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Project Title - Phase II 6-month Cyclic Progesterone/Spironolactone pilot Therapy Trial in Polycystic Ovary Syndrome—pre-post, single-arm feasibility study

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Abstract:

Androgenic Polycystic Ovary Syndrome (PCOS) creates physical/emotional burdens in 4-20% of premenopausal women living with PCOS (WLWP) including: few menstruations per year, subfertility, hirsutism/acne and low quality of life by validated PCOS Questionnaire (PCOSQ). Combined hormonal contraceptives (CHC), the current standard-of-care, improve PCOSQ only 16%; with stopping CHC, benefits disappear within 6-months. We hypothesize too-fast brain/luteinizing hormone (LH) pulses cause PCOS. Progesterone (P4) slows LH when testosterone (FreeT) is normal. Combining two approved medications, Cyclic P4 with anti-androgen, Spironolactone (Sp), will likely provide effective, durable benefits. WLWP lit-review suggested significant P4 7-14-day benefits. A 6-month prospective feasibility study of CyclicP4/Sp is necessary.

Overall purpose: Perform a pilot study in 40 WLWP on Cyclic P4 (300 mg/bedtime, 14 days/month) plus Spironolactone (Sp, 200 mg/d).

Feasibility: Assess longitudinal within-WLWP 6-month changes in: 1) PCOSQ; 2) FreeT, HbA1c, and LH; plus 3) WLWP's acceptance of CyclicP4/Sp.

Methods: Single-centre, prospective, longitudinal single cohort study. Recruit ~7-8 WLWP/month over 4-6 months; 85% retention. Eligible WLWP have physician-diagnosed PCOS, are 1-month off CHC/metformin, ages 19-40 (avoiding adolescence/perimenopause), HbA1c <6.4% (no diabetes) and commit to non-hormonal contraception, if needed.

Recruitment uses online, internet, strategic ads and tear-tab posters. Measures at 0 and 6 months: as above plus K+ (safety assessment). Menstrual Cycle Diary monitoring (flow, adherence), 3 visits, monthly telephone/emails for support and assessment of any adverse effects. Statistical analysis: changes by paired T-test.

Summary: A feasibility 6-month study of CyclicP4/Sp will facilitate a CIHR-funded RCT of CHC versus this innovative and likely beneficial treatment for WLWP.

Overall Objectives—to answer this

Research question: *Does 6-months Cyclic Progesterone (CyclicP4) and Spironolactone (Sp) treatment of women living with androgenic PCOS improve their PCOS-specific quality of life?*

To assess, in women ages 19-40 with physician-diagnosed androgenic polycystic ovary syndrome (PCOS) taking open-label cyclic progesterone (CyclicP4, 300 mg at bedtime [hs]) and daily spironolactone (Sp 200 mg at hs) for six months/cycles, ***the within-woman* changes *in***:

1. **Primary Objective**--Health-Related Quality of Life (HRQoL) change is measured within-woman with androgenic PCOS by the PCOS-Questionnaire© (PCOSQ) instrument comparing the total score, and domain-specific scores between Phase 0 (screening, random cycle day) and the early follicular phase of Cycle 7 (study end) after 6-months' treatment with cyclic progesterone (CyclicP4) and 5-months' treatment with spironolactone (Sp) therapies.
2. ***Secondary Objectives*:**
   a) Serum luteinizing hormone (LH) measured by conventional laboratory methods and salivary free testosterone (FreeT) measured by liquid chromatography/mass spectroscopy (LC/MS-MS) in a quantitative aliquot (derived from three separate mornings' samples) given the pulsatility of hormones in saliva) changes are compared within-woman between Phase 0 and Cycle 7.
   b) Salivary FreeT change measured by liquid chromatography /mass spectroscopy (LC/MS-MS) is compared within-woman between Phase 0 and Cycle 1 early follicular phase after 14 days of CyclicP4 taken in late Phase 0 to bring on menstruation.
   c) Weight (kg, measured using balance beam), height (m, measured using weight scale stadiometer), waist circumference (cm, measured using NIH method), blood pressure (seated, automated mean of three readings), body mass index (kg/m^2), blood HbA1c (measured using standard laboratory methods), tobacco and alcohol use measured using CaM*os* questionnaire, and physical activity changes within-woman assessed between Cycle 1 and Cycle 7.
   d) Menstrual cycle lengths, variability (predictability), flow (assessed by counts of soaked standard-sized pad/tampons or by menstrual cup semi-quantitative measures) and Menstrual Cycle Diary© (Diary) experience changes recorded within-woman are compared between Phase 0 data and the mean of experiences from Cycles 1-6, and also compared between Phase 0 and Cycle 6.
   e) Estradiol, progesterone, cortisol and DHEA saliva data within-woman changes measured using high-throughput liquid chromatography-tandem mass spectrometry from Phase 0 to Cycle 1 (after one 14-day CyclicP4 exposure) and from Phase 0 to Cycle 7.
   f) Women's Perceived Change questionnaire on acne and sleep quality, scored on a Likert Scale (-5 to 0 to +5), assessed in the follicular phase of Cycle 7.
   g) Generic HRQoL changes assessed within-woman using the SF-36 instrument domain scores and mental and physical summary scores from Phase 0 to Cycle 7.
   h) SF-36 HRQoL domain and summary scores at Phase 0, and also at Cycle 7, are compared with most recent SF-36 data in age- and BMI range-similar local population-based women from the regional population-based BC Canadian Multicentre Osteoporosis Study (CaM*os*) cohort.
   i) W Serum C-reactive protein (hsCRP) and albumen (assessing inflammation) by local laboratory methods, Leukocyte Telomere Length (LTL) and mitochondrial DNA (assessments of genetic

aging, by UBC Cote lab-specific methods), and Anti-Mullerian Hormone (AMH, assessment of reproductive reserve) by the best method available (in archived serum stored at -70°C) in 2022-2023 changes will be measured and compared within-woman from Phase 0 to Cycle 7.

