (53, 55). This suggests that daytime and nighttime LH frequency may be differentially affected by experimental maneuvers.

On the basis of the above observations, we have considered the possibility that *daytime* (or non-sleep-associated) LH frequency is acutely responsive to changes in P concentrations in women, even though nighttime frequency is not. This would be consistent with the notion that CNS control of *nocturnal* LH frequency may override hormonal control, at least in the short-term. We would like to test this hypothesis by using a protocol identical to the earlier JCM016 protocol except that the timing of the sampling and P (and PBO) administration is altered by exactly 12 hours (e.g., P and PBO are administered at 0600 h instead of 1800 h).

We will also study women with PCOS in this protocol, primarily to assess for potential abnormalities of acute negative feedback by P (i.e., the rapidity with which P slows LH pulse frequency). But as a secondary aim, we will also assess for abnormalities of *positive* feedback (i.e., P-induced increases of LH amplitude and mean LH).

As mentioned above, studies demonstrate that the GnRH pulse generator is relatively resistant to the negative feedback effects of longer-term administration of P in adults with PCOS (48, 49). In women taking combined oral contraceptives, LH (GnRH) pulse frequency is increased in PCOS compared to controls (49). Similarly, in a study where 7 days of exogenous P and E2 were given (to achieve physiologic concentrations) to women with PCOS and controls, LH pulse frequency was suppressed by 60% in controls, but by only 25% in PCOS (48). Similar findings are observed prenatally-androgenized sheep (56), an animal model of PCOS. In adult PCOS, this relative resistance of the GnRH pulse generator to P negative feedback appears to be mediated by T excess, as it is reversed by the androgen-receptor blocker flutamide (57). Mouse studies also reveal that DHT impairs the ability of P to slow GnRH firing rate (58). This effect of androgens may reflect reduced basal and E-induced P receptors in the hypothalamus (59). Thus, we expect that P will be less effective (in reducing LH frequency) in PCOS compared to controls.

We are unaware of any studies investigating the *positive* feedback effects of P in PCOS, a potentially important factor contributing to ovulatory dysfunction. As described above, our earlier study (50) disclosed a rapid (within 4 h) and pronounced augmentation of gonadotropin secretion after oral P
administration in E2-pretreated women. Similar findings have been described previously (60-62). These effects of P on gonadotropin secretion appear to be related, at least in part, to augmentation of GnRH-stimulated release of gonadotropins from pituitary gonadotropes (62, 63). On the basis of these data, it is generally held that the late follicular rise in P—which begins ~12 h before the LH surge (61, 64)—is an
important mechanism contributing to the midcycle LH surge. However, relative resistance to the *positive* feedback effects of P could represent another mechanism by which ovulatory function is reduced in PCOS. That is, we hypothesize that LH amplitude and mean LH will not increase after P administration to the degree observed in controls.

Hypothesis to be Tested

We propose two primary hypotheses: (1) administration of progesterone (at 0600 h) to normally cycling adult women during the follicular phase will result in a demonstrable suppression of daytime LH (and by inference GnRH) pulse frequency within 12 hours; (2) administration of progesterone (at 0600 h) to women with PCOS (during the follicular phase) will result in less suppression of daytime LH pulse frequency than in ovulatory women without PCOS. We will also assess the acute effects of progesterone on mean LH, LH pulse amplitude, and mean FSH. A secondary hypothesis is that LH augmentation after progesterone administration will be less in PCOS compared to normal controls.

Study Design: Biomedical

1. Will controls be used?Yes

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

This is a cross-over study; thus, when evaluating the effects of progesterone vs. placebo, subjects will serve as their own controls. Also, the data from women with PCOS will be compared to data in normal women (controls).

2. What is the study design?

Double blind until data analysis (progesterone concentrations are measured, which will disclose whether progesterone or placebo was given).

3. **Does the study involve a placebo?**Yes

►IF YES, provide a justification for the use of a placebo

LH secretory characteristics can be influenced by various factors such as time of day, stress, etc. Thus, defining LH secretory characteristics in the absence of progesterone is important for valid inference regarding the effects of progesterone.

Human Participants

Ages: 18-35
Sex: **Female**
Race: **All Races**

Subjects- see below

INSTRUCTIONS: For question 1-4 below insert an exact #. Ranges or OPEN is not allowed. This # should be the maximum # you expect to need to enroll (i.e. sign consent) If you are only collecting specimens the number of participants should equate to the # of specimens you need. If you are collecting only data from a chart review the number should designate the number of subjects whose medical records you plan to review. Age/ Sex/Race criteria should designate the demographics of participants from whom you will obtain the specimen/data.

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

Twelve ovulatory subjects and twelve women with PCOS are needed to complete the protocol.
(Update at time of 5-year renewal April 2017: we have completed study of all required controls, and we are nearing completion of study in women with PCOS.)

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

We anticipated that approximately one-third would fail screening, and that one-third would either dropout or withdraw from the study. Since the study inception, 81 subjects have been enrolled in the study. To date, 59 of those subjects enrolled have either dropped out or been withdrawn from the study, ~ 73%.

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

N/A – this is a single site study

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

90 subjects will sign a consent form under this protocol. *(Notably, we will stop recruiting when we have obtained complete and interpretable data for 12 women with PCOS and 12 controls).* (Update at time of 5-year renewal April 2017: we have completed study of all required controls, and we are nearing completion of study in women with PCOS.)

5. Provide an estimated time line for the study.

We anticipate 100% enrollment in two years and 50% completion of data analysis in five years.
(Update at time of 5-year renewal April 2017: We anticipate completion of the study no later than 2019.)

Inclusion/Exclusion Criteria

1. List the criteria for inclusion

Criteria for inclusion:

- Subjects will be healthy women in two groups: (1) women with regular menstrual cycles and no evidence of hyperandrogenism, and (2) women with PCOS (defined as clinical/biochemical evidence of hyperandrogenism plus oligomenorrhea, but with no evidence for other endocrinopathies).
- Subjects will be 18-35 years old; we use a cutoff age of 35 years because early menopause at this age is very rare, and the risk of DVT with estrogen use may increase beyond this age.
- Subjects will be willing to strictly avoid pregnancy (using non-hormonal methods) during the time of study and must be willing and able to provide informed consent.

2. List the criteria for exclusion

Criteria for exclusion:

- We will exclude women with a history of any disorders that may potentially be complicated by hormonal treatment, such as DVT and breast, ovarian, or endometrial cancer.
- We will exclude women with any other cancer diagnosis and/or treatment (with the exception of basal cell or squamous skin carcinoma) unless they have remained clinically disease free (based on appropriate surveillance) for five years.
- Women with anemia (hematocrit $\leq$ 36% and/or a hemoglobin level <12 g/dl) will be treated with iron for a maximum of 2 sequential months before the 1st admission and/or before the 2nd admission. If they remain anemic after 2 sequential months of ferrous gluconate (325 mg bid), they will then be excluded from further participation in the study.
- Women with a history of any disorders that may potentially be complicated by long-term iron supplementation, such as hemochromatosis and polycythemia vera, will be excluded.
- Women with a significant history of cardiac or pulmonary dysfunction (e.g., known or suspected congestive heart failure; known or suspected coronary atherosclerosis; asthma requiring systemic intermittent corticosteroids; etc.) will be excluded.
- Women with liver enzymes, alkaline phosphatase, or bilirubin > 1.5 times upper limit of normal (confirmed on repeat) will be excluded, with the exception that mild bilirubin elevations will be accepted in the setting of known Gilbert's syndrome.
- Abnormal sodium or potassium concentrations (confirmed on repeat); bicarbonate concentrations <20 or >30 (confirmed on repeat)
- Women with abnormal renal function (i.e., serum creatinine > 1.4) will be excluded (confirmed on repeat)
- Pregnant and breast-feeding women will be excluded.
- A history of allergy to progesterone or estradiol will constitute grounds for exclusion.
- Women with a BMI greater or equal to 40 kg/m^2.
- Virilization
- A total testosterone > 150 ng/dl in women with PCOS (which suggests the possibility of a virilizing neoplasm) (confirmed on repeat)
- Elevated DHEAS (mild elevations may be seen in PCOS, and elevations < 1.5 times the upper limit of normal will be accepted in PCOS) (confirmed on repeat)
- Follicular 17-hydroxyprogesterone > 300 ng/dl, which suggests the possibility of congenital adrenal hyperplasia (if elevated during the luteal phase and there is a concern about the possibility of congenital adrenal hyperplasia, the 17-hydroxyprogesterone may be collected during the follicular phase, or >60 if oligomenorrheic). *NOTE:* If a 17-hydroxyprogesterone > 300 ng/dl is confirmed on such repeat testing, an ACTH stimulated 17-hydroxyprogesterone < 1000 ng/dl will be required for study participation.
- A previous diagnosis of diabetes, a fasting glucose ≥ 126 mg/dl, or a hemoglobin A1c > 6.5%

- Abnormal TSH (subjects with adequately treated hypothyroidism, reflected by normal TSH values, will not be excluded; or, for a new diagnosis of hypothyroidism, further study will at the least be delayed pending appropriate treatment) (confirmed on repeat)
- Abnormal prolactin (mild elevations may be seen in PCOS, and elevations < 1.5 times the upper limit of normal will be accepted in this group) (confirmed on repeat)
- Evidence of Cushing's syndrome by history or physical exam

Due to the amount of blood being drawn in the study, subjects with body weight < 110 lbs. will be excluded from the study

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

Being a study of reproductive hormonal effects on the hypothalamic-pituitary unit, subjects must not take hormonal medications (e.g., oral contraceptives) or other medications known to affect the reproductive axis for 90 days prior to the study and during the study.

