IRB-HSR# 21649: Relative desirability of metformin vs. birth control pills in treating PCOS in women of later reproductive age.

NCT03905941

PROTOCOL

Background

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

Polycystic ovary syndrome (PCOS) is a highly prevalent reproductive disorder characterized by hyperandrogenism (HA) (e.g., hirsutism, acne, alopecia), oligo/anovulation, and subfertility (1). About 6 to 20% of reproductive aged women are affected by PCOS depending on the diagnostic criteria used (1, 2). PCOS is associated with metabolic syndrome, obesity, insulin resistance, type 2 diabetes mellitus (DM), psychosocial distress and poor quality of life, and possibly cardiovascular (CV) disease (1,3).

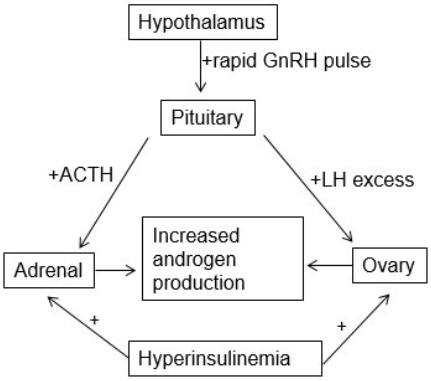

Figure 1. Etiological factors of HA.

In young women with PCOS, many factors contribute to HA, including: 1) rapid gonadotropin-releasing hormone (GnRH) pulse frequency, which favors luteinizing hormone (LH) secretion, and LH excess promotes ovarian androgen production; 2) abnormal ovarian steroidogenesis; 3) abnormal adrenal steroidogenesis; and 4) hyperinsulinemia (related to insulin resistance), which augments LH-stimulated ovarian and ACTH-stimulated adrenal androgen production (1) (Fig. 1).

The classic features of PCOS (HA, oligo/amenorrhea, subfertility) have been the primary foci of PCOS therapies in young women. However, the classical manifestations of PCOS change favorably with aging. For example, women with PCOS in their 40s may experience improvement in biochemical and clinical HA (4) in addition to improvement in ovulatory function (5). In contrast, the metabolic manifestations of PCOS may worsen over the same time frame. Data to guide therapeutic approaches for older reproductive aged women with PCOS are largely lacking.

Large observational studies have shown that serum androgen levels decrease gradually, with improvement in menstrual cycle regularity, ovulation, and fertility, as women with PCOS age (beginning at around 37 years of age) (2,6,7,8). Despite this age-related amelioration in HA, androgen levels remain higher compared to age-matched control women (2,6,9). Pinola et al. examined the change in androgen profile from age 18 to >50 years in women with and without PCOS. Although free androgen index (FAI), calculated free testosterone (T), androstenedione (A4) levels remained higher than normal women in all age groups, the difference in androgen levels between women with PCOS and normal controls *narrowed* with increasing age (e.g., the gap for T decreased by approximately 70%) (2).

Use of oral contraceptives in aging women and its risks

Oral combined hormonal contraceptives (OCs) are effective in reducing HA in PCOS by suppressing LH secretion and increasing hepatic synthesis of SHBG. OCs are first line medical therapy for treating the classic symptoms of PCOS (e.g., hirsutism, menstrual irregularity) until

menopause (1,10). However, OCs can elevate blood pressure (BP) and triglyceride levels, and OCs have been associated with venous thromboembolism (VTE) and increased risk of myocardial infarction and ischemic stroke (11,12,13,14,15). Given the high prevalence of metabolic syndrome in women with PCOS, the adverse CV risks of OCs could possibly be exaggerated in women with PCOS, especially as they get older. Obesity is common among women with PCOS, and obesity increases the risk of OC-associated VTE (16). In addition, metabolic syndrome is associated with increased risk of arterial thrombosis at baseline (17,18,19). According to the medical eligibility criteria for OC use, the risks of OCs outweigh the benefits when multiple CV risk factors are present (20,21), and this may be increasingly relevant as women with PCOS get older. While OCs offer reliable contraception, other contraceptive options with lower CV risks exist. Similarly, while OCs offer reliable protection from anovulation-related endometrial hyperplasia, other options exist (e.g., progestin-only pills, progestin IUDs)—and this could possibly be less of a concern in women with PCOS who experience improved ovulatory function with age.

When using OCs in clinical practice, the risk-benefit ratio should be considered carefully in each individual. In early reproductive aged women with PCOS, the likely benefits of OCs usually outweigh the possible risks, and the use of OCs is generally favored as the first-line treatment for PCOS. However, it is unclear if this continues to be the case in later reproductive aged women with PCOS.

Metformin as a potential therapy for improving HA, anovulation, and other metabolic risk factors in aging PCOS women

Therapies to reduce hyperinsulinemia have been studied and used clinically for the treatment of PCOS. Metformin is an oral drug that is primarily used to treat type 2 DM. Metformin is also used as a therapy for PCOS given its ability to reduce hyperinsulinemia (22). Indeed, metformin is the insulin sensitizing drug for which the efficacy and safety profile has been most rigorously studied in PCOS. Metformin can have direct and indirect effects on ovarian and adrenal steroidogenesis, lowering androgen levels (23,23,25,26). Metformin may also reduce LH secretion and increase hepatic SHBG synthesis, thus improving biochemical HA (22,27). In addition to improvement of biochemical HA, metformin can improve ovulatory function in young women with PCOS (28). Regardless, metformin is not generally considered a first-line monotherapy in PCOS because OCs are superior for the treatment of the classic symptoms of PCOS. However, whether metformin can treat PCOS adequately in older women (when HA and menstrual function naturally improve) is not known.