j) Diary Phase 0, and also Cycle 6 records will be compared with similar-aged normative premenopausal data (archived from the 1-year Prospective Ovulation Cohort, n=53, Prior NEJM 1990) using non-parametric methods and principal components analysis

k) Changes within-woman in all measures between Phase 0 and Cycle 7 will be assessed using regression methods for an influence related to how long (in months, up to 1-year) since women in this trial stopped taking combined hormonal contraceptives (CHC) or metformin treatments.

l) Serum potassium (K+) measured by standard laboratory methods at Cycle 7 and determined to be, or not be, within or less than 10% above the local laboratory reference range.

m) Potential adverse effects of Sp assessed using the presence/absence of abnormal/unpredictable menstrual timing and flow in Cycle 2.

n) Women's willingness to continue taking CyclicP4/Sp therapy assessed using a 7-part Likert scale at Cycle 7; women's preference assessed on a 7-part Likert scale for CHC vs CyclicP4/Sp therapy in women who had previously taken CHC as a PCOS treatment.

o) Assessment of QBT in the Screening period and salivary progesterone (P4) and estradiol (E2) in saliva samples obtained during the middle of cyclic progesterone treatment will assist in validating morning temperature (QBT analysis) versus the ratio of P4/E2.

p) Testosterone, progesterone and cortisol levels pre- and post- within-woman analysis using blood spot analysis.

3. ***Safety Objectives*:** Sp use and risk of abnormal flow (timing and amount) when started after two episodes of P4 cycle; potassium (K+) level within or above the reference range or in unsafe levels in Cycle 7 (after 5 months of spironolactone in a dose ≤ 200 mg/d)
4. ***Feasibility* Objectives:** Willingness to continue to take CyclicP4/Sp therapy (7-part Likert scale); preference (if past CHC therapy) for CHC versus (vs) CyclicP4/Sp (7-part Likert)

Rationale and Background

The **rationale** for this pilot[1], single arm, single centre, prospective 6-month (6-mo) open-label interventional study is the *need to provide **therapy choices** for women with **androgenic PCOS*.**

As a **background**, women with PCOS comprise 4-20% of menstruating women[2] and ~10% of adult women in population-based data have *androgenic* PCOS[3]. Healthcare providers (HCP) diagnose PCOS in adult women (>2 years [y] since menarche[4]) who meet ≥2 of 3 criteria plus other causes must also be eliminated[5,6]:

1) Oligomenorrhea;
2) Clinical (hirsutism/acne) or biochemical (high Free Testosterone, FreeT) Androgen Excess;
3) Typical cysts and larger ovary by ultrasound.

Living with androgenic PCOS is distressing[7]; its facial hair, acne and infertility issues challenge a women's gender image[8]. Women with PCOS often have decreased HRQoL[9], depression and/or anxiety[10]. CeMCOR has documented importantly (>5-unit lower domain score[11]), universally, lower generic HRQoL (via SF-36) in local women with PCOS despite more education than in population- based, age-similar local controls[12]. SF-36 domain scores ranged from -5.8- -19.9 (mean -11.6) lower[12] **(*Appendix Fig 1*).** Combined hormonal contraceptives (CHC)[13] are the current PCOS standard-of-care; they improve menstrual regularity, androgen excess (hirsutism/acne) plus PCOS-Q health related quality of life (HRQoL) by 16% for fertility-seeking women with PCOS[14]. An internet survey (2015-16) of >1300

European/N American women with PCOS found the following ***top concerns*:**

1) difficulty losing weight;
2) subfertility;
3) abnormal menstrual cycles, and
4) hirsutism/acne[15].

Continuous CHC, however, in an RCT was only effective for concerns 3 and 4[14]; it did not aid in important weight loss nor in increased live birth rate post-clomiphene[16]. Also, based on prospective data, within 6- months of stopping CHC, its clinical benefits for women with PCOS, except less acne, had virtually disappeared[17]. CHC are convenient, readily available and women and healthcare providers (HCP) are comfortable with them. However, not all women with PCOS find CHC acceptable or tolerable. ***Women with PCOS need effective non-CHC treatment options.***

Our 2019, similar *unfunded* WHRI Catalyst application was positively assessed. Reviewers wrote: "a very innovative proposal that is highly relevant to women's health" since "current treatment does not meet 2 of top 4 concerns, including infertility"; and that it promised "women with androgenic PCOS will benefit." Since then, we have gained more pertinent knowledge. We conducted a review of the literature on PCOS and micronized P4 therapy[18] that showed cyclic P4 caused predictable flow with lower LH and T levels; vaginal P4 in letrozole ovulation induction also improved live birth rates[18]. We further documented the 6- months within-woman daily Menstrual Cycle Diary[19] (Diary) change in a woman living with androgenic PCOS volunteer taking cyclic P4 her physician had prescribed; she had significantly decreased fluid retention, breast tenderness and cervical mucus **(*Appendix Fig 2*)**[20].

We also launched (July 15, 2020) an unfunded PCOS treatment survey to assess WLWP's preferences and willingness to volunteer for research (www.cemcor.ubc.ca) to which about 800 women provided input. We are in the process of analyzing these data. Finally, Besins© are providing an arms-length donation of the oral micronized progesterone needed for Cyclic P4 for this 6- month, single-arm interventional trial. In addition to these recent data, Dr. Prior has past 20-year, 200- woman strong clinical experience effectively treating women with androgenic PCOS with *Cyclic P4* ("luteal phase replacement" since ovulatory disturbances are very common) *plus Spironolactone* (Sp, anti- androgen therapy that is effective, safe and inexpensive).