Statistical Considerations

1. **Is stratification/randomization involved?**Yes

►IF YES, describe the stratification/ randomization scheme.

The subjects will be randomized to receive either progesterone or placebo during the first CRU admission. This randomization will be generated by the CRU biostatistician using the PROC PLAN procedure of SAS version 9.3 (SAS Institute Inc., Cary NC).

►IF YES, who will generate the randomization scheme?

__X___ UVa Statistician. Jim Patrie

2. What are the statistical considerations for the protocol?

This is a randomized, placebo-controlled, cross-over study.

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

Sample size/statistical power: The primary endpoint will be the change in LH pulse frequency (over 10 h) attributable to P. The null hypothesis is that 10-h (1000-2000 h) LH frequency after P administration will be the same as 10-h (1000-2000 h) LH frequency after placebo administration. Estimates of within-subject variability utilized in the calculation (column 2 of table below) were based on our preliminary data. Assuming that 12 women in both groups complete both CRU admissions (placebo admit, P admit), we list in column 3 (table) the estimates for the minimum detectable absolute difference between the mean change in 10-h LH pulse frequency that would provide ≥ 80% statistical power.

Endpoint	Within-subject standard deviation	Minimum detectable P effect on LH frequency
Change in LH pulse-interval (min)	20.6	16.7
Change in # of LH pulses / 10-h	1.24	1.0

4. What is your plan for primary variable analysis?

Data analysis plan: 10-h LH pulse frequency will be analyzed using hierarchical mixed-effects ANOVA models. Each ANOVA model will be structured to examine whether exogenous P affects LH frequency. In the ANOVA model, "subject" will be a blocking factor, while the treatment condition (placebo, P) will be modeled as highest within-subject level factor, and the measurement assessment time (10-h baseline sampling period, 10-h post-treatment sampling period) will be modeled as the lowest within-subject level factor. Treatment by measurement assessment time interaction will also be modeled. We will use linear-contrasts of the ANOVA least-squared means to test our a priori research hypothesis. Specifically, we will test whether the change in mean LH frequency from the baseline state to the post-

treatment state differed depending on whether placebo or P was administered at 0600 h. 95% confidence interval construction (for the estimation of the true magnitude of P effect) will be based on the t-distribution. Traditional residual diagnostics will be conducted to evaluate model goodness of fit. If LH frequency data fail to adhere to equal variance and normality assumptions of linear mixed-effects models, the data will be analyzed using a generalized linear mixed-effect-model in a similar format, with the exception that LH pulses as will be modeled as counts utilizing either a Poisson or a Negative-Binomial distribution. Similar statistical procedures will be used to compare the effects of P in controls vs. PCOS: in the ANOVA model, PCOS status (no, yes) will be a between-subject factor, while the treatment (placebo, progesterone) will be a within-subject factor.

5. What is your plan for secondary variable analysis?
Secondary outcome variables (30-min measurements of P; 2-h measurements of P, FSH, E, T; LH pulse amplitude) will be analyzed via hierarchical mixed-effect ANOVA models (models/procedures identical to those 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?

Interventions in this research study are not expected to directly benefit the volunteers, and all interventions (e.g., collection of blood, data, administration of estradiol and progesterone) are being done solely to answer a research question and generate generalizable knowledge.

Studies under this protocol will be performed in normally cycling women and in women with PCOS from 18 to 35 years old. Criteria for PCOS will be (a) clinical and/or biochemical evidence of hyperandrogenism, (b) oligomenorrhea, and (b) the absence of clinical or biochemical evidence of other potential causes of hyperandrogenism and/or oligomenorrhea. After informed consent is obtained, all potential subjects will undergo a screening history and physical exam. Subjects will need to fast for a minimum of 8 hours prior to screening blood draw. After informed consent is obtained, blood tests (~ 16 cc) will be drawn at 0800-0900 h as follows: LH, FSH, progesterone (P), estradiol (E2), total testosterone, SHBG, 17-OHP, androstenedione, DHEA-S, beta-hCG, TSH, prolactin, CBC, chemistry and liver panels, hemoglobin A1c, fasting insulin, and fasting glucose. Additionally, BOD POD® will be used to measure total fat mass, fat free mass, and percent body fat. Waist and hip circumference will be measured. If a low hematocrit and low hemoglobin with a low or low-normal MCV (i.e., likely related to iron deficiency) is seen, we will offer 1 month of iron treatment (ferrous gluconate 325 mg bid) with a subsequent recheck of hematocrit and/or hemoglobin. If hematocrit and/or hemoglobin are still low, we will offer 1 more month of iron treatment (ferrous gluconate 325 mg bid) with a subsequent recheck of hematocrit and hemoglobin. Only volunteers with a hematocrit ≥ 36% or a hemoglobin level ≥12 g/dl (after a maximum of 2 sequential months of iron treatment) will be allowed to proceed with frequent blood sampling (see below). Women with anemia will be treated with iron for a maximum of 2 sequential months before the 1st admission and/or before the 2nd admission. If they remain anemic after 2 sequential months of ferrous gluconate (325 mg bid), they will then be excluded from participation in the study.

If three months have elapsed between (a) the subject's most recent safety labs and (b) an overnight admission, a CBC, chemistry and liver panel will be obtained prior to the overnight admission (or on admission) to exclude anemia and any other exclusion criteria.

This study follows a crossover design, with assessment of the acute effects of P and placebo (individually) on GnRH pulse frequency; subjects will be randomized to receive either P or placebo during the first overnight admission, with subsequent overnight study occurring during a subsequent cycle.

Women will begin E2 patches (0.1 mg/d per patch, 2 patches [delivering a total of 0.2 mg/d] placed on the abdomen and changed every 2 d) on the evening of day 4-8 of the study cycle (controls) or $\geq$ 4 days post-menses (PCOS). These patches will be continued for a total of 4 d, with overnight admission occurring on day 3 of E2 administration. Exogenous E2 administration will standardize hypothalamic exposure to E2 and help ensure the presence of sufficient hypothalamic P receptors.

Four to 5 days before a scheduled overnight admission, subjects will come to the CRU or alternate UVA clinical unit for an outpatient blood draw for P and beta-HCG (2 cc). If 30 days will have elapsed between (a) the most recent hemoglobin and hematocrit and (b) the scheduled overnight admission, a hemoglobin and hematocrit will also be drawn at this time (1cc). Note: A hematocrit ≥ 36% or hemoglobin ≥ 12 g/dl in the month before overnight admission is required to participate in the scheduled inpatient (frequent sampling) protocol.

After 3 d of E2 administration, women will undergo a 24-h sampling study in the CRU, alternate UVA hospital unit, or off-site hotel. Estradiol administration (E2 patches) will continue throughout the overnight admission. Subjects will be admitted to the CRU, alternate UVA hospital unit, or hotel at 1800 h (2 h prior to sampling). Beginning at 2000 h, blood will be obtained through an indwelling i.v. forearm heparin lock over a 24-h period as follows: LH every 10 min (1 ml); P every 30 min (1 ml); FSH, E2, and T every 2 h (assays to be run in same samples as LH and P). SHBG, fasting insulin, and fasting glucose (i.e., fasting since 2200 h) will be run on the 0600 h sample (extra 2 cc drawn). (Subjects will fast from 2200 to 0600 h.) After 10 h of sampling (i.e., at 0600 h), either oral micronized P (100 mg p.o.) suspension or placebo suspension will be administered (according to randomization). (NOTE: the study coordinator will pick up the P/placebo from the research pharmacy to send to the CRU, alternate UVA hospital unit, or hotel the evening before administration.) With exogenous P, we aim to achieve mean plasma P concentrations 4-8 ng/ml. Subjects will not be allowed to sleep during the day (i.e., from 0600 to 2200 h). Subjects will be encouraged to sleep from 2200 to 0600. When possible, sleep will be formally evaluated (extraoculograms, electroencephalograms, wrist actigraphy, etc.) [footnote 1]. At the completion of sampling, E2 patches will be discontinued. Volunteers will be discharged on oral iron (325 mg BID). We will ask women to eat only the food provided by the CRU staff.

Subjects will undergo another overnight study identical to the first (including pretreatment with E2, outpatient blood draw 4-5 days before admission, etc.), except that oral P will be exchanged for placebo or *vice versa* in accordance with the crossover design. (Subjects will again begin E2 patches on the evening of cycle day 4-8 [controls] or $\geq$ 4 days post-menses [PCOS].) In this way, we will be able to standardize any change in GnRH pulse frequency after P administration to any change in GnRH pulse frequency after placebo administration.