IGT and type 2 DM in PCOS are more prevalent in PCOS compared to normal age-matched population (29); and metformin may have protective effects in this regard. In patients with IGT, metformin reduces progression to type 2 DM by 31% (30), and it reduces CV mortality in patients with type 2 DM (31). Although there is no high-quality evidence that addresses the efficacy of metformin in preventing DM or CV events in PCOS, in one meta-analysis, metformin significantly reduced fasting glucose, fasting insulin and homeostatic model assessment (HOMA) index in PCOS (32). Metformin also lowers LDL and triglyceride levels and increases HDL levels (33,34,35).

In addition, a randomized controlled study recently suggested that the changes in quality of life (QoL) between metformin and OCs are comparable in young women with PCOS (3). This study, however, may have been underpowered to detect small differences in QoL, and the questionnaire used to assess QoL was not specific to PCOS. Moreover, since both biochemical HA, clinical HA, and ovulatory function naturally improve with aging in PCOS, it is plausible that such a QoL comparison could have different results in older vs. younger women with PCOS. A therapy's impact on QoL is an important factor to incorporate in any patient-centered decision-making process. However, to our knowledge, there have been no studies that compared the impacts of PCOS therapy options (e.g., OC vs. metformin) on QoL in older women with PCOS.

Since HA and menstrual function appear to improve with age, the effectiveness gap between OCs vs. metformin, which is evident in young women with PCOS, may narrow with age. Similarly, since CV risks of OCs increase with aging, the risk profile gap between these therapies may widen in later reproductive years. However, the relative efficacy of OC vs. metformin has not been studied in older women with PCOS (Fig.3).

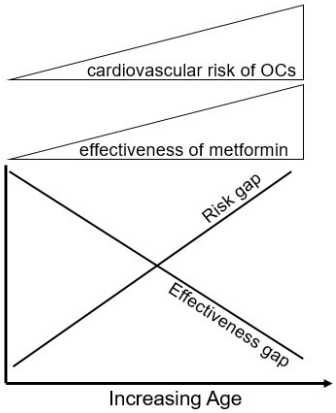

Figure 3. Proposed relationship between age and relative efficacy of MF (vs. OC) for treating PCOS.

What we know about the benefits and risks of OCs primarily relates to use for contraception in younger women without PCOS; in general, we must extrapolate these data to women with PCOS. (Of note, the published medical eligibility criteria for OC use are predicated on the notion that OCs are primarily used for contraception, and this may not fully apply to some women with PCOS.) When we apply the same risk-benefit considerations to older women with PCOS, we are extrapolating a step further. We do not have a clear understanding about how the risk-to-benefit ratio changes with aging in PCOS. Similarly, what we know about the relative benefits of OCs vs. metformin in PCOS are largely based on studies of younger women with PCOS. We proposed to study the relative desirability of OCs vs. metformin in late-reproductive age women with PCOS, and how these treatments impact pathophysiology, patient satisfaction, and various CV risk factors. High-quality evidence on how different therapy options affect QoL in older women with PCOS do not exist, yet QoL is an important issue for medical providers to consider when delineating treatment options. Data generated in this study will help delineate therapy options with the most favorable patient-centered outcomes.

Objectives/Hypothesis

Objectives: The objective of the study is to determine the relative patient-desirability of metformin vs. OCs—as measured by QoL assessments—in older reproductive aged women with PCOS.

Hypothesis: In light of their respective effects on the classic and metabolic facets of PCOS, metformin will provide non-inferior patient satisfaction compared to OCs in later reproductive age women with PCOS.

Primary outcome measures: The primary outcome is health-related QoL (HRQoL) via PCOS Questionnaire (PCOSQ). Our rationale for this is two-fold. First, we view QoL as a critically important and highly patient-relevant factor that should be carefully assessed in therapeutic studies. Second, we expect metformin and OC to have divergent effects on a number of important endpoints, but it remains unclear how we would summarize multiple important changes with a single objective endpoint. (That is, it is unclear how we would appropriately weigh different effects—for example, is the change in free T more relevant than the change in 2-hour glucose during OGTT?) With HRQoL, such determinations are made by subjects themselves, which guarantees relevance.

Secondary outcome measures: Short Form (SF)-36 score, General Anxiety Disorder (GAD)-7 score, total T concentrations, calculated free T concentrations, sex hormone binding globulin, LDL cholesterol level, HDL cholesterol level, triglyceride level, blood pressure, weight, body mass index, waist-to-hip ratio, Matsuda index, fasting insulin, fasting glucose, 2-hour glucose during oral glucose tolerance test, hemoglobin A1c, and Framingham risk score.

Study Design: Biomedical

1. Will controls be used?

No (but subjects will serve as their own controls in this crossover study)

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

Answer/Response: N/A

2. What is the study design?

Example: case series, case control study, cohort study, randomized control study, single-blind, double-blind, met-analysis, systematic reviews, other. You may also view the IRB-HSR Learning Shot on this topic to help you answer this question. (http://www.virginia.edu/vpr/irb/learningshots/Writing_protocol_June09/player.html

Randomized, controlled, double-blinded, crossover study.

3. Does the study involve a placebo?

Yes, the study involves placebo pills.

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

The placebo pills in this study are not being used as controls (vs. treatment). The subjects will receive both OCs and metformin during the study, but they will receive these as single treatments in sequence (e.g., OC for 6 months then metformin for 6 months, or metformin for 6 months then OC for 6 months). OCs are given once daily while metformin is given twice daily. To help facilitate blinding to treatment allocation, we will provide dummy placebo pills for the alternate therapy. Thus, subjects receiving metformin will also receive a dummy placebo pill (for OC) to be taken once a day. Similarly, subjects receiving OC will receive dummy placebo pills (for metformin) to be taken twice a day.