Women with androgenic PCOS on these two therapies, in Prior's clinical experience, developed ovulatory normal- length cycles, lost acne and with longer therapy, beard hair, felt better so they exercised more and ate better with resultant weight loss; they could eventually stop all therapy. They were also able to spontaneously (when off spironolactone) become pregnant. Prior thus learned the key role of P4. Sometimes women with PCOS would briefly "relapse" to anovulation, irregular cycles and the return of androgen excess when exposed to increased stress or weight change. These mild changes were readily reversed when re- treated with Cyclic P4.

Why would Cyclic Progesterone and Spironolactone be effective for androgenic PCOS?

Because scientific evidence suggests that deficient progesterone is the key to resolving the fundamental physiological and metabolic imbalances in PCOS, the rapid pulse rate of GnRH and LH. Although the origin of PCOS is multifactorial with contributions from genetic, *in utero* androgen exposure, inflammation, insulin resistance and environmental variables[21], the clinical syndrome appears to begin with ***abnormally fast pulsing hypothalamic GnRH***[22]. The GnRH quick pulses (stimulated by afferents from Kisspeptin and inadequate GABA-A feedback) lead to similarly pulsing LH that increases ovarian thecal testosterone (T) production, suppresses FSH, and prevents ovulation. The result is abnormally high FreeT production[22], tonic or elevated estrogens, and few cycles with most having ovulatory disturbances. It is likely that these changes in turn lead to insulin resistance, obesity[23], decreased fertility and increased risk for endometrial cancer. Higher insulin levels/resistance also increase FreeT

production[24], with inflammation[25] and obesity both contributing[21]. Once begun, androgenic PCOS creates a self- perpetuating, 'vicious cycle'. Other advantages Cyclic P4 for PCOS has are: regular cycles, eliminate the risk of endometrial cancer, indirectly decrease clinical acne and hirsutism through decreased T to dihydrotestosterone (DHT)(Bruchovsky N, Rennie PS, Batzold FH, et al. Kinetic parameters of 5 alpha-reductase activity in stroma and epithelium of normal, hyperplastic, and carcinomatous human prostates. *J Clin EndocrinolMetab* 1988;67(4):806-16.) and because of slower LH pulses, and finally, improved sleep (Schussler P, Kluge M, Yassouridis A, et al. Progesterone reduces wakefulness in sleep EEG and has no effect on cognition in healthy postmenopausal women. *Psychoneuroendocrinology* 2008;33(8):1124-31) and probably decreased anxiety (Dennerstein L, Spencer-Gardner C, Gotts G, et al. Progesterone and the premenstrual syndrome: a double blind crossover trial. *British Medical Journal* 1985;290:1617-21).

Since progesterone normally slows GnRH and LH pulsing at the menstrual midcycle[26], **treatment with CyclicP4 may be effective PCOS therapy.** However, the higher FreeT levels in PCOS appear to interfere with P4's LH pulse-slowing actions[22]. In women with PCOS on CyclicP4, adding an anti-androgen such as spironolactone (Sp), allowed luteal phase P4 levels to effectively slow LH pulses to normal[27] with resulting lower LH and FreeT levels. P4 itself also lowers androgen *activity* by competing for the 5- alpha reductase enzyme that converts T to its active skin metabolite, dihydrotestosterone (DHT)[28].

Despite the promising new information, none of the PCOS studies we recently reviewed gave oral micronized P4 for longer than a single (usually 7-12) day cycle[18]; it is not yet known whether 200 mg of Sp will be effective for women with PCOS having body mass index (BMI) values >30; if Sp given after a 14-day cycle of P4 followed by withdrawal flow will prevent abnormal bleeding patterns[29], and how high K+ will be elevated, and whether it, as in treatment of acne[30], will be without serious risk. We previously studied quality of life in women with PCOS on no, or no specific, therapy, using the appropriate generic SF-36 HRQoL tool for population comparison[12]. We found that HRQoL was statistically lower than age-similar population-control data (see Appendix). Data are still needed for androgenic PCOS on this innovative Cyclic P4/Sp therapy using PCOSQ, the PCOS-Specific HRQoL instrument[7]. We have performed a pilot study of cyclic oral micronized progesterone (300 mg at bedtime for 14 days/cycle; Cyclic P4) and showed that high-estrogen related symptoms (such as fluid retention and breast tenderness) improved over six months[20]. In addition, it is not clear whether the recent use of CHC or metformin will interfere with or potentiate potential beneficial effects of CyclicP4 and Sp. Thus, there are important data that are needed before it will be possible to plan an appropriately powered ***RCT comparing this new PCOS therapy versus (vs) CHC.*** As Dr. Helena Teede, leader of a PCOS possible international stakeholder consortium[13] wrote (2018/09/01): "*I. . .strongly state the need for a study such as Prior and colleagues are proposing."*

Study hypothesis and aims

Our ***hypothesis*** is that CyclicP4/Sp will effectively increase the total PCOSQ score during 6-mo of therapy (Primary objective). We also *hypothesize* that women will elect to continue Cyclic P4/Sp and prefer it to CHC (Feasibility objective). Clinical experience suggests that Sp started after one or more cycle of P4 treatment will not cause flow problems, and will not dangerously increase K+ (Safety objectives).