If the subject does not have hematocrit $\geq$ 36% or hemoglobin $\geq$ 12 g/dl shortly before the second scheduled overnight admission, we will offer 1 month of iron treatment (ferrous gluconate 325 mg bid) with a subsequent recheck of hematocrit and/or hemoglobin. If hematocrit and/or hemoglobin are still low, we will offer 1 more month of iron treatment (ferrous gluconate 325 mg bid) with a subsequent recheck of hematocrit and/or hemoglobin. Only volunteers with hematocrit $\geq$ 36% or hemoglobin level $\geq$ 12 g/dl (after a maximum of 2 sequential months of iron treatment) will be

[1] Update at time of 5-year renewal April 2017: With regard to the formal sleep evaluation, we have had difficulties obtaining formal polysomnography because of scheduling difficulties with a sleep technician, primarily related to (a) limited availability of sleep technicians for CRU studies and/or (b) increased competition for sleep technicians. For this reason, we have been unable to perform formal sleep studies for the past several years. However, we will routinely perform wrist actigraphy. We will use the Motionlogger Basic-L wrist actigraphy unit (Ambulatory Monitoring, Inc.), which is a watch-like device that contains an accelerometer. This device captures subject movement data, which allows estimation of sleep periods. This watch-like device will be worn on the wrist by the research participant during the CRU admission. There are no known risks associated with wrist actigraphy.

allowed to proceed with the second overnight admission. If three months have elapsed between (a) the subject's most recent safety labs and (b) the second overnight admission, a CBC, chemistry and liver panel will be obtained prior to the overnight admission (or on admission) to exclude anemia and any other exclusion criteria.

The study will end after the second overnight admission. Subjects will be asked to continue oral iron supplementation for at least 30 d after this last overnight admission.

ADDITIONAL NOTE: As this is a study to determine the effects of acute progesterone administration on LH hormone regulation, it is important to study subjects during the follicular phase (a time when progesterone should be low, < 1.0), rather than during the luteal phase (when progesterone is high). For ovulatory control subjects, the timing of the luteal phase is predictable, so admission usually occurs around menstrual cycle day 7-11 (during the follicular phase). However, subjects with polycystic ovary syndrome have infrequent/irregular ovulation and infrequent/irregular luteal phases, so it is harder to predict when they will be in the luteal phase. For this reason, overnight admission is scheduled at a time that is ≥ 7 days since their last menses began; and a progesterone level is checked within 1-5 days before the overnight admission, to help rule out the possibility that women will be studied during the luteal phase (i.e., when progesterone levels are high). Following completion of the second overnight admission, samples will be assayed in the Center for Research in Reproduction Ligand Assay Core Lab for progesterone and LH. If it is found that a subject had a high progesterone level (> 1.0 ng/mL) prior to administration of progesterone/placebo syrup at 6am during either of their study admissions, the subject will be asked to repeat that particular study admission at a time when they are in the follicular phase of their cycle (progesterone < 1.0 ng/mL). The subject would be asked to wait the appropriate 8 weeks before scheduling the repeat study admission, so as not to put the subject at undue risk for anemia. The exact timing of the repeat study admission will depend on the subject's menstrual cycle. We would obtain all of the appropriate safety labs prior to studying the subject for a final study admission (to ensure subject safety). And to prevent a repeat occurrence of catching the subject in the wrong phase of her cycle, a progesterone would be checked one to two days before admission. Importantly, in such a situation, subjects would not be obligated to repeat the study admission, and they would receive $300 for completing the study even if they do not repeat the admission. However, if they indeed complete the extra admission, we would offer additional compensation of $100 for completion of this extra study admission, consistent with compensation for each overnight admission under this protocol.

2. List the procedures, in bullet form, that will be done for <u>RESEARCH PURPOSES</u> as stipulated in this protocol.ALL

3. Do you confirm that, except for blood draws through a peripheral site, that all invasive procedures will be performed by a licensed health care provider under the supervision of an MD?
NA, this study only involved blood draws by a licensed health care provider within the UVA hospital system.

4. Will you be using data/specimens in this study that were collected previously, with the use of a research consent form, from another research study?No

5. Will any of the procedures listed in item # 2 have the potential to identify an incidental finding? This includes ALL procedures, assessments and evaluations that are being done for <u>RESEARCH PURPOSES</u> that may or may not be considered investigational. Yes

►IF YES, *check one of the following two options:*

__X___The examination(s) utilize(s) the same techniques, equipment, etc., that would be used if the subject were to have the examination(s) performed for clinical care. **There exists the potential for the discovery of clinically significant incidental findings.**

- The PI takes full responsibility for the identification of incidental findings:

- The PI will inform the subjects verbally of all incidental findings that are of clinical significance or are of questionable significance**.**
- A follow-up letter describing the finding should be provided to the subject with instructions to either show the letter to their PC or if the subject has no PCP, the subject should be instructed to make an appointment at UVa or at the Free Clinic**.**

6. Do any of the procedures listed above, under question # 2, utilize any imaging procedures for <u>RESEARCH PURPOSES</u>?No

7. Will you be using viable embryos?No

8. **Will you be using embryonic stem cells?**No

9. **If this protocol involves study treatment, explain how a subject will be transitioned from study treatment when they have completed their participation in the study.** NA

10. Will your study involve measures used to screen or assess for depression and/or suicidality <u>for research purposes?</u> No

11. **Where will the study procedures be done?**

_X___ UVa , but not medical center facilities: Clinical Research Unit (Barringer)

12. If the study involves medical risk and study procedures will be done outside of the UVa Medical Center what is your plan to protect the subjects in case of a medical emergency?

_____ MD, RN, onsite during procedures
__X___ Individual trained in CPR on site during procedures
__X__ AED and Individual trained to use it onsite
_____ Call 911
__X__ Other : Call Study staff

13. Are any aspects of the study kept secret from the participants? No

14. Is any deception used in the study?No

Family History/Pedigree

1. What kind of information is being sought?

Family history (no questionnaire is being used).

2. What identifiers will be recorded with any of the following info (e.g. names, initials, relationship such as mother, father, brother, sister, random number)?Relationship status.

3. Will HIPAA identifiers will recorded?No

4. Does any of the information sought potentially expose the subject or a family member to additional risk?
No

Specimens

Specimen Information

1. Describe the type of specimen to be used: Blood

2. Will the specimen be obtained BEFORE a subject has signed a consent form? No

3. Will you be using discarded specimens? No

►**IF NO, where will blood be drawn?**
__X___ in the clinical research unit (CRU)

►**IF NO, who will draw the blood?**
__X___ a member of the study team who is an individual licensed to practice medicine or osteopathy, a nurse practitioner, or a physician assistant employed by UVa School of Medicine

►**IF NO, and taking a blood sample, will blood be taken more than 2 times/week?**Yes

►**IF NO, and taking a blood sample, check the option(s) below which match the subject population.**
__X___ healthy, non-pregnant adults who weigh at least 110 pounds.
__X___ Amount will NOT exceed 550 cc in an 8 week period
_____ Amount to exceed 550 cc in an 8 week period

►**IF NO, will blood ONLY be obtained via a peripheral blood stick?**Yes

Specimen Labeling

1. What information/ HIPAA identifiers will be on the specimen label when it is given to the study team (from clinical labs or other source outside the study team) and/or what information will you put on the specimen?
Name, medical record #, CRR protocol #, date of birth, time (date and clock hour) drawn

2. If the specimen is given to the study team with information on the label will you delete any of the information on the specimen label? No

3. Will any additional data be linked to the specimen by way of a code? No

4. Will the analysis on the specimen be done soon (within 24 hours) after it is collected?
Yes and No. See the explanation below.

Samples from the screening exam that are analyzed by UVA clinical labs will generally be run within 24 hrs.

►**IF NO, where will the specimen be stored until analysis is done?**

Some samples from the screening exam (e.g., TSH, LH, FSH, P, Testosterone, E_2 and insulin) and all samples from the inpatient admissions will be analyzed and stored in the UVA Center for Research in Reproduction Ligand Core lab Suhling Building, Rooms 6918 & 6921, IBC #132-02.

Specimen Shipping

1. Do you plan to ship any specimens outside of UVA?No

Specimen Security

The following security precautions will be implemented:

- Specimens will be kept in a locked freezer/ or locked room

__X___ Specimens will be stored with HIPAA identifiers. Access to the freezer/room will be limited to authorized personnel. Specimens with HIPAA identifiers will never be shared outside of UVa without the written permission of the subject.

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 subject s.

1.2 How will you define serious adverse events?

__X___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?

Do not change this answer

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 studies
- 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?

Do not change this answer

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 regulation**s**. 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?