Human Participants

Ages: 40-49 yo
Sex: Female
Race: All

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

2. **Describe expected rate of screen failure/ dropouts/withdrawals from all sites.**
   20%

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

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

Inclusion/Exclusion Criteria

1. **List the criteria for inclusion**
   - Women with PCOS aged 40-49 years. Subject is considered to have PCOS if she has current or verifiable history of: a) clinical and/or biochemical evidence of hyperandrogenism plus b) oligomenorrhea or irregular menstruation (substantially inconsistent menstrual cycle length). Subjects with fewer than 10 menses/year or average menstrual cycle length >35 days are allowed to participate if they have a compelling past history of oligomenorrhea (average menstrual cycle length >45 days or fewer than 9 menses/year) or irregular menstruation.
   - Screening safety labs within normal reference ranges although mild abnormalities that are common in obesity and/or hyperandrogenism will not be grounds for exclusion (see exclusion criteria).
   - Subjects must be willing and able to provide written informed consent.
   - Willingness to strictly avoid pregnancy (using non-hormonal methods) during the time of the study.

2. **List the criteria for exclusion**
   - Postmenopausal status
   - Biochemical evidence for perimenopause as defined by an anti-Mullerian hormone <0.5 ng/mL. As an alternative, cycle day 3 FSH > 9 IU/L (with concomitant estradiol level >80 pg/mL), if this testing is available, will serve as evidence of perimenopause status. NOTE: If FSH >9 IU/L on screening (but it is not cycle day 3), FSH and estradiol will be repeated on cycle day 3 (36, 37).
   - History of hysterectomy and/or bilateral oophorectomy

- BMI ≥ 40 kg/m^2
- Inability to comprehend what will be done during the study or why it will be done.
- Being a study of older women with PCOS, children and men will be excluded.
- Pregnancy or lactation within the past 6 months. Subjects with a positive pregnancy test will be informed of the result by the screening physician.
- History of (or clinical evidence for) Cushing's syndrome or adrenal insufficiency.
- History of congenital adrenal hyperplasia or 17-hydroxyprogesterone (17-OHP) >200 ng/dL, which suggest the possibility of congenital adrenal hyperplasia. 17-OHP will be collected during follicular phase, or ≥ 40 days since last menses if oligomenorrheic. NOTE: if a 17-OHP >200 ng/dL and is confirmed on repeat testing, an ACTH-stimulated 17-OHP <1000 ng/dL will be required for study participation.
- Total testosterone >150 ng/dL, which suggests the possibility of virilizing neoplasm.
- DHEA-S greater than the upper limit of normal range (mild elevations may be seen in PCOS, and elevations < 1.5 times the upper limit of normal will be accepted in these groups).
- Virilization
- Diagnosis of diabetes mellitus (DM), fasting glucose ≥ 126 mg/dL, or a hemoglobin A1c of ≥ 6.5%.
- Abnormal thyroid stimulating hormone (TSH). Subjects with stable and adequately-treated hypothyroidism, reflected by normal TSH values, will not be excluded.
- Moderate to severe hyperprolactinemia. Mild prolactin elevations may be seen in PCOS, and elevations < 1.5 times the upper limit of normal will be accepted in this group.
- Persistent liver abnormalities, with the exception that mild bilirubin elevations will be accepted in the setting of known Gilbert's syndrome. Mild transaminase elevations may be seen in obese women, so elevations <1.5 times the upper limit of normal will be accepted in this group.
- Persistent hematocrit <36% and hemoglobin <12 g/dL. Note: If a low hematocrit and/or 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 twice daily) with a subsequent recheck of hematocrit and/or hemoglobin.
- Abnormal sodium, potassium, or bicarbonate concentrations or elevated creatinine concentration.
- Significant history of pulmonary dysfunction (e.g., asthma or COPD requiring intermittent systemic corticosteroid, pulmonary hypertension, etc.).
- History of known or suspected congestive heart failure.
- History of known or suspected ischemic heart disease or cerebrovascular disease.
- History of hypertension.
- History of uncontrolled/untreated dyslipidemia. Subjects with stable and adequately treated dyslipidemia reflected by normal lipid panel values will not be excluded.
- History of complicated valvular heart disease (e.g. pulmonary hypertension, risk of atrial fibrillation, history of subacute bacterial endocarditis)
- History of stroke
- Current cigarette smoking (of any amount)

- History of severe cirrhosis or liver tumor (e.g. hepatocellular adenoma or malignant hepatoma).
- Use of anticonvulsants, rifampicin or rifabutin therapy. The interaction of these drugs with OCs will not be harmful to the subjects, but it will reduce the effectiveness of OCs.
- History of venous thromboembolism (e.g. deep venous thrombosis (DVT), pulmonary embolism (PE)).
- Personal history of blood clotting disorders (e.g., protein C, protein S, positive antiphospholipid antibodies).
- First-degree relative history of blood clotting disorder, unless the same disorder has been formally excluded for the study subject.
- History of migraine headaches.
- History of breast, ovarian, or endometrial cancer.
- Note: If endometrial thickness on transvaginal ultrasound is >8 mm in the proliferative (follicular) phase or >14 mm in the secretory (luteal) phase, the subject will be referred to a gynecologist for further evaluation (38). These particular subjects will be required to obtain a clearance from their gynecologist to participate in this study.
- Note: Any abnormal labs may be repeated to exclude a lab error.
- No medications known to affect the reproductive system can be taken in the 2 months prior to screening and in the 3 months prior to the study. Such medications include oral contraceptive pills, metformin, progestins, glucocorticoids, anti-psychotics, and/or mood stabilizers that are known to cause hormone abnormalities.

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

Other than the study medications, no medications known to affect the reproductive system (as listed in "Exclusion Criteria") can be taken during the study.

Statistical Considerations

1. Is stratification/randomization involved?

Yes

►IF YES, describe the stratification/ randomization scheme.

INSTRUCTIONS:
The stratification factors and/or the randomization plan should be identified. If there is no randomization component or important patient characteristics that will be used in treatment allocation or data analysis, a statement to this effect should be included.