Our ***aims for this pragmatic trial*** (as outlined in **Objectives**) are to obtain new data with the least disturbance of women's lives in an open-label, prospective 6-mo interventional study of treatment with CyclicP4/Sp in 40 women, of all BMI values, with healthcare provider (HCP)-diagnosed androgenic PCOS, to track changes in the PCOS-Q (1º outcome), FreeT and LH levels as well as changes in weight, waist circumference, blood pressure, estradiol, cortisol, and DHEAS levels and self- perceived

acne and sleep.

We have decided, we will treat all women with cyclic progesterone for two weeks before their official start of treatment (in order to ensure the timely entry of women with oligo-amenorrhea into this 1-year funded study). Therefore, we have made Phase 0 in addition to the screening HbA1c blood test, the time when we will draw all baseline bloods (before Cyclic P4).

We are also adding science-based objectives to document changes in leukocyte telomere length (LTL) which is a genetic marker of cellular aging and be lower in women with PCOS than controls[30a] or not[30b], and LTL may inversely relate to high sensitivity C-reactive protein (CRP). The ratio of CRP to albumen (that respond in opposite directions to inflammation) may be an accurate marker of the presence of PCOS in the community[25]. We will also learn the role of BMI[31] related to PCOSQ changes on these therapies.

Our *primary overall purpose* is to assess the effectiveness of 6-mo CyclicP4/Sp in improving the lives of women living with PCOS (by assessing the PCOSQ) and to provide essential data for planning an adequately powered RCT that will formally compare CHC vs CyclicP4/Sp. We will also learn changes in key hormonal values (FreeT, LH and HbA1c), assess its safety by K+ levels and flow and experience changes by therapy-indicated daily electronic fillable PDF Menstrual Cycle Diary© tools [***Appendix Fig 3***].

Study design, methodology and analysis plan

Design: this is a pre-post, pilot (Phase II) prospective 6-mo, single arm, open-label interventional study
Methods: Our *primary outcome* is the 6-mo change in PCOSQ, the validated PCOS-specific HRQoL tool[7], that is sensitive to change and comprehensively covers all primary issues for women with PCOS except acne[31,32].

Interventions: oral micronized progesterone (P4) 300 mg taken at bedtime (a dose that keeps the serum P4 at/above the luteal phase level for 24 hours)[33] and is sufficient to decrease LH pulsatility[22]. We will prescribe P4 cyclically 14 days/month or for cycle days 14-27 of a normal-length menstrual cycle (***CyclicP4***). In addition, all women with androgenic PCOS will start spironolactone (**Sp**) 200 mg/bedtime *in the* ***second cycle***.

Given the male fetus teratogenic effects of Sp, to be eligible, all participants at pregnancy risk must agree to conscientiously use some form of ***non-hormonal contraception***: condom/vaginal spermicide or copper IUD. There is evidence that metformin improves cycle regularity and lowers androgen levels even in normal-weight, non-diabetic women with PCOS[24]. We will include women 1-m *off metformin* and, to avoid needing it since it may confuse CyclicP4 & Sp effects on PCOSQ in WLWP[32], we will screen for increased risks for type 2 Diabetes Mellitus (T2DM) and exclude women with HbA1c >6.4%[13] who are at increased diabetes risk.

Participants will be **eligible** if they are women with PCOS diagnosed by a physician and their PCOS is androgenic per PCOS/Androgen Excess Society criteria[4]. *We will enroll 40 eligible women.* (This is feasible for to recruitment in 4-6 months; we anticipate it providing adequate power since an RCT with significant PCOSQ results had similar #/arm[14]). Eligible women must be ages 19-40 to be at or above the BC age of majority and are unlikely to be within 2-years of menarche[6]. We will screen those between the ages of 35-40 for Very Early Perimenopause as this is when PCOS experiences change[34].A participant will be ineligible if they score 3 or more new Early perimenopausal symptoms/experiences and/or have night sweats. Women with previous or recent CHC or metformin treatment are eligible, provided CHC or metformin has been discontinued for ≥1 month (although we will encourage at least three months off, if possible). All will be screened and have a normal HbA1c. Since women having all BMI values are

eligible, we will assess it for a potential influence on our objectives.

Women with PCOS will be **ineligible** if seeking immediate fertility, do not commit to using non-hormonal contraception if at risk for pregnancy, are pregnant, currently taking reproductive hormones, are unwilling to stop CHC or metformin for a month before enrolling, or to record daily Diary experiences. Further, individuals will be ineligible if they are currently breastfeeding and have been for less than six months (so they will not have difficulty waking for a night feeding), and if they have a history of migraines with aura and/or neurological signs and symptoms since the brain sensitivity of these women may mean starting or stopping progesterone could trigger a migraine.

We will assign each participant a unique number. No identifying data will leave the PI's locked office in a locked file thus securing name, contact and code data.

Recruitment will be from the Metro Vancouver region by the CeMCOR's proven successful and cost-effective methods[35]. We will emphasize social media recruitment. CeMCOR has a pandemic safety plan in place for remote or in-person trial conduct. We anticipate 7-8 recruited women/m and an 85% continuation rate based on past studies. We will attempt to replace any women who drop-out/discontinue.

Tools: To assess these data's generalizability at baseline, elements of the Canadian Multicentre Osteoporosis Study (***CaMos Questionnaire****)* will be interviewer-administered for comparison with local population-based CaM*os* controls[12]. Participants at enrolment visit (in Cycle 1) will have weight, height, waist circumference (NIH method) and automated blood pressure (BP) measured and complete the validated ***PCOSQ***[7] and SF-36; we will repeat these measures at 6-mo. (For consistency, all women will be provided the PCOSQ and SF-36 and be asked to complete these in their own homes in Phase 0 and at the final visit. Also, at the end of this trial, participants will record changes in acne and sleep (graded -5 to +5) by the ***Women's Perceived Change Questionnaire*** (WPCQ)[36] as well repeat the CaM*os* questionnaire food frequency/diet and physical activity and habits (alcohol, cigarette) sections and answer feasibility questions about their preferences.