___X__ 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?

__X___NA- No outside sponsor

1.7 What is the definition of a data breach?

Do not change this answer

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**
Risks associated with venipuncture	
Discomfort	__X__Occurs frequently ____Occurs infrequently ____Occurs rarely ____Frequency unknown
Bruising	__X__Occurs frequently ___Occurs infrequently ____Occurs rarely ____Frequency unknown
Infection	____Occurs frequently ____Occurs infrequently ____Occurs rarely __X__Frequency unknown, but likely to be very rare
Blood clot at the site of intravenous catheter insertion	____Occurs frequently ____Occurs infrequently __X__Occurs rarely ____Frequency unknown
Risks of frequent blood draw	
Anemia	____Occurs frequently ____Occurs infrequently ____Occurs rarely __X__Frequency unknown
Risk to Fetus	Unknown risk to fetus
Risk of Progesterone	
Fluid retention	May occur with longer-term P administration, but this would not be expected after one dose of P.
Sleepiness	____Occurs frequently ____Occurs infrequently __X__Occurs rarely ____Frequency unknown
Mood swings	Occurs with longer-term P administration, but rare after one dose of P.
Mild vaginal bleeding	Generally occurs shortly after discontinuation of a one-week course of exogenous P and E2. We do not expect the same to occur after only one dose of P.
Risk of Estradiol	
Local skin irritation at the site of E2 patch application	May occur in approximately 15-20% of subjects
Deep Vein Thrombosis	____Occurs frequently

	____Occurs infrequently ____Occurs rarely __X__Frequency unknown, however occurs very rarely with long-term estrogen use (as with oral contraceptive pills), so would expect to be exceedingly rare with the short term administration in this protocol.
Mild nausea	____Occurs frequently __X__Occurs infrequently ____Occurs rarely ____Frequency unknown
Swelling or breast tenderness	____Occurs frequently __X__Occurs infrequently ____Occurs rarely ____Frequency unknown
Hot flashes	____Occurs frequently ____Occurs infrequently __X__Occurs rarely ____Frequency unknown
Risk of Iron Supplementation	
nausea	____Occurs frequently __X__Occurs infrequently ____Occurs rarely ____Frequency unknown
constipation	____Occurs frequently __X__Occurs infrequently ____Occurs rarely ____Frequency unknown
dark or black stools	__X_Occurs frequently ____Occurs infrequently ____Occurs rarely ____Frequency unknown
Risk for early termination of study participation if subject becomes pregnant	Subjects are not expected to have decreased infertility as a result of study medications (i.e., this short course of physiologic E2 and P will not provide contraception). Therefore, sexually active subjects may get pregnant during this protocol. If the volunteer becomes pregnant, the study and all study medications will be discontinued.
Reproductive Risks *Specify potential 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:

- Risks of venipuncture will be minimized by using proper sterile technique

- Risk of anemia will be reduced in the following manner: A hemoglobin and hematocrit will be obtained and reviewed prior to each CRU sampling study, and only volunteers with a hematocrit ≥ 36% or a hemoglobin ≥ 12 will be allowed to proceed with the frequent sampling study. The one exception to this rule is that those subjects documented to have a hematocrit ≥ 36% or Hemoglobin ≥ 12 g/dl in the month before overnight admission will not be required to have a repeat hematocrit and hemoglobin on overnight admission.

- Risk of fetal exposure to study drugs will be reduced by having a serum pregnancy test obtained and reviewed prior to each overnight sampling study. If the volunteer is pregnant, the study and all study medications will be discontinued.

- A history and physical will be performed at screening. Women with a history of any disorders that may potentially be complicated by long-term iron supplementation, such as hemochromatosis and polycythemia vera, will be excluded. Women with a significant history of cardiac or pulmonary dysfunction (e.g., known or suspected congestive heart failure; known or suspected coronary atherosclerosis; asthma requiring systemic intermittent corticosteroids; etc.) will be excluded. We will exclude women with a history of any disorders that may potentially be complicated by hormonal treatment, such as DVT and breast, ovarian, or endometrial cancer.

- Risk from use of estradiol patch will be reduced by instructing subjects to rotate sites of their E2 patch to avoid skin irritation and/or rash.

- Risk from iron supplementation 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.

- A comprehensive metabolic panel will be performed at screening. Women with liver enzymes, alkaline phosphatase, or bilirubin > 1.5 times upper limit of normal (confirmed on repeat) will be excluded, with the exception that mild bilirubin elevations will be accepted in the setting of known Gilbert's syndrome. Women with abnormal renal function (i.e., serum creatinine > 1.4) will be excluded. Women with abnormal sodium, potassium, or bicarbonate concentrations <20 or >30 (confirmed on repeat) will be excluded.

- If three months have elapsed between (a) the subject's most recent safety labs and (b) an overnight admission, a CBC, chemistry and liver panel will be obtained prior to the overnight admission (or on admission) to exclude anemia and any other exclusion criteria.

- Subjects who are asked to repeat a study admission at a time when they are in the follicular phase of their cycle will be asked to wait the appropriate 8 weeks to schedule the repeat study admission so as not to put the subject at undue risk for anemia. All appropriate safety labs will be obtained prior to studying the subject for a final study admission to ensure subject safety.

- Subjects will have frequent (at least every 10 minutes) contact with CRU nursing personnel and will be asked to report any unusual symptoms occurring during the protocol.

To minimize the risk of having >500 ml of blood drawn within 48 hours:

A hemoglobin level will be drawn within 30 days prior to the visit at which the blood will be drawn. The blood will not be drawn if the levels do not meet the criteria below:

- Hematocrit at least 36% and/or a hemoglobin level at least 12 g/dl

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

__X___At subject, PI or sponsor's request (see below)

- If the volunteer is pregnant, the study and all study medications will be discontinued.
- Estradiol will be discontinued and the study stopped if side effects (e.g., nausea, breast tenderness) become problematic.
- If subjects remain anemic (HCT < 36% and hemoglobin < 12) after 2 sequential months of ferrous gluconate (325 mg bid), they will then be excluded from participation in the study.

_____Treatment would be stopped if the subject had a serious adverse event deemed related to study, or study drug will be increased if the subject tolerates dosing

_____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?

INSTRUCTIONS;

- These are called stopping rules for early termination of the entire study.
- List criteria regardless of whether the study is sponsored or not.
- Be sure to include any criteria for which the UVa PI would halt the study at UVa.
- Check all that apply.

__X___Per IRB, PI, DSMB, or sponsor discretion

Two (2) subjects having serious, unexpected related or possibly related Adverse events.

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

__X___Other: If a severe adverse event occurs within 24 hours after administration of progesterone/placebo.

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?

►IF NO, what criteria will be used? No

__X___Only adverse events deemed related/possibly related to study

_____Only adverse events that are deemed serious

_____Only adverse events that are deemed related AND serious

3.2 How will adverse event data be collected/recorded? Check all that apply

__X___Paper AE forms/source documents

__X___Spreadsheet: paper or electronic

_____Database Specify name/type of database Answer/Response:

3.3. How will AEs be classified/graded? Check all that apply

_____World Health Organization Criteria (WHO)

_____NCI Common Toxicity Criteria, Version 2.0/ NCI Common Terminology Criteria, Version 3.0

_____NCI CTCAE Version 4.0

__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? *Check all that apply.*

__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?

__X___ End of study drug/device/intervention/participation

_____ 30 days post study drug/device/intervention

_____ 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 *An internal event is one that occurs in a subject enrolled in a UVa protocol*	IRB-HSR	Within 24 hours	IRB Online and phone call www.irb.virginia.edu/
Internal, Serious, related/possibly related, Unexpected adverse event	IRB-HSR	Within 7 calendar days from the time the study team received knowledge of the event. *Timeline includes submission of signed hardcopy of AE form.*	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 *The IRB-HSR only requires that MAJOR violation be reported, unless otherwise required by your sponsor, if applicable.* **OR** **Enrollment Exceptions** *See definition- only allowed if there is a commercial sponsor or a DSMB that has granted the enrollment exception.*	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* *Go to 3rd bullet from the bottom.*
Data Breach	The UVa Corporate Compliance and Privacy Office ITC: if breach involves electronic data	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	UVa Corporate Compliance and Privacy Office- Phone 924-9741 **ITC:** Information Security Incident Reporting procedure, http://www.itc.virginia.edu/security/reporting.html

	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	time the incident is identified. IMMEDIATELY.	UVa Police-Phone- (434) 924-7166

4. How will the endpoint data be collected/recorded. Check all that apply

__X___Protocol specific case report forms

_X___Source documents

5. Data and Safety Oversight Responsibility

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

__X___DSMB/ DSMC

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

Members of the study team may NOT also be members of the DMSB.

__X___Other- William Evans, MD; Mark DeBoer, MD; Guofen Yan, PhD. (This internal (UVA) DSMB has monitored this study since its inception.

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

Check all that apply.

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

__X___All adverse events

__X___Unanticipated Problems

__X___Protocol violations/Issues of noncompliance

_____Audit results

_____Application of dose finding escalation/de-escalation rules

These should be outlined under 2.4.

_____Application of study designed stopping/decision rules

_____Early withdrawals

_____Whether the study accrual pattern warrants continuation/action

_____Endpoint data

_____Other: Specify Answer/Response:

5.4 **How often will aggregate review occur?**

For additional information on aggregate review see:
www.virginia.edu/vpr/irb/hsr/continuations.html#aggreview

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

_____Per Enrollment/Events

__X___Annually

_____Semi-Annually

_____Quarterly

_____Monthly

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

A copy of these reports must be sent to the IRB if applicable as soon as they are received by the PI. Do not wait until the next continuation to submit them to the IRB.