Stratification factors: These are pretreatment patient characteristics which could be balanced across treatment arms by design or may be used to determine starting dose or treatment allocation.

If randomization is going to be used, the details of the randomization plan should be described.

The description should include:

--the method and timing of randomization
--the type of randomization scheme that will be used in the study
--whether or not the randomization masked/blinded/if so, then to whom is it masked/blinded
--who has access to the randomization scheme

Treatment allocation in this study (OCs vs. metformin) is determined by the randomization schedule (known only to Investigational Pharmacy), and we do not find out about the treatment allocation while the study is ongoing. That is, the study is effectively blinded to the research subject and the investigators at the time of study. However, after the data analysis is complete, treatment allocation will be unblinded. Given the GI side effects that may be associated with metformin and the predictable bleeding pattern that usually occurs with OCs (every 28 days), there is a possibility that subjects will be able to guess the treatment they are receiving. One way to enhance the level of blinding would be to provide progestin therapy (while taking metformin) on a monthly basis to induce withdrawal bleeding every 28 days. However, the addition of progestin would confound our ability to assess metformin as a monotherapy. Also, even if episodic progestin were to be given together with metformin, we still could not eliminate the possibility that GI side effects would indicate likely treatment allocation. Due to the nature of the interventions, we acknowledge that full blinding may not be possible. We will ensure that study personnel (investigators, CRU nurses, and statistician) will remain blinded to treatment allocation until data analysis is complete. To help facilitate blinding to treatment allocation, we will provide dummy placebo pills for the alternate therapy. Thus, subjects receiving metformin will also receive a dummy placebo pill (for OC) to be taken once a day. Similarly, subjects receiving OC will receive dummy placebo pills (for metformin) to be taken twice a day. At the end of the study, we will also ask the subjects the order of treatments they think they received.

The subjects will be randomized to the order of which drug is taken first, either OCs or metformin. This randomization will be generated by a Department of Public Health Sciences biostatistician using the PROC PLAN procedure of SAS version 9.3 (SAS Institute Inc., Cary NC).

►**IF YES, who will generate the randomization scheme?**

_____ Sponsor
__x___ UVa Statistician. Insert name Answer/Response: James Patrie, M.S.
_____ UVa Investigational Drug Service (IDS)
_____ Other*:* Specify Answer/Response:

2. What are the statistical considerations for the protocol?

The objectives section and the statistical section should correspond, and any objective for which analysis is unfeasible should be deleted. Also, the estimates and non-statistical assumptions of the statistical section should be supported by discussion in the background section.

The answer to this question should include:
--Study Design/Endpoints
--Recap of study objectives and endpoint definitions. An assessment of how study objectives will be assessed by identifying & defining which endpoints will be used to assess each component of the study objectives.
--The study design should include contingencies for early stopping, interim analyses, stratification factors (If applicable), and any characteristics to be incorporated in analyses.
--The power/precision of the study to address the major study endpoint(s), the assumptions involved in the determination of power/precision.
--If statistical hypothesis testing is included then specify the null and alternative hypotheses, the test statistic, and the type I and II error rates
--If precision of an estimate, then provide a definition for precision
--If other, then specify

As a two treatment and two period crossover study, the primary goal of this study will be to determine the relative desirability of metformin vs. OCs in treating classical PCOS symptoms and metabolic parameters in late reproductive-age women with PCOS. In late reproductive-age women with PCOS, metformin will provide non-inferiority patient satisfaction (vs. OCs) with regard to the womens' perceived health-related quality of life. If 73 late reproductive-age women with PCOS complete the study protocol, we expect to have at least an 80% chance of rejecting the null hypothesis that with respect to health-related quality of life metformin therapy is inferior OCs therapy, in favor of the alternative that with respect to health-related quality of life metformin therapy is non-inferior to OCs therapy. Health-related quality of life will be assessed via the participants' post-therapy (i.e. post metformin therapy and post OCs therapy) responses to the 5 domains of questions of the PCOS Questionnaire (PCOSQ). Each PCOSQ domain will require the participants to select a value between 1 and 7, which reflects the participant's subjective post-therapy opinion about how the therapy impacted a particular aspect of the participant's health-related quality of life. Under the null hypothesis we assume that the mean *intra-subject* difference between the post-metformin-therapy questionnaire domain score and the post-OCs-therapy questionnaire domain score will differ by greater than the *non-inferiority* margin of 0.5 units in favor of the use of OCs therapy to treat PCOS in late reproductive age women, with the alternative that the mean *intra-subject* difference between the post-metformin-therapy and post-OCs-therapy questionnaire domain scores is 0.5 units or less. To conduct the power analysis, the PAIREDMEANS statement of the POWER procedure of SAS version 9.4 was used. As statement input, we assumed that the true underlying mean *intra-subject* difference between the post-metformin-therapy questionnaire domain score and the post-OCs-therapy questionnaire domain score is 0 units, and the *null difference* (i.e. the non-inferiority margin) is 0.5 units in favor of the use of OCs therapy to treat PCOS. In the PAIREDMEANS statement, we specified the inter-subject variability in the post-therapy PCOSQ domain score to be 1.7 units irrespective of the therapy, and we specified the *intra-subject* correlation between the post metformin-therapy questionnaire domain score and the post-OCs-therapy questionnaire domain score to conservatively be at least than 0.5.

Hypothesis testing: Under the null hypothesis it will be assumed that the mean *intra-subject*

difference between the post metformin therapy PCOSQ domain score and the post OCs therapy PCOSQ domain score is greater than the non-inferiority margin of 0.5 units in favor of the use of OCs therapy to treat PCOS in late reproductive-age women, with the non-inferiority alternative hypothesis that the mean *intrasubject* difference between the metformin therapy PCOSQ domain score and OCs therapy PCOSQ domain score is 0.5 units or less. A linear contrast of means will be utilized to test this hypothesis and a one-sided upper 95% confidence limit will be derived for the mean *intra-subject* difference between the metformin therapy PCOSQ domain score and OCs therapy PCOSQ domain score (i.e. mean ΔOCs – metformin). If the one-sided upper 95% confidence limits does not exceed 0.5 units, the null hypothesis will be rejected.