Laboratory testing will include morning, anytime in the cycle (Phase 0 cycle days 1-6 assessments and in the early follicular phase of the 7th cycle:

Screening HbA1c—following informed consent, but before the start of the study in Phase 0. At the same time women will also have untreated, no CyclicP4 assessment of ***Serum LH_0*** and will collect 3 salivary samples pooled for an *untreated* ***salivary FreeT (Phase 0) and post-Cyclic P4 in Cycle 1.***

HbA1c is by standard clinical methods at VGH lab. If the HbA1c is >6.4 they will be ineligible; that participant's study # will be retired. This test will be repeated in the early follicular phase Cycle 7.

Serum LH (by standard, monitored Vancouver General Hospital [VGH] laboratory methods)

FreeT obtained in Phase 0, follicular phase of Cycles 1 and 7 from morning-collected saliva and analyzed by the reliable, specific, new liquid chromatography mass spectrometry (LC/MS-MS) method of Gao[37]. These, obtained at home, will be stored in the home freezer for just over six months. Given the pulsatility of steroids[26] three saliva samples will be obtained over 3 days and quantitatively pooled into a single analytic sample. At the end of the study we will ship all analytic saliva samples, labelled with ID# and date (middle of the 3 samples), to Germany on dry ice for analysis. Returned data will be by coded date-specific hormonal data (***cortisol***, ***estradiol*** **and** ***DHEA*** as bonus descriptive measures) via password-protected email. Each woman's total samples will be analyzed in the same batch.

Progesterone, Cortisol, and Testosterone (by Blood Spot Analysis) *obtained during Phase 0 and follicular phase of Cycle 7 for all newly enrolled participants, and in the Cycle 7 time point for all yet-to-complete participants will be analyzed by the method recently developed and validated in Dr. Michael Chen's UBC-affiliated lab in Victoria, B.C.* The coordinator will perform the finger-prick and drops of blood will be used to fill 4 1-cm circles on a special filter card designed for this purpose. Each card will have the ID# and date, be air-dried, then stored in a zip-lock bag until mailed in a batch to the Victoria laboratory.

(Note this is necessary because the salivary progesterone, and testosterone assays have not yet been validated.)

Serum K+ (VGH laboratory—standard methods) final post 5-m on Sp in the follicular phase (Cycle 7). Other serum measurements will be obtained and frozen for later systematic analysis with Phase 0, Cycle 1 and 7 samples in the same analytic batch. AMH will use the most sensitive current method (not yet determined). LTL and mitochondrial DNA will be tested at UBC in Dr. Helene Cote's lab.

Validation of salivary progesterone versus mean morning luteal phase temperature

The liquid chromatography-tandem mass spectroscopy method for measuring salivary **progesterone[37] has yet to be validated in relation to ovulation**. We ***need*** this validation to interpret the results of our recently completed Menstrual cycles and Ovulation Study (MOS2); we also need it to interpret this PCOS study.

In MOS2 we can compare the first morning (basal) mean luteal phase temperature during the luteal phase of normally ovulatory cycles by Quantitative Basal Temperature (QBT). We can use QBT as the gold standard and compare the luteal phase mean temperature versus the ratio of salivary P4 divided by salivary E2. (The reason for the ratio of two ovarian steroids rather than just P4 is that, although P4 raises core temperature, E2 ether decrease that P4 effect or independently lowers basal temperature.)

In this PCOS study we can collect saliva samples (3 days in a row) during the middle of the 14-day Cyclic Progesterone therapy. Therefore, we can measure the mean P4-treated temperature (over 14 days) with the ratio of P4/E2 in this second dataset.

Women with PCOS are not normal, and it would be ideal to study a second cohort of normal women for validation. But, because this validation is urgent to interpret MOS2 data, women with PCOS during Cyclic Progesterone should be a valid sample. We are recruiting women with androgenic PCOS who will need screening (Phase 0) with a normal HbA1c, collect untreated Diary data and have their baseline serum and salivary hormone testing. To bring on flow in a timely manner, we will also be giving them cyclic progesterone during Phase 0. This provides the possibility to use them as a second sample for validation. The coordinator will meet potential participants coming for blood work, will provide them with a digital thermometer (batch standardized) and enough tubes for collecting saliva during the early and late part of that month. She can also, then or earlier, teach them to record the Menstrual Cycle Diary© and collect basal temperature. We will provide them with the Phase 0 Diary (**Figure 3**) that includes a spot for QBT recording, and indicates when the 3-day saliva samples should be collected during Cyclic Progesterone therapy.

Analysis plan: Our pilot study's purpose is to provide treatment-related estimates of changes and variability in key outcomes. The statistical analysis plan will be written by Dr. Joel Singer (biostatistician/epidemiologist) in consultation with the clinical team.

We will assess all changes from baseline within-woman by paired T test; multiple regression will be used to assess the association of the final PCOSQ with baseline PCOSQ, BMI and physical activity, and recent CHC/metformin use at baseline. We will describe the PCOSQ overall percentage change (for comparison with CHC[14])[7]. As previously[12], we will describe baseline demographic and anthropometric variables for women with androgenic PCOS vs population-based regional similar-aged CaM*os* controls. We will describe cycle lengths and variability changes. We will analyze ordinal Diary variables with non-parametric statistics and can compare with initially normally ovulatory women[38],[39] using SPSS for all analyses. Statistics will be performed by Drs. Shirin/Goshtasebi with direction from and support by Dr. Singer.