___X__Annually

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

__X___Part of IRB-HSR continuation status form

Payment

INSTRUCTIONS:

What is the difference between compensation and reimbursement?

A reimbursement is used when the subject is paid back for travel expenses such as mileage, lodging, food while traveling. Receipts or mileage must be submitted for a reimbursement.

Compensation is "payment" for things such as time, discomfort, inconvenience.

Total possible compensation should reflect the true value of the total possible dollar amount per participant for one year involvement in the study whether it be cash, check, gift card, goods, etc. or a combination of these items.

Retention "Gifts"- gifts may be given to a subject periodically during the study to remind them they are in the study. Sponsors may provide such items as water bottles, birthday cards etc. to the subject. NOTE: Cash or gift cards are NOT allowed as retention items.

1. Are subjects being reimbursed for travel expenses?

INSTRUCTIONS:

- If subject will NOT submit receipts for actual expenses (e.g. hotel, food, you MUST answer this NO.
- If subjects will have mileage/distance traveled, calculated and confirmed *via Mapquest for example, this questions should be answered YES
- Reimbursements must be paid with Oracle Expenditure types found under the Travel Heading.
- For instructions on how to process a reimbursement please see "Goods and Services Procurement Guide" at http://www.procurement.virginia.edu/main/. You may also call the Procurement Help Desk at 924-4212.
- The money will not be reportable to the IRS as income, but will be withheld if the subject owes money to the state.

Yes

►IF YES, explain rate/ amount/ upper limits of reimbursements.

When round trip travel exceeds 50 miles (one way), we will make available travel reimbursement in the amount of $25 per trip. (Total travel reimbursement not to exceed $75 for the entire study).

►IF YES, Do you confirm you are aware of the following procedures to follow for reimbursements?

INSTRUCTIONS

- Subject will submit receipts for actual expenses (e.g. hotel, food)
- Reimbursements must be paid with Oracle Expenditure types found under the Travel Heading.
- For instructions on how to process a reimbursement see "Goods and Services Procurement Guide" at http://www.procurement.virginia.edu/main/. You may also call the Procurement Help Desk at 924-4212. The money will not be reportable to the IRS as income, but will be withheld if the subject owes money to the state.
- Reimbursements may not be done with gift cards

Yes

- Subject will submit receipts for actual expenses (e.g. hotel, food, actual mileage).
- Reimbursements must be paid with Oracle Expenditure types found under the Travel Heading. *For instructions on how to process a reimbursement see "Goods and Services Procurement Guide" at http://www.procurement.virginia.edu/main/. You may also call the Procurement Help Desk at 924-4212. The money will not be reportable to the IRS as income, but will be withheld if the subject owes money to the state.*

- Reimbursements may not be done with gift cards

2. Are subjects compensated for being in this study? Yes

►IF YES, answer the following questions (2a-2d).

2a. What is the maximum TOTAL compensation to be given over the duration of the protocol?
$300.00 in most circumstances (although total compensation of $400 is possible [see below])

2b. Explain compensation to be given.
Subjects will receive $100 for each completed inpatient admission (normally, the study involves 2 inpatient admissions), and a $100 bonus for completing all the admissions and outpatient visits in the study. If a subject completes the first two study admissions and is asked by the study team to repeat one of those overnight studies, she will be compensated an additional $100 for this extra overnight admission.

2c. Is payment pro-rated?
Yes. Subjects completing only one of two inpatient study admissions will receive $100.

2d. Is money paid from UVa or State funds (including grant funds) or will items such as gift cards be distributed through UVa?

INSTRUCTIONS
Examples of when to say no:

- Researcher is using their own personal funds to compensate participants.
- Compensation is coming from a UVa Foundation and therefore not subject to UVA financial policies and procedures.

Examples of when to say yes:

- Sponsor, via a grant or contract, sends money to OSP/ SOM Grants and Contracts office to cover cost of compensation to be given to subjects. Subjects are then paid via Oracle system
- UVA researcher purchases gift cards for distribution to subjects and there is NO outside sponsor.
- Sponsor purchases gift cards/ debit cards and sends to UVa for study team to distribute to the subjects.

Yes

►IF YES, answer the following questions [2d(i)-2d(ii)].

2d(i). How will the researcher compensate the subjects?

___X__ Check issued to participant via UVA Oracle or State system

2d(ii). Which category/ categories best describes the process of compensation?

__X___ All compensation will be made via check issued to participant via UVA Oracle or State system

The preferred method

Risk/ Benefit Analysis

1. What are the potential benefits for the participant as well as benefits which may accrue to society in general, as a result of this study?

There are no direct benefits for the participant as a result of this study. The investigators judge that the risk of participating in this study is minimal and that the information obtained may help to further elucidate the mechanisms involved in modulation of the GnRH pulse generator in health and disease. Volunteers may rarely receive the diagnostic benefits of laboratory evaluation without charge. Also, the information obtained via this study will be critical to obtain while pursuing our hypotheses regarding (1) the development of ovulatory cycles during puberty and (2) the emergence of neuroendocrine abnormalities in adolescents destined to develop PCOS. These hypotheses may be used in developing therapeutic interventions for hyperandrogenemic adolescents, a condition felt to represent a forerunner of PCOS.

2. Do the anticipated benefits justify asking subjects to undertake the risks?

NA. There are no direct benefits for the participant as a result of this study. The risk/benefit ratio is acceptable.

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APPENDIX: Legal/Regulatory

Recruitment

The following procedures will be followed:

- Finders fees will not be paid to an individual as they are not allowed by UVa Policy.
- All recruitment materials will be approved by the IRB-HSR prior to use. They will be submitted to the IRB after the IRB-HSR has assigned an IRB-HSR # to the protocol.
- Only those individuals listed as personnel on this protocol will recruit and or conduct the consenting process with potential subjects.

Retention Incentives

Any item used by the sponsor/ study team to provide incentive to a subject to remain in the study, other than compensation identified in the Payment section, will be submitted to the IRB for review prior to use. The IRB-HSR will provide the study team with a Receipt Acknowledgement for their records. Retention incentive items are such things as water bottles, small tote bags, birthday cards etc. Cash and gift cards are not allowed as retention incentives.

Clinical Privileges

The following procedures will be followed:

- Investigators who are members of the clinical staff at the University of Virginia Medical Center must have the appropriate credentials and been granted clinical privileges to perform specific clinical procedures whether those procedures are experimental or standard.
- The IRB cannot grant clinical privileges.
- Performing procedures which are outside the scope of the clinical privileges that have been granted may result in denial of insurance coverage should claims of negligence or malpractice arise.
- Personnel on this protocol will have the appropriate credentials and clinical privileges in place before performing any procedures required by this protocol.
- Contact the Clinical Staff Office- 924-9055 or 924-8778 for further information.

Sharing of Data/Specimens

Data and specimens collected under an IRB approved protocol are the property of the University of Virginia. You must have "permission" to share data/ specimens outside of UVa other than for a grant application and or publication. This "permission" may come in the form of a contract with the sponsor or a material transfer agreement (MTA) with others. A contract/ MTA is needed to share the data outside of UVa even if the data includes no HIPAA identifiers and no code that could link the data back to a HIPAA identifier.

- No data will be shared outside of UVa, beyond using data for a grant application and or publication, without a signed contract/MTA approved by the SOM Grants and Contracts office/ OSP or written confirmation that one is not needed.
- No specimens will be shared outside of UVa without a signed contract/MTA approved by the SOM Grants and Contracts office/ OSP or written confirmation that one is not needed.

Prisoners

If the original protocol/ IRB application stated that no prisoners would be enrolled in this study and subsequently a subject becomes a prisoner, the study team must notify the IRB immediately. The study team and IRB will need to determine if the subject will remain in the study. If the subject will remain in the study, the protocol will have to be re-reviewed with the input of a prisoner advocate. The prisoner advocate will also have to be involved in the review of future continuations, modifications or any other reporting such as protocol violations or adverse events.

Prisoner- Individuals are prisoners if they are in any kind of penal institution, such as a prison, jail, or juvenile offender facility, and their ability to leave the institution is restricted. Prisoners may be convicted felons, or may be untried persons who are detained pending judicial action, for example, arraignment or trial.
For additional information see the OHRP website at http://www.hhs.gov/ohrp/policy/populations/index.html

Compensation in Case of Injury

If a subject requests compensation for an injury, the study team should notify the IRB-HSR (924-9634/2439847) the UVa Health System Patient Relations Department (924-8315). As a proactive courtesy, the study team may also notify UVa Health System Patient Safety and Risk Management (924-5595).

On request, the study team should provide the Risk Management Office with the following information/documents:

- Subject Name and Medical Record Number
- Research medical records
- Research consent form
- Adverse event report to IRB
- Any letter from IRB to OHRP

Subject Complaints

During a research study, the study team may receive complaints from a subject. If the study team is uncertain how to respond to a complaint, or is unable to resolve it with the subject, the study team may contact the IRB-HSR (924-9634/243-9847), the UVa Health System Patient Relations Department (924-8315).