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

In the PAIREDMEANS statement we specified an one-sided α=0.05 upper tailed non-inferiority test. Based with this set of PAIREDMEANS statement input parameters, we determined that if 73 late reproductive-age women complete the crossover study protocol, we should have at least 0.80 statistical power to reject the null hypothesis that with respect to treating PCOS in late reproductive-age women, health-related quality of life while on metformin therapy is inferior to health-related quality of life while on OCs therapy. With adjustment for the drop out/ineligibility rate of 20%, we target to enroll 88 subjects for the study. We anticipate that we will recruit 1-2 subjects every month.

4. What is your plan for primary variable analysis?

Include primary outcome(s)/predictor variable(s), statistical methods/models/tests to be employed, or descriptive summaries as appropriate. If not applicable, please provide explanation.

Per PCOS questionnaire (PCOSQ) domain, the post-therapy health-related quality of life scores will analyzed via a linear mixed model (LMM), in which the LMM will be specified in accordance with a 2 treatment by 2 period crossover design***. LMM specification***: The LMM dependent variable will be the PCOSQ domain score, and the LMM independent variables will the therapy (i.e. metformin versus OCs), the sequential temporal order of the interventions (i.e. metformin$\rightarrow$ OCs, or OCs$\rightarrow$ metformin), and the crossover study period (first period, second period). The random effects of the LMM will be specified so that the domain score component of variability attributed to *within-sequence* variability will be separated from the residual component of domain score variability, and thereby increasing the statistical power of the hypothesis test.

5. What is your plan for secondary variable analysis?

Include the following: --Secondary outcome(s)/predictor variables, statistical methods/models/tests to be employed, or descriptive summaries as appropriate. If not applicable, please provide explanation. --For phase III studies, the power/precision of the study to address the secondary objective(s).

The secondary variable analysis will be performed using the method that is identical to the primary variable analysis.

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

IF YES, what is their name?
James Patrie, M.S.

7. Will data from multiple sites be combined during analysis?
No

INSTRUCTIONS: IF YES, answer the following questions

7(a). Does the study involve randomization?
Answer/Response:

IF YES, will randomization be done at each site or among sites?
Answer/Response:

7(b). Has the sample size calculation considered the variation among sites?
Answer/Response:

7(c). When combining the data from multiple sites to assess the study results, is the effect of the treatment to be tested (or the association to be tested) assumed to be the same across sites or vary among sites? What is the modelling strategy?
Answer/Response:

7(d). Is there a common protocol used in all sites?
Answer/Response:

IF NO, how will differences among sites, such as those related to the implementation, inclusion criteria, patient characteristics, or other sites characteristics, be considered to assess the study results?
Answer/Response:

Study Procedures-Biomedical Research

1. What will be done in this protocol?

INSTRUCTIONS:
This should include everything that will be done as part of this protocol. Do not repeat information that is included in other sections such as Background or Hypothesis sections.
This section should include an indication of which research interventions if any offer a prospect for direct benefit and which interventions (invasive measurements, collection of blood, tissue, data, surveys, etc.) are being done solely to answer a research question and generate generalizable knowledge. If the interventions done solely for research purposes are associated with greater than minimal risk they need to be justified. Describe and justify any control and experimental arm and include method, dose, and duration of drug administration. Reference any claim of clinical equipoise if applicable.

If you are obtaining specimens or data, provide information regarding the type of specimen/data, amount of specimen needed and how the specimen/data will be obtained and what analysis will be done with the specimen/data.

Special note for studies with waiver of consent/waiver of documentation of consent: Include a statement regarding how subjects will be recruited. For other studies this information is captured in Recruitment does not need to be duplicated in this section.

Interventions in this research study can directly benefit the volunteers since the study medications are commonly and widely used to treat PCOS, but all interventions are being done solely to answer a research question and generate generalizable knowledge.

We will recruit women with PCOS aged 40-49 years. The subjects will be recruited from primary care, endocrine and gynecology clinics at the University of Virginia (UVA) Health System and other local clinics. We will also use other advertisement methods including informational letters to local physicians, fliers/brochures at medical clinics, study advertisements on UVA websites, Facebook, PCOS support websites, local newspapers and magazines.

Screening procedures (visit 1):

Informed consent will be carefully obtained from interested parties. Participants will undergo detailed screening procedures to identify exclusion criteria and to ensure good general health. Such procedures include:

- Detailed medical history and chart review. Medical history will include data on menstrual history, race and ethnicity, symptoms of HA, reproductive history, thorough review of past medical history and family history (to ensure eligibility criteria). When appropriate and if granted with permission, patient charts for previous laboratory and medical history data will be reviewed to obtain information about general health and hormonal status. Data from subjects' electronic records will be reviewed to verify their history of PCOS if needed (and if permission is granted to do so). In some women with PCOS, the clinical manifestations of this condition (i.e. clinical or biochemical HA, menstrual irregularity, polycystic ovary morphology) can improve as they age. As such, we expect that there will be instances we will want to verify their history by reviewing lab results, physician's notes, etc.
- Physical exam (including Ferriman Gallwey score).
- Vital signs (height, weight, blood pressure and heart rate)
- Blood samples collected while fasting at 0800-0900 h will be tested for complete blood count, comprehensive metabolic panel (electrolytes, glucose, renal and liver function), hemoglobin A1c, LH, FSH, progesterone (P4), estradiol (E2), total T, SHBG, 17-OHP, androstenedione, DHEA-S, AMH, beta-hCG, thyroid stimulating hormone (TSH), cortisol, prolactin, lipid panel, and fasting insulin (about 17 ml). These laboratory tests will exclude anemia, pregnancy, and other hormone abnormalities. A subject will be considered to have PCOS if she has current or verifiable history of: a) clinical and/or biochemical evidence of HA plus b) oligomenorrhea, but without evidence for other causes of HA and/or oligomenorrhea (see Inclusion/Exclusion criteria).
- Body composition analysis (total fat mass, percent body fat) using BOD POD® will be performed.
- Waist and hip circumference will be measured.