Anticipated results

Based on past retention and the eagerness with which women with PCOS seem to desire a new therapy, we anticipate that ≥36 women will complete this study. We will replace any losses to follow-up to ensure this number completes. Their data will provide an estimate of the expected changes in PCOSQ score/domains to enable an adequately powered comparative RCT with CHC (see below). Keeping the

Diary may improve women's self-efficacy; as women with PCOS feel better, they also may improve lifestyle habits and exercise (assessed by a few repeat CaM*os* questions at the final visit). In addition to quality-of-life measures, we also anticipate improvements in cycle length and regularity, acne, sleep[40], weight[23], waist circumference (WC) and WC/height, HbA1c and BP[41].

Safety related results and amelioration

Although there is no specific Health Canada PCOS indication for Cyclic Progesterone nor for Spironolactone, the standard, BC College of Physicians and Surgeon's approved (2020) drug monographs for "Progesterone" and "Spironolactone" https://www.cpsbc.ca/library (Accessed Nov 10, 2020) specifically describe progesterone as commonly used for irregular cycles, or luteal phase insufficiency that are present in most women with PCOS, in similar doses, and as cyclic therapy. In addition, spironolactone is commonly used for acne vulgaris and hirsutism as well as PCOS in usual clinical practice.
The dose ranges recommended for both progesterone and spironolactone include the doses for this study. For these reasons, Health Canada instructions and the Chair of the Clinical Research Ethics board both indicate we do not need to obtain Health Canada, Clinical Trials Administration approval for this study. This study is now listed on the ISRCTN registry with study ID ISRCTN99343883. We will (with signed permission from each participant) write to their primary healthcare provider informing her/him of the study, the purpose, rationale, the medications being prescribed, their doses and durations for each. We will invite them to ask any questions or relate any concerns to Dr. J. C. Prior, PI, providing her contact information.

We anticipate no difficulties for women taking Cyclic Progesterone (P4). However, if a woman might be sleep deprived, we will re-enforce the importance of starting CyclicP4 on an evening following which it is possible to sleep in the next morning. Also, they should take the progesterone just as they are ready to fall asleep to avoid dizziness. We will further tell women that occasionally they may experience increased estrogen-related effects (breast tenderness, cervical mucus, fluid retention) for the first cycle of progesterone. All of these transient issues are resolved by the second cycle.

When starting spironolactone (Sp), we will warn women to increase their fluid intakes to compensate for initial, mild ~1-week-long Sp-related diuretic effects. Although abnormal vaginal bleeding is a side-effect of Sp, since we will start it only after they have experienced withdrawal flow following two cycles of P4, based on extensive previous clinical experience we expect Sp to cause no abnormal flow. Sp, because of its mineralocorticoid-blocking effects, raises serum potassium (K+) values. We expect only mild increases to the upper limit of normal K+ values and will monitor for this at study end. The most serious adverse effect with spironolactone is potential feminization of a male fetus should the woman become pregnant. All women who are sexually active with a man in this study are required to agree to conscientious use of a non-hormonal contraceptive method. We are providing condoms and vaginal spermicide for those women needing pregnancy protection. If women are sexually active with a man/men and unable or unwilling to use the barrier/spermicide, they will be required to get a copper IUD inserted before they are eligible.

Study timeline

In mid-July 2020, we posted (www.cemcor.ubc.ca) an ethics-approved androgenic PCOS therapy survey. On this, women indicate where they live—local, potentially interested women will thus become aware of this study.
Our main project ethics applications and other administrative requirements were completed by February 2021, but delay in the approval of a donation agreement between UBC and Besins Healthcare International meant a delay until June-July in achievement of progesterone shipment and hiring of a coordinator. Recruitment will begin as soon as ethics approvals, pharmaceutical supplies (CyclicP4 from Europe,

stored and managed by Pure Integrative Pharmacy, prescribed by Dr. Marshall Dahl) and study personnel are in place. We aim to recruit, study and complete the trial within a year; we have obtained extension of the study to October 2022.

Participant-specific timeline

Each interested woman will contact us by email or telephone. We will email them the consent form and, if >1 month off CHC and/or metformin, will teach them accurate and convenient Diary-keeping (http://www.cemcor.ca/resources/daily- menstrual-cycle-diary) and provide them with the electronic PDF fillable forms for Phase 0 and Cycle 1. If they agree to consent verbally, we will telephone-screen for eligibility and if eligible, invite them to the laboratory (bringing their signed consent) for a screening blood test for HbA1c and LH_0. At the time of the laboratory screening, we will provide them with the $Cycle_0$ 3 tubes for saliva sampling (and instructions) for FreeT and the PCOSQ and SF-36 forms. We will ask them to also record two weeks of untreated Diary data (Phase 0). They will also complete the at-home PCOSQ and SF-36 forms while on no therapy. We will then have Pure Integrative Pharmacy courier two weeks of CyclicP4 that they will take to bring on flow, while continuing to record dairy data for Phase 0.

If their HbA1c result is <6.4% (eligible), and we have their analyzed LH (Sample 0), we will instruct them to continue monitoring using the PCOS Study Menstrual Cycle Diary Cycle 1 form. We will provide instructions for taking CyclicP4 which they will begin once they have two weeks of recording Phase 0 Diary-records that we will collect and review with them at Visit 1.

All research personnel will have had two COVID-19 vaccinations more than two weeks before. Using physical distancing and masks (if they have not had two COVID-19 vaccinations), enrolled women will have in-person **Visit 1** (initial, at which they will bring their completed PCOSQ and SF-36 forms), **Visit 2** (3-months) and **Visit 3** (final) visits (UBC-CeMCOR space 4th floor 2775 Laurel St.). At Visit 1 we will provide their original HbA1c and LH results and go over their Phase 0 Diary data. We will review their completed PCOSQ1 and SF-361 data to ensure completeness.