Request for Research Records from Search Warrant or Subpoena

If the study team receives a request for research records from a search warrant or subpoena, they should notify UVa Health Information Services at 924-5136. It is important to notify them if information from the study is protected by a Certificate of Confidentiality.

APPENDIX: Drug Information

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

Micronized progesterone suspension (i.e., Progesterone USP, micronized, for prescription compounding [NDC 39822-6000-3] Mfg: Spectrum Chemicals and Laboratory Products). This protocol is under IND# 64, 126.

We will also be using commercially available estradiol patches (0.1 mg/d), which are provided by the UVAHS research pharmacy. This protocol is under IND# 64, 126.

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

John C. Marshall

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

APPENDIX: Recruitment

Recruitment includes identifying, review of records to determine eligibility or any contact to determine a potential subjects interest in the study.

*The UVa HIPAA covered entity is composed of the UVa VP Office of Research, the Health System, School of Medicine, School of Nursing, Nutrition Services (Morrisons), the Sheila C. Johnson Center, the Exercise and Sports Injury Laboratory and the Exercise Physiology Laboratory.

1. How do you plan to <u>identify</u> potential subjects?

- To "identify" a potential subject refers to steps you plan to take to determine which individuals would qualify to participate in your study. This does NOT include steps to actually contact those individuals.

- If your study involves more than one group of subjects (e.g. controls and cases or subjects and caregivers) note below which groups are being identified by the given method.
- Check the methods you plan to utilize:

a.____ Chart Review/ Clinic Schedule Review/ Database Review from a database established for health care operations (departmental clinical database) or an Improvement Project (*e.g. Performance Improvement, Practice Improvement, Quality Improvement*).
If you plan to obtain data from the UVa Enterprise Data Warehouse (EDW) please see option b below.

DHHS: Study team requests Waiver of Consent to identify potential subjects.

HIPAA: Allowed under Preparatory to Research if PHI to be accessed.

IMPORTANT
Keep in mind that PHI in the medical record may only be accessed by individuals who work under the UVa HIPAA covered entity; which means they meet one of the following criteria:
--a UVa student working in the UVa HIPAA Covered Entity*
--a faculty or staff member in a PAID appointment in the UVA HIPAA Covered Entity*

b____ Review of a database that was established to keep data to be used for future research such as the CDR, departmental research database or use of data from a separate current active research protocol.
If you plan to obtain data from the UVa Enterprise Data Warehouse (EDW) you are required to submit your request to the CDR. The CDR staff will work with the EDW to obtain the data you need.

DHHS: Study team requests Waiver of Consent to identify potential subjects.

HIPAA: Allowed under Preparatory to Research if PHI to be accessed.

IMPORTANT
Keep in mind that PHI in the medical record may only be accessed by individuals who work under the UVa HIPAA covered entity; which means they who meet one of the following criteria:
--a UVa student working in the UVa HIPAA Covered Entity*
--a faculty or staff member in a PAID appointment in the UVA HIPAA Covered Entity*

The information from which you are obtaining potential subjects must also have an IRB protocol approval. If this item is checked, enter the IRB # below.

IRB# _____

If obtaining information from the Clinical Data Repository (CDR) insert IRB # 10797

c. ____ Patients UVa health care provider supplies the UVa study team with the patients contact information without patients' knowledge.

DHHS: Study team requests Waiver of Consent to identify potential subjects.

HIPAA: Allowed under Preparatory to Research if PHI will be shared by the health care provider.

IMPORTANT
Keep in mind that PHI may only be given to individuals who work under the UVa HIPAA covered entity; which means they meet one of the following criteria:
--a UVa student working in the UVa HIPAA Covered Entity*
--a faculty or staff member in a PAID appointment in the UVA HIPAA Covered Entity*

d. ____ Patient obtains information about the study from their health care provider. The patient contacts the study team if interested in participating. (Health care provider may or may not also be the a member of the study team)

DHHS: NA
HIPAA: Allowed under Health Care Operations

If this choice is checked, check 3d-INDIRECT CONTACT below.

e. _X___ Potential subjects will not be directly identified. They will respond to an advertisement such as a flyer, brochure etc.

If this choice is checked, check 3d- INDIRECT CONTACT below.
DHHS & HIPAA: NA

f. _____ Potential subjects have previously signed a consent to have their name in a registry/database to be contacted for future studies of this type.

IRB# of registry/ database: _____

DHHS & HIPAA: NA

g. ____ Other: Specify Answer/Response:

If item # a, b or c is checked above and if this protocol involves the use of protected health information do you confirm the following to be true? n/a

- The use or disclosure is sought solely to review protected health information as necessary to prepare the research protocol or other similar preparatory purposes.
- No PHI will be removed from the UVa covered entity.
- The PHI that the researcher seeks to use or access is necessary for the research purposes.

2. How will potential subjects be contacted?

To "contact" a potential subjects refers to the initial contact you plan to take to reach a potential subject to determine if they would be interested in participating in your study. This may include direct contact by such methods as by letter, phone, email or in-person or indirect contact such as the use of flyers, radio ads etc.

If your study involves more than one group of subjects (e.g. controls and cases or subjects and caregivers) note below which groups are being contacted by the given method.

Check the methods below you plan to utilize:

a. ____ Direct contact of potential subjects by the study team via letter, phone, direct e-mail. Members of study team ARE NOT health care providers of patients. Information will not be collected from psychotherapy notes.

Note: Letter, phone, direct email scripts must be approved by IRB prior to use. See IRB-HSR Website for templates.

DHHS/HIPAA: Study team requests a Waiver of Consent and Waiver of HIPAA Authorization to contact potential subjects.

IMPORTANT:
Keep in mind that if PHI was collected during the identification phase that contact with potential subjects may only be performed by individuals who work under the UVa HIPAA covered entity; which means they meet one of the following criteria:

- a UVa student working in the UVa HIPAA Covered Entity*
- a faculty or staff member in a PAID appointment in the UVA HIPAA Covered Entity*

b. ____ Potential subjects will be approached while at UVa Hospital or Health Clinic by a person who is NOT a member of their health care team. Information will not be collected from psychotherapy notes.

DHHS & HIPAA: Study team requests a Waiver of Consent and a Waiver of HIPAA Authorization to contact potential subjects.

IMPORTANT:
Keep in mind that contacting individuals in a clinical setting may only be performed by individuals who work under the UVa HIPAA covered entity; which means they meet one of the following criteria:
a UVa student working in the UVa HIPAA Covered Entity*
a faculty or staff member in a PAID appointment in the UVA HIPAA Covered Entity*

You should share the following information with the potential subject:

- Your name
- Who you are: physician, nurse etc. at the University of Virginia.
- Why you want to speak with them
- Ask if you have their permission to explain the study to them
- If asked about how you obtained their information use one of the following as an option for response.
    - DO NOT USE THIS RESPONSE UNLESS YOU HAVE OBTAINED PERMISSION FROM THEIR UVa PHYSICIAN: Your doctor, Dr. insert name wanted you to be aware of this research study and gave us permission to contact you.
    - We obtained your information from your medical records at UVa.
    - Federal regulations allow the UVa Health System to release your information to researchers at UVa, so that we may contact you regarding studies you may be interested in participating. We want to assure you that we will keep your information confidential.
- IF THE PERSON SEEMS ANGRY, HESITANT OR UPSET, THANK THEM FOR THEIR TIME AND DO NOT ENROLL THEM IN THE STUDY. YOU MAY ALSO REFER THEM TO THE IRB-HSR AT 924-9634.

c.____Direct contact of potential subjects by the study team by approaching in person at UVa or via letter, phone, direct e-mail. Members of study team contacting potential subjects ARE health care providers of patients.

If you are not approaching them in person but using a letter, phone call or direct email please note that the letter, phone, direct email scripts must be approved by IRB prior to use.
See IRB-HSR Website for templates.

DHHS: Study team requests a Waiver of Consent to contact potential subjects
HIPAA: Allowed under Health Care Operations.

d.__X__ Indirect contact (flyer, brochure, TV, broadcast emails, patient provided info about the study from their health care provider and either the patient contacts study team or gives their healthcare provider permission for the study team to contact them.)

The indirect method used (flyer, brochure, TV, broadcast emails) must be approved by the IRB prior to use. The IRB does not need to review any type of script to use when the potential subject responds to the indirect method.

DHHS & HIPAA: NA

e. ____ Potential subjects are not patients. The study does not include obtaining subjects health information. Subjects will be contacted directly via email, phone, letter or presentation in group setting with consent then obtained individually in a private setting.

If you are not approaching them in person but using a letter, phone call or direct email please note that the letter, phone, direct email scripts must be approved by IRB prior to use.
See IRB-HSR Website for templates.

DHHS: Study team requests a Waiver of Consent to contact potential subjects.
HIPPA: NA

3. **Will any additional information be obtained from a potential subject during "prescreening"?**

Pre-screening for IRB purposes is the term used to describe activities PRIOR to obtaining Informed Consent and may not include any research procedures.