Unless otherwise stated, all blood samples will be assayed at the Center for Research in Reproduction's Ligand Assay & Analysis Core. The following labs will be analyzed at UVA Clinical laboratory: CBC, CMP, lipid panel, HgA1c, pregnancy test (beta-hCG). An additional single sample (additional 1 ml) will be obtained to measure T by liquid chromatography-tandem mass spectrometry (LC-MS/MS; Mayo Clinic Laboratories). However, our immunoassay for total T values historically correlated very well with measurement of total T by LC-MS/MS; Mayo Clinic Laboratories (39, 40). For testosterone by LC-MS-MS, samples must be drawn in *plain, red-top tubes* (serum gel tubes are not acceptable as they may cause interference in the assay). These samples will be sent to the Mayo Clinic (via the UVA Health System, main hospital lab) for analysis. These samples must remain refrigerated until analysis.

If a low hematocrit and/or 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 twice daily) with a subsequent recheck of hematocrit and/or hemoglobin.

Pre-Study (visit 2) Outpatient visit (transvaginal ultrasound visit)
Once the subjects meet the screening eligibility criteria, subjects will return to have transvaginal ultrasound performed to assess for endometrial wall thickness. If endometrial thickness is >8 mm in the proliferative (follicular) phase or >14 mm in the secretory (luteal) phase the subject will be referred to a gynecologist for further evaluation (38). These particular subjects will be required to obtain a clearance from their gynecologist to participate in this study. We will also assess for ovarian antral follicle counts. For any incidental findings, we will refer to their primary gynecologists or primary care provider for further evaluation prior to continuing with or excluding from the study.

Randomization: Subjects will be randomized to a treatment during visit 3.

Outpatient medication dispense and oral glucose tolerance test visit (visit 3):
If the transvaginal ultrasound does not show any concerning ovarian and endometrial abnormality that precludes the subjects from being in the study, their study medications (metformin or oral contraceptive pills) and oral micronized progesterone pills will be dispensed. If it has been greater than 3 months since comprehensive metabolic panel has been checked, we will repeat this before starting study medications. Beta-hCG will be measured to rule out pregnancy.

Oral glucose tolerance test will be performed during this visit. Baseline glucose and insulin levels will be checked (about 1 ml). Subjects will then drink 75 g oral glucose solution and blood will be drawn for 30-, 60-, 90-, 120- minute insulin and glucose levels (about 1 ml each). This is a standard oral glucose tolerance test and will also allow us to calculate a Matsuda index (an estimate of insulin sensitivity). Subjects will also fill out PCOSQ, SF-36, GAD-7 scale questionnaires (as baseline).

Subjects will be randomized to either receive metformin or low dose OC (i.e., containing 20 mcg ethinyl estradiol dose]) for a total of 6 months, and they will crossover to the other treatment

for the following 6 months. We will use metformin 1000 mg twice daily; metformin is commonly used for management and prevention of type 2 diabetes with minimal side effects. The risks of side effects can be reduced further when the dose is titrated slowly. Subjects will start metformin 500 mg daily and increase in increments of 500 mg weekly (e.g. 1st week: 500 mg daily; 2nd week: 1000 mg daily; 3rd week: 1500 mg daily; 4th week: 2000 mg daily). We will use a low-estrogen dose combined OC (ethinyl estradiol 20 mcg / norethindrone acetate 1 mg). Among available OC formulations, we believe that this combination of low dose ethinyl estradiol (20 mcg) and this particular progestin (lower metabolic risk compared to levonorgestrel and lower venous thromboembolism [VTE] risk compared to third- and fourth-generation progestins) is preferable. We are using the lower dose of ethinyl estradiol to minimize the risk of VTE.

Prior to beginning study medication for the first time, subjects will take oral micronized progesterone 200 mg daily at bedtime for 10 days to induce menstrual bleeding. This is to minimize the risk of endometrial hyperplasia that may potentially occur if subject is randomized to metformin first and if the subject doesn't have regular menses while on metformin. This same maneuver will be unnecessary immediately prior to OC use since OC use will effectively prevent endometrial hyperplasia.

During the study, we will also measure salivary progesterone levels twice weekly to assess for ovulatory rate, which will be an important endpoint to assess while a subject is taking metformin (we expect that ovulation should not occur while on OCs). Subjects will be given salivary collection kits to collect the saliva samples at home. Saliva will be collected twice weekly after fasting for 60 min and after rinsing with water, and at least 12 hours after using mouth rinses containing alcohol (41, 42). Subjects will be instructed to store the saliva samples in a home freezer until collected by study coordinator at least every 3 months. Subjects will be encouraged to bring the saliva samples back sooner if possible.

Outpatient safety visit every 3 months (visits 4 and 6)

For safety surveillance, blood will be drawn to measure electrolyte levels, renal function, liver function, and pregnancy tests (about 3 ml) every 3 months after being on the study medication. Every 3-month follow-up visit will also allow us to assess the general health of subjects and to reduce drop-out. Subjects will also return their saliva samples and pick up new saliva collection kits.