We will make monthly email/telephone contact ("how are you?" also answering any questions and systematically asking about any adverse events), provide any needed support and ask them to email their completed diaries before coming for their medication refills at 3-months.

Women with PCOS will have a Visit 3 (final) in the early 7th cycle bringing completed PCOSQ2 and SF-362 forms (reviewed for completeness), be asked a couple of short CaM*os* questions (about diet, habits and exercise), report their perceived changes in acne and sleep, have weight, waist circumference and BP measured again, have laboratory testing (LH, HbA1c and K+), have collected 3 days of saliva (bringing all their collected, frozen specimens) and bring/provide their final completed Diaries. We will also ask a final questionnaire, if they have previously used CHC, that assesses women's preferences, on a 7-point Likert scale[42], for CyclicP4/Sp vs CHC. We will similarly assess their likelihood of continuing CyclicP4/Sp therapy.

We should know the primary **study results** by the fall of 2022 so we can re-apply for CIHR in 3/2023 for our long- planned one-year comparative RCT of CHC vs CyclicP4/Sp followed by a 6 months post trial no-drug documentation.

Once analyzed, we will immediately communicate study results with PCOS study participants via an email notification and link to password-protected articles specifically related to this study on www.cemcor.ubc.ca. Online articles will be posted on the CeMCOR website for general women's knowledge about PCOS after data are officially presented and/or published.

Relevance to the health of women in British Columbia

Women with androgenic PCOS have had no major innovation in therapy for at least 3 decades. We believe results of this trial will allow them the potential to opt for something other than CHC and may prevent the need for expensive and often difficult fertility interventions. This 6-mo, open-label interventional study using a within-woman pre-post design is piloting one of two arms of a proposed future CIHR-funded RCT comparing CHC, the standard-of-care therapy for PCOS, with Cyclic Oral Micronized Progesterone and Spironolactone.

Encouraging data from science and clinical experience support CyclicP4/Sp. Should this pilot study show what we expect—better PCOSQ quality of life including emotional, weight, hirsutism, cycles and fertility domains, it will have provided the first evidence supporting a potentially important new therapy for PCOS. This new therapy might be life-changing for the majority of women now living with androgenic PCOS in B.C.

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APPENDIX

Dissemination plan for the results

This pilot includes embedded knowledge translation since we have co-investigators who are trained in family medicine (AG), obstetrics and gynecology (SS), public health (JS), pharmacology (JY) and endocrinology (MD and JCP) as well as community partners who are women living with PCOS (FM, NY).

We will initially privately share pilot study results with participating women using a password-protected section of the popular CeMCOR website (www.cemcor.ubc.ca) that received 3,500-5,000 page-views a day from over 180 countries world-wide. We will upload a number of short reports, notifying each via email of new postings as we have successfully done following a CIHR-funded RCT of progesterone for perimenopausal hot flushes.

We will submit the results to the annual international Endocrine Society conference; we expect a podium or plenary poster presentation. We will present the new data to UBC and BC academic medical lectures, in particular, for Women's Health Research Institute rounds, VCHRI's C2E2 and family practice monthly or weekly "rounds."

We will submit the results of this phase II pilot study for publication, possibly in the open access Journal Medical Internet Research (impact factor 5.43) or a higher ranked, also open access publication or others such as: *J. Clin Endocrinol Metab, Human Reproduction, Clinical Endocrinol or Fertility Sterility.* As soon as the data are in press, we will seek public media (newspapers/CBC's *The National* and other Canadian and worldwide (*The Conversation*) reporting as well as creating social media posts highlighting the results of this innovative new therapy for women living with androgenic PCOS.

We will create new CeMCOR online articles about PCOS describing the new therapy, how it works and what to expect. This website garners 3,500-5,000 page-views/day from >180 countries. Finally, we will post CeMCOR articles for Healthcare Providers to aid them in understanding Cyclic Progesterone/ Spironolactone therapy for PCOS, detailing how to prescribe it, what to expect in the responses for women with androgenic PCOS and what evidence suggests that the potential benefits and risks may be.

Future goals outlining how the catalyst grant will facilitate funding and research

The purpose of this pilot is to provide estimates of the baseline PCOS-Q and its 6-month change and standard deviation of change in 40 women taking Cyclic Progesterone (CyclicP4) and Spironolactone (Sp). This will provide data necessary to design an adequately powered randomized comparative trial versus combined hormonal contraceptives (CHC), the standard of care for PCOS. Our intent, once we have these data is to again apply to the Canadian Institutes for Health Research to do a planned 1:1 randomized, 1.5-year prospective comparative study with a final six months' observational phase off all therapy for all participants so we can assess the relative and absolute durability of improvements on each therapy. The data from this pilot are also essential for assessing: 1) Sp response by BMI; 2) effects of previously discontinued CHC or metformin; 3) adverse effects; 4) women's preferences and willingness to continue Cyclic P4/Sp therapy. We anticipate re-applying to CIHR in March 2023.

Figures

Figure 1. The Z-scores (showing portions of a standard deviation) of SF-36 HRQoL domain and component scores of 93 local premenopausal women with PCOS versus 56 age-similar women from the randomly sampled BC Canadian Multicentre Osteoporosis Study population-based regional controls.