The activities may involve pre-screening of potential subjects over the telephone or in person is generally performed to determine their initial eligibility for, and, interest in a study and is a common strategy in the recruitment process.
Questions appropriate for pre-screening address the specific inclusion/exclusion criteria for the study and other issues of suitability, for example, an individual's ability to come to the research site multiple times.
It is not appropriate at this point in the process (i.e. prior to obtaining informed consent/enrollment) to gather information that is not directly related to assessing eligibility and suitability (e.g. obtaining complete medical histories, obtaining blood specimens for lab tests).
An additional telephone script is not required, for this pre-screening process, in addition to any scripts required under Recruitment question # 2.

Yes

IF YES, submit any documents that will be used to collect pre-screening information so that the IRB may confirm what questions will be asked.
NOTE: To comply with HIPAA regulations only the minimum necessary information may be collected at this time. This means that only questions pertaining to the Inclusion and Exclusion Criteria may be asked.

IF YES,
DHHS: study team requests a Waiver of Documentation of Consent for Pre-screening questions.

HIPPA:
HIPAA does not apply if:
--no PHI is collected or
--if PHI is collected from a potential subject by an individual from a department that is not part of the HIPAA covered entity.

HIPAA does apply if the collection occurs by individuals* who work in a department that is part of the HIPAA covered entity.

In this case the collection will be covered under Health Care Operations/

These individuals are those that meet one of the following criteria:
--a UVa student working in the UVa HIPAA Covered Entity*
--a faculty or staff member in a PAID appointment in the UVA HIPAA Covered Entity*

IF YES, Will any of the questions involve health information?Yes

IF YES, will you collect HIPAA identifiers with the health information?Yes

IF YES, which HIPAA identifiers will be recorded?
Name, MR#, date of birth, age, postal address, telephone number, email address (if applicable)

Do you confirm that health information with HIPAA identifiers will not be shared outside of UVa until a consent form is signed or only shared in a de-identified manner?
Yes

4. **Do you plan to ask the subjects to do anything, other than answering questions, for the study prior to signing a consent?**

For example: come to the first visit fasting, stop taking medications that may be an exclusion criteria, change diet. As this is still part of pre-screening one is not allowed to gather information that is not directly related to inclusion/exclusion criteria or other issues of suitability (e.g. is person able to come to UVa for multiple visits)

NOTE:
Only those members of the study team with a DEA# (license to prescribe drugs) are allowed to determine if a potential subject may be asked/informed to stop taking a drug which is an exclusion criteria.
It is recommended that the potential subject notify their health care provider if they plan to stop a prescription drug.

Yes

►**IF YES, explain in detail what you will ask them to do**.

We will request that subjects come to the screening visit while fasting for at least 8 hours. We will tell potential subjects that only subjects who have taken no hormonally-active medications for 2 months prior to the screening visit (and 3 months prior to inpatient study) will be eligible for participation. However, we will strongly recommend that potential subjects do not stop medications without first consulting with their personal physicians

Tips to Study Team
You must document their verbal consent in the study records.
If a subject is asked to stop taking a drug, document the date and name of the person on the study team giving the verbal order to stop medications (again- must be a person with a DEA#).

DHHS: Study team requests the use of Verbal Consent (Waiver of Documentation of Consent) for minimal risk screening procedures.

HIPPA:
If the individual, obtaining consent, works under the HIPAA Covered Entity this is covered under Health Care Operations
If the individual obtaining consent does not work under the HIPAA covered entity, HIPAA does not apply.

5. How will the consenting process take place with either the prospective subject, the subject's legally authorized representative or parent/legal guardian of a minor (if applicable)?

HIPPA:
If the individual, obtaining consent, works under the HIPAA Covered Entity consenting is covered under Health Care Operations.

If the individual obtaining consent does not work under the HIPAA covered entity, HIPAA does not apply.

Potential subjects will be given a consent form (via mail or e-mail) to read at their leisure. If the potential subject desires to be involved in the study, a consent and screening visit will be scheduled. At the beginning of this visit, the study will be discussed and any questions will be answered. If the potential subject decides to enter the study, they will then sign the consent form, and study screening procedures will begin thereafter.

6. Will subjects sign a consent form for any part of the study? Yes

7. Will the study procedures be started the same day the subject is recruited for the study?
Yes

►IF YES, explain in detail why the subject cannot be given more time to make a decision to consent.
Potential subjects are given a copy of the consent form prior to their screening visit. Subjects are instructed to review the consent form, and are encouraged to ask any questions prior to scheduling a screening visit. At the screening visit, subjects are lead through a discussion of the consent form. Subjects are given the opportunity to ask any additional questions during this portion of the screening visit. After the study team has read through the entire consent form with the subject, they are then given the opportunity to sign the consent form, decline to enroll in the study, or take more time to review the consent form before making a decision whether or not to enroll. If the subject decides to enroll in the study on the day of the screening visit,

then we will proceed with the study procedures outlined in the study visit above, unless the subject objects.

Therefore, the study team feels that the subject is under no pressure to give consent at the screening visit without having ample opportunity to review the consent form. In addition, subjects are given the option to decline consent or delay study procedures at any point during this initial visit.

►**IF YES, explain in detail what will be done to assure the potential subject has enough time to make an informed decision.**
Potential subjects are given a copy of the consent form prior to their screening visit. Subjects are instructed to review the consent form, and are encouraged to ask any questions prior to scheduling a screening visit. At the screening visit, subjects are lead through a discussion of the consent form. Subjects are given the opportunity to ask any additional questions during this portion of the screening visit. After the study team has read through the entire consent form with the subject, they are then given the opportunity to sign the consent form, decline to enroll in the study, or take more time to review the consent form before making a decision whether or not to enroll.

8. Is there the potential to recruit economically or educationally disadvantaged subjects, or other vulnerable subjects such as students or employees?Yes

IF YES, what protections are in place to protect the rights and welfare of these subjects so that any possible coercion or undue influence is eliminated?
Potential subjects are given the opportunity to read the consent at their leisure prior to the study staff knowing if they are economically or educationally disadvantaged or a student or employee of Uva.

9. Do you need to perform a "dry run" of any procedure outlined in this protocol? No

10. Is the study regulated by the Department of Defense (DoD)?No

APPENDIX: Pharmacy-Investigational Drugs/Biologics

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

Micronized progesterone suspension (i.e., Progesterone USP, micronized, for prescription compounding [NDC 39822-6000-3]

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

__X___ Inpatient Unit: *Subjects will be given medication at 0600h during one of their inpatient study admissions (randomized with placebo).*

3. What dose will be utilized in this study?

100 mg

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

Subjects will be given a single dose of 100 mg progesterone suspension during the study. Subjects will receive either progesterone or placebo during each inpatient admission at 0600h. If subjects receive placebo during the first inpatient admission, they will receive progesterone during the second inpatient admission (and *vice versa*).

5. What will be the duration of dosing in this study?
Subjects will be given a single dose of progesterone during the study.

6. What route of administration will be utilized? PO

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

7b. Diluents
___X__ Standard

7c. Stability after prepared
__X___ Standard

7d. Special storage requirements
__X___ Standard

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?
Missed doses are not expected in this study, since the subject will receive this dose during their inpatient study admission.

11. Will a comparator (active or placebo) be utilized in the protocol? Yes

►IF YES, comparator is:
__X___ Placebo*:* At each inpatient admission, subjects will receive either a single dose of progesterone or a single dose of placebo, determined by randomization. The study is set up as a cross-

over design, such that subjects receiving placebo during admission 1, will receive progesterone at admission 2, and *vice versa*. Placebo suspension is made up with the same formulation as the micronized progesterone suspension minus the active ingredient.

12. Does this study involve research on a drug, biologic, supplement or food additive? yes

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

IF YES, answer questions 13a-13f
You may reference the non-IRB protocol to answer these questions.

13a. Describe pertinent animal data that is available regarding the toxicity/safety of this drug.
There are no specific data regarding animal toxicity/safety of the UVAHS's progesterone suspension.

13b. Describe pertinent human data that is available regarding the toxicity/safety of this drug.
There are no published data regarding human toxicity/safety of the UVAHS's progesterone suspension. However, the progesterone used to formulate the suspension (i.e., Progesterone USP, micronized, for prescription compounding [NDC 39822-6000-3] Mfg:Spectrum Chemicals and Laboratory Products) is FDA approved. Of course, progesterone is a natural sex-steroid, and we administer a short-course (< 1 day) of physiologic amounts in this protocol. We have used this progesterone suspension in other protocols, and we have thus far administered the progesterone suspension to > 50 subjects. The only adverse event that we felt was unusual (but can occur with progesterone) was mild somnolence with longer term use (several days): the somnolence abated when the progesterone dose was decreased. Other uncommon reported adverse events, which were not unexpected, involved temporary irregular menstrual bleeding.

13c. Have there been any human deaths associated with this drug?
None known.

13d. In how many humans has this drug been used previously?
We have thus far administered the progesterone suspension to > 50 subjects.