Outpatient visit after completion of each study medication at 6 and 12 months (visits 5 and 7)

After 6 months after each study medication, we will assess blood pressure, weight, waist-to-hip ratio, average intermenstrual cycle length (in the previous 3 months), Ferriman-Gallwey score (as a measure of hirsutism), estimated cardiovascular risk (Framingham risk score), HRQoL using both PCOSQ and SF-36, and severity of anxiety using GAD-7 scale. Body composition analysis (total fat mass, percent body fat) using BOD POD® will be performed. Blood samples will be collected for total T, SHBG, progesterone, HgA1c, LDL- cholesterol, HDL-cholesterol, and triglycerides (about 9 ml), in addition to the three month safety labs and pregnancy test. An oral glucose tolerance test will be repeated as described for the baseline visit. Of note,

testosterone concentrations will also be obtained with measurement by LC-MS/MS; Mayo Clinical Laboratories at the end of each study as well. Specifically, 1 ml (additional) blood will be taken. For testosterone by LC-MS-MS, samples must be drawn in *plain, red-top tubes* (serum gel tubes are not acceptable as they may cause interference in the assay). These samples will be sent to the Mayo Clinic (via the UVA Health System, main hospital lab) for analysis. These samples must remain refrigerated until analysis. Subjects will also return their saliva samples (both 6 month and 12 month visits) and pick up new saliva collection kits (for 6 month visit only). Subjects will be given the second study medication at the end of the 6 month visit.

At the completion of the entire study (i.e after taking both medications), we will ask the subjects which treatment they preferred and which treatment they think is more beneficial.

Note: The subjects will keep a diary of when the medications were taken. The subjects will be asked to bring the bottle and the medication diary at visits 4, 5, 6 and 7. The clinical coordinator will also contact the subjects every 2-4 weeks to remind them about study procedure compliance (e.g. taking medications daily).

2. **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.**

   At the conclusion of study, subjects will be advised to contact their medical provider (e.g. primary care provider or endocrinologist or gynecologist) for further management of PCOS.

Subject Compliance with Study Procedures

1. **Explain how the study team will monitor the subject for compliance with the study procedures.**

   (e.g. study team will administer study drug/ study interventions, study drug inventory of dispensed and returned drug, diary etc.)

   Answer/Response: The Center for Research in Reproduction clinical coordinator will dispense the medications at the outpatient visits. The subjects will keep a diary of when the medications were taken. The subjects will be asked to bring the bottle and the medication diary at visits 4, 5, 6, and 7. The clinical coordinator will also contact the subjects every 2-4 weeks to remind them about study procedure compliance (e.g. taking medications daily).

2. **Describe criteria for when a subject is considered to be non-compliant with study procedures.**

   The subjects will be considered to be non-compliant with the study procedures if the subject returns more than 20% of the study drug, and/or if the subject misses any of the follow-up visits. However, the subjects will be given an opportunity to reschedule a follow-up appointment within 2-3 weeks of the scheduled visit when unexpected events occur (e.g. family emergency, weather, etc.).

Bibliography

1. McCartney C, Marshall J. Polycystic ovary syndrome. *N Engl J Med* 2016;375:54-64.
2. Pinola P, Piltonen T, Puurunen J, Vanky E, Sundstrom-Poromaa I, Stener-Victorin E, et al. Androgen profile through life in women with polycystic ovary syndrome: a nordic multicenter collaboration study. *J Clin Endocrinol Metab* 2015;100(9):3400-3407.
3. Altinok ML, Ravn E, Andersen M,Glintborg, D. Effect of 12-month treatment with metformin and/or oral contraceptives on health-related quality of life in polycystic ovary syndrome. *Gynecol Endocrinol* 2018;Epub ahead.
4. Alsamarai S, Adams J, Murphy M, Post M, Hayden D, Hall J, et al. Criteria for polycystic ovarian morphology in polycystic ovary syndrome as a function of age. *J Clin Endocrinol Metab* 2009;94(12):4961-4970.
5. Welt C. Lifecycle of polycystic ovary syndrome (PCOS): from in utero to menopause. *J Clin Endocrinol Metab* 2013;98(12):4629-4638.
6. Winters S, Talbott E, Guzick D, Zborowski J, McHugh K. Serum testosterone levels decrease in middle age in women with the polycystic ovary syndrome. *Fertil Steril* 2000;73(4):724-729.
7. Elting M, Korsen T, Rekers-Mombarg L, Schoemaker J. Women with polycystic ovary syndrome gain regular menstrual cycles when ageing. *Hum Reprod* 2000;15(1):24-28.
8. Hudecova M, Holte J, Olovsson M, Sundstrom Poromaa I. Long-term follow-up of patients with polycystic ovary syndrome: reproductive outcome and ovarian reserve. *Hum Reprod* 2009;24(5):1176-1183.
9. Markopoulos M, Rizoas D, Valsamakis G, Deligeoroglou E, Grigorious O, Chorousos G, et al. Hyperandrogenism in women with polycystic ovary syndrome persists after menopause. *J Clin Endocrinol Metab* 2011;96(3):623-631.
10. Legro R, Arslania S, Ehrmann D, Hoeger K, Murad M, Pasquali R, et al. Diagnosis and treatment of polycystic ovary syndrome: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab* 2013;98(12):4565-4592.
11. De Melo A, Dos Reis R, Ferriani R, Vieira C. Hormonal contraception in women with polycystic ovary syndrome:choices, challenges, and noncontraceptive benefits. *Open access J Contracept* 2017;8:13-23.
12. Curtis K, Mohllajee A, Martins S, Peterson H. Combined oral contraceptive use among women with hypertension: a systematic review. *Contraception* 2006;73(2):179-188.
13. Dragoman M, Curtis K, Gaffield M. Combined hormonal contraceptive use among women with known dyslipidemias:a systematic review of critical safety outcomes. . *Contraception* 2015;94(3):280-287.
14. Khader YS, Rice J, John L, Abueita O. Oral contraceptives use and the risk of myocardial infarction: a meta-analysis. *Contraception* 2003;68:11-17.
15. Baillargeon JP, McClish DK, Essah PA, Nestler JE. Association between the current use of low-dose oral contraceptives and cardiovascular arterial disease: a meta-analysis. *J Clin Endocrinol Metab* 2005;90(7):3863-70.
16. Pomp E, le Cessie S, Rosendaal F, Doggen C. Risk of venous thrombosis: obesity and its joint effect with oral contraceptive use and prothrombotic mutations. *Br J Haematol.* 2007;139(2):289-296.