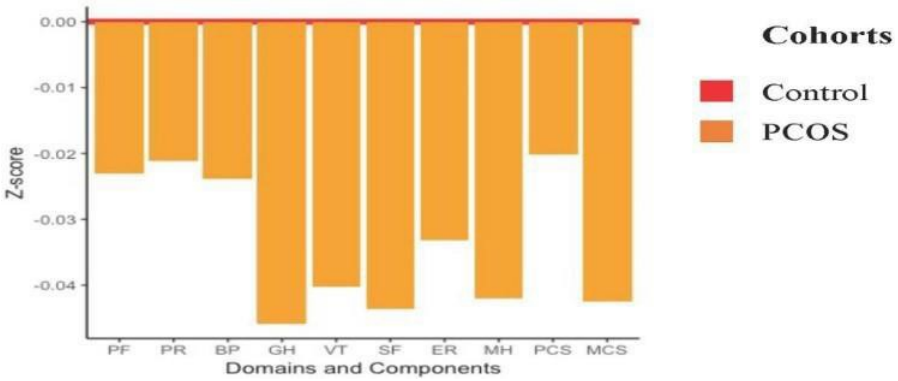

Figure 2. Within-woman PCOS's Diary Experiences Changes on Cyclic Oral Micronized Progesterone Over Six Cycles. Mean (SD) (reported for all cycle days) of the selected Menstrual Cycle Diary© experiences during cycles 1 (yellow) and 6 (red) in a single, within-woman analysis using Wilcoxon Signed Ranks Test[20]. *indicates a difference at P < .05. Note that this is now published: Shirin, S.; Murray, F.; Goshtasebi, A.; Kalidasan, D.; Prior, J.C. Cyclic Progesterone Therapy in Androgenic Polycystic Ovary Syndrome (PCOS)—A 6-Month Pilot Study of a Single Woman's Experience Changes. Medicina **2021**, 57, 1024. https://doi.org/10.3390/medicina57101024

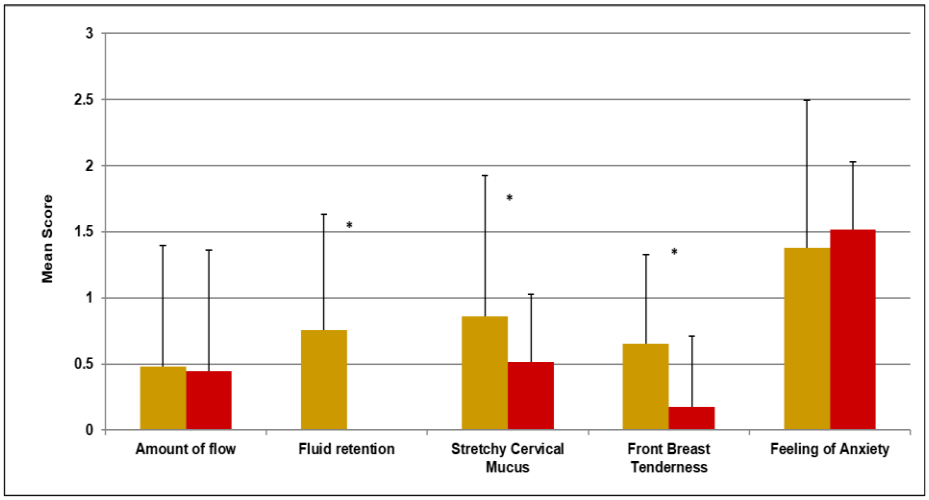

Figure 3 The Menstrual Cycle Diary© adapted for Phase 0 (screening) of this PCOS Pilot Study shows the days of the month or cycle days of taking Cyclic Progesterone to bring on menstrual flow and has a line for recording the first morning basal temperatures (needed for validation). This fillable PDF will also allow us to record pill counts that, with returned medications, will assess adherence.

Phase II 6-month Cyclic Progesterone/Spironolactone Pilot Therapy Trial in Polycystic Ovary Syndrome - pre-post one-arm feasibility study

Menstrual Cycle Diary - Cycle 1

Participant ID: _____ Month: _____ Year: _____

Cycle Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Date																															
Tampons +/- pads (normal size-soaked/day																															
Menstrual Cup Flow 1*																															
Menstrual Cup Flow 2*																															

Record 0 = none, 1 = minimal, 2 = moderate, 3 = moderately intense, 4 = very intense

Amount Flow																															
Cramps																															
Breast Sore: Front																															
Breast Sore: Side																															
Fluid Retention																															
Mucus secretions																															
Constipation																															
Headache																															
Sleep Problems																															
Feeling Frustrated																															
Feeling Depressed																															
Feeling Anxious																															

Record M = much less, L = a little less, U = usual, Y = a little increased, Z = much increased

Appetite																															
Breast Size																															
Interest In Sex																															
Feeling Of Energy																															
Feeling Of Self-Worth																															
Outside Stresses																															
Cyclic Progesterone (300mg at bedtime) take 3/day for 14 days. Record # of capsules taken																															
Saliva Sample (Add ✓ on 3 days/first week)	☐	☐	☐	☐	☐	☐	☐																								
Comments (feeling sick, poor sleep, etc.)																															

***Menstrual Cup Flow: Please record flow (ml) whenever you empty your cup. Use the scoring outlined below (ie. flow between 7.5 and 15ml = C)**

Flow Scoring Choices

0	A	B	C	D	E	F
0ml	0 – 7.5ml	7.5ml	7.5 – 15ml	15ml	15 – 30ml	30ml

Phase II 6-month Cyclic Progesterone/Spironolactone Pilot Therapy Trial in Polycystic Ovary Syndrome - pre-post one-arm feasibility study

Menstrual Cycle Diary - Cycles 2-6 Cycle #_____

Participant ID: _____ Month: _____ Year: _____

Cycle Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Date																															
Tampons +/- pads (normal size-soaked/day																															
Menstrual Cup Flow