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

14. Do the following criteria apply? Check all that 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:

- Regarding micronized progesterone suspension, the IND was required solely because the micronized progesterone is formulated/constituted by our investigational pharmacy. No risk is expected on the basis of the way the progesterone suspension is made (and, at worst, minimal potential risk is associated with progesterone administration). The non-progesterone ingredients of the suspension are innocuous. The progesterone used to formulate the suspension is FDA-approved. The progesterone (in the micronized progesterone) is chemically identical to progesterone made by ovaries, and the achieved concentrations will be less than that observed in the luteal phase of the normal menstrual cycle. Our group has used this formulation successfully in numerous clinical research protocols.
- Regarding transdermal estradiol use: Transdermal estradiol is being used to standardize estradiol levels prior to progesterone/placebo administration. The doses employed will achieve physiological levels appropriate to the late follicular phase (i.e., estradiol levels will not be supraphysiological). The estradiol used in this study is chemically identical to estradiol made by ovaries, and the achieved concentrations will be similar to that observed in the late follicular phase of the normal menstrual cycle. Our group has used transdermal estradiol successfully in numerous clinical research protocols. With this in mind, we believe that the use of transdermal estradiol in this study will be associated with minimal potential risk (at worst).

__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

Privacy Plan

The following procedures must be followed.

- The data will be secured per the Data Security Plan of this protocol.
- Only investigators for this study and clinicians caring for the patient will have access to data. They will each use a unique login ID and password that will keep confidential. The password should meet or exceed the standards described on the Information Technology Services (ITS) webpage about *The Importance of Choosing Strong Passwords.*
- Each investigator will sign the University's Electronic Access Agreement forward the signed agreement to the appropriate department as instructed on the form.

If you currently have access to clinical data it is likely that you have already signed this form. You are not required to sign it again.

- UVa University Data Protection Standards will be followed http://www.virginia.edu/informationsecurity/dataprotection.
- If identifiable data is transferred to any other location such as a desktop, laptop, memory stick, CD etc. the researcher must follow the University's "Electronic Storage of Highly Sensitive Data Policy". Additional requirements may be found in the University's Requirements for Securing Electronic Devices.
- If identifiable data is taken away from the UVa Health System, Medical Center Policy # 0218 will be followed.
- Data will be securely removed from the server/drive, additional computer(s), and electronic media according to the University's Electronic Data Removal Policy.
- Data will be encrypted or removed if the electronic device is sent outside of UVa for repair according to the University's Electronic Data Removal Policy.
- If PHI will be faxed, researchers will follow the Health System Policy # 0194.
- If PHI will be emailed, researchers will follow the Health System Policy # 0193 and University Data Protection Standards .
- Data may not be analyzed for any other study without additional IRB approval.
- If you are using patient information you must follow Health System Policy # 0021.
- Both data on paper and stored electronically will follow the University's Record Management policy and the Commonwealth statute regarding the Destruction of Public Records.

If you have a question or concerns about the required security standards contact ISPRO at it-security@virginia.edu

Summary of Requirements to Comply with UVa Health System, Medical Center and University Policies and Guidance as noted above:

Highly Sensitive Data is:
-personal information that can lead to identity theft if exposed or
-data that reveals an individual's health condition and/or history of health services use.
Protected Data (PHI) a type of Highly Sensitive Data, is data combined with a HIPAA identifier
Identifiable Data under HIPAA regulations is considered to be *Highly Sensitive Data at UVa.*
A **Limited Data Set** (LDS) under HIPAA regulations is considered to be *Moderately Sensitive Data* at UVa. The only HIPAA identifiers associated with data: dates and or postal address information limited to town or city, state, and zip code.
Will not include subjects age if older than 89 or subjects DOB if older than 89.

Highly Sensitive Data (Identifiable Health Info per HIPAA)	**Moderately Sensitive Data (Limited Data Set and De-identified data per HIPAA)**
General Issues	*General Issues*
Discussions in private Do not share with those not on the study team or those who do not have a need to know.	Do not share with those not on the study team or those who do not have a need to know
Password protect	Password protect
Physically secure (lock) hard copies at all times if not directly supervised. If not supervised hard copies must have double protection (e.g. lock on room OR cabinet AND in building requiring swipe card for entrance).	Physically secure (lock) hard copies at all times if not directly supervised.
For electronic documents turn off File Sharing; turn on firewalls; use up to date antivirus and antispyware; delete data securely.	For electronic documents turn off File Sharing; turn on firewalls; use up to date antivirus and antispyware; delete data securely.
Encrypt See Encryption Solutions Guidance *Files on Health System Network drives are automatically encrypted. If not stored there it is study teams responsibility to make sure data are encrypted.*	
If device sent out for service or repair, encrypt or remove data AND contract for repair using a UVa Purchase order.	If device sent out for service or repair, encrypt or remove data AND contract for repair using a UVa Purchase order.
Store files on a network drive specifically designated for storing this type of data, e.g. high-level security server/drives managed by Information Technology Services or the “F” and “O” managed by Heath Systems Computing Services. You may access it via a shortcut icon on your desktop, but you are not allowed to take it off line to a local drive such as the desktop of your computer (e.g. C drive) or to an individual Use Device*. May access via VPN	
Do not share with sponsor or other outside group before consent is obtained or the IRB has granted appropriate approvals and contract/ MTA is in place	Do not share with sponsor or other outside group before consent is obtained or the IRB has granted appropriate approvals and contract/ MTA is in place
If collected without consent/ HIPAA authorization will NOT be allowed to leave UVa HIPAA covered entity unless disclosure is approved by the IRB and the disclosure is tracked in EPIC	If collected without consent/ HIPAA authorization will NOT be allowed to leave UVa HIPAA covered entity unless disclosure is approved by the IRB and an MTA is in place prior to sharing of data

Highly Sensitive Data **(Identifiable Health Info per HIPAA)**	**Moderately Sensitive Data** **(Limited Data Set and De-identified data per HIPAA)**
Electronic Data Collection & Sharing	*Electronic Data Collection & Sharing*
(e.g. smart phone app, electronic consent using tablet etc.) MUST consult with ISPRO or Health System Web Development Office: 434-243-6702 ▪ University Side: IT-Security@virginia.edu ▪ Health System: Web Development Center:	
Individual-Use Device	*Individual-Use Device*
Do not save to individual-use device* without written approval of your Department AND VP or Dean. If approval obtained, data must be password protected and encrypted.	
Do not save an email attachment containing HSD to an individual use device (e.g. smart phone)	
E Mail	*E Mail*
Do not share via email with Outlook Web/ or forward email using other email vendors like Gmail/ Yahoo	
Do not send via email on smart phone unless phone is set up by Health System	
Email may include name, medical record number or Social Security number only if sending email to or from a person with * HS in their email address. *NOTE: VPR & IRB staff do not meet this criteria!*	In addition to sharing LDS, may include initials if persons sending and receiving email work within the UVa HIPAA covered entity.**
FAX	*FAX*
Verify FAX number before faxing	Verify FAX number before faxing
Use Fax Cover Sheet with Confidentiality Statement	Use Fax Cover Sheet with Confidentiality Statement
Verify receiving fax machine is in a restricted access area	Verify receiving fax machine is in a restricted access area
Verify intended recipient is clearly indicated	Verify intended recipient is clearly indicated
Recipient is alerted to the pending transmission and is available to pick it up immediately	Recipient is alerted to the pending transmission and is available to pick it up immediately

Highly Sensitive Data (Identifiable Health Info Per HIPAA	**Moderately Sensitive Data (Limited Data Set and Deidentified data per HIPAA**
Electronic Data Collection & Sharing	*Electronic Data Collection & Sharing*
(e.g. smart phone app, electronic consent using tablet etc…) MUST consult with ISPRO or Health System Web Development Office: 434-243-6702 University Side: IT-Security@virginia.edu Health System: Web Development Center: Contract must include required security measures.	
May be Stored in Qualtrics MAY NOT be stored in places like UVaBOX, UVa Collab or QuestionPro May also NOT be stored I n non-UVA licensed cloud providers, such as Dropbox, Google Drive, SkyDrive, Survey Monkey etc..	May be stored in places like UVaBox, UVaCollab, Qualtrics May NOT be stored in non-UVa licensed cloud providers, such as Dropbox, Google Drive, SkyDrive, Survey Monkey, etc.
LOST OR STOLEN	*LOST OR STOLEN*
Must report in accordance with protocol in accordance with the Information Security Incident Reporting Policy Any data breach will also be reported to the IRB of record in the report meets the criteria of an Unanticipated Problem	Must report in accordance with protocol/ in accordance with the Information Security Incident Reporting Policy. Any data breach will also be reported to the IRB of Record if the report meets the criteria of an Unanticipated Problem.

** Individual Use Device – examples include smart phone, CD, flash (thumb) drive, laptop, C drive of your computer,*
***The UVa HIPAA covered entity is composed of the UVa VP Office of Research, the Health System, School of Medicine, School of Nursing, Nutrition Services (Morrison's), the Sheila C. Johnson Center, the Exercise and Sports Injury Laboratory and the Exercise Physiology Laboratory.*