17. Glueck C, Papanna R, Wang P, Goldenberg N, Sieve-Smith L. Incidence and treatment of metabolic syndrome in newly referred women with confirmed polycystic ovarian syndrome. *Metabolism* 2003;52(7):908-915.
18. Apridonize T, Essah P, Iuorno M, Nestler JE. Prevalence and characteristics of the metabolic syndrome in women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 2004;90(4):1929-1935.
19. Grundy S, Cleeman J, Daniels S, et.al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 2005;112(17):2735-2752.
20. World Health Organization. Medical eligibility criteria for contraceptive use. *5th ed.: World Health Organization.* 2015.
21. Curtis KM, Tepper NK, Jatlaoui TC, et. al. Medical eligibiltiy criteria for contraceptive use, 2016, MMWR Recomm Rep 2016; 65:1.
22. Palomba S, Falbo A, Zullo F, Orio FJ. Evidence-based and potential benefits of metformin in the polycystic ovary syndrome:a comprehensive review. *Endocr Rev* 2009;30(1):1-50.
23. Mansfield R, Galea R, Brincat M, Hole D, Mason H. Metformin has direct effects on human ovarian steroidogenesis. *Fertil Steril* 2003;79(4):956-962.
24. Attia G, Rainey W, Carr B. Metformin directly inhibits androgen production in human thecal cells. *Fertil Steril* 2001;76(3):517-524.
25. La Marca A, Morgante G, Paglia T, Ciotta L, Cianci A, De Leo V. Effects of metformin on adrenal steroidogenesis in women with polycystic ovary syndrome. *Fertil Steril* 1999;72(6):985-989.
26. Nestler J, Jakubowicz D. Decrease in ovarian cytochrome P450c17a activity and serum free testosterone after reduction of insulin secretion in polycystic ovary syndrome. *N Engl J Med* 1996;335:617-623.
27. Genazzni A, Chierchia E, Rattighieri E, Santagni S, Casarosa E, Luisi M, et al. Metformin administration restores allopregnanolone response to adrenocorticotropic hormone (ACTH) stimulation in overweight hyperinsulinemic patients with PCOS. *Gynecol Endocrinol* 2010;26(9):684-689.
28. Nestler J. Metformin for the treatment of the polycystic ovary syndrome. *N Engl J Med* 2008;358:47-54.
29. Legro R, Kunselman A, Dodson W, Dunaif A. Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. *J Clin Endocrinol Metab* 1999;84(1):165-169.
30. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM, Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 2002;346(6):393-403.
31. UK Prospective Diabetes Study Group. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes. *Lancet* 1998;352(854):865.
32. Salpeter SR, Buckley NS, Kahn JA, Salpeter EE. Meta-analysis: metformin treatment in persons at risk for diabetes mellitus. *Am J Med* 2008;121(2):149-157.

33. Palomba S, Falbo A, Russo T, Manguso F, Tolino A, Zullo F, et al. Insulin sensitivity after metformin suspension in normal-weight women with polycystic ovary syndrome. *J Clin ENdocrinol Metab* 2007;92(8):3128-3135.
34. Fleming R, Hopkinson Z, Wallace A, Greer I, Sattar N. Ovarian function and metabolic factors in women with oligomenorrhea treated with metformin in a randomized double blind placebo-controlled trial. *J Clin Endocrinol Metab* 2002;87(2):569-574.
35. Ng EH, Wat NM, Ho PC. Effects of metformin on ovulation rate, hormonal and metabolic profiles in women with clomiphene-resistant polycystic ovaries: a randomized, double-blinded placebo-controlled trial. *Hum Reprod.* 2001;16(8):1625-1631.
36. Seifer DB, Baker VL, Leader B. Age-specific serum anti-Mullerian hormone values for 17,120 women presenting to fertility centers within the United States. *Fertil Steril.* 2011;95(2):747-750.
37. Freeman EW, Sammel MD, Gracia CR, Kapoor S, Lin H, Liu L, Nelson DB. Follicular phase hormone levels and menstrual bleeding status in the approach to menopause. *Fertil Steril* 2005;83(2)383-392.
38. Feldman S. Evaluation of the endometrium for malignant or premalignant disease. Up To Date, 2018; Topic 3232, Version 21.0.
39. **Kim SH**, Lundgren JA, Bhabhra R, Collins JS, Patrie JT, Burt Solorzano CM, Marshall JC, McCartney CR. Progesterone-mediated inhibition of the GnRH pulse generator: differential sensitivity as a function of sleep status. *J Clin Endocrinol Metab.* 2018; 103(3):1112-1121. PMID: 29300925. PMCID In process.
40. Legro RS, Schlaff WD, Diamond MP, Coutifaris C, Casson PR, Brzyski RG, et.al. Total testosterone assays in women with polycystic ovary syndrome: precision and correlation with hirsutism. *J Clin Endocrinol Metab.* 2010;95(12):5305-13.
41. Gandara BK, Leresche L, Mancl L. Patterns of salivary estradiol and progesterone across the menstrual cycle. Ann N Y Acad Sci. 2007;1098:446-50.
42. Codner E, Eyzaguirre FC, Iniguez G, Lopez P, et.al. Ovulation rate in adolescents with type 1 diabetes mellitus. Fertil Steril. 2011;95 (1): 197-202.e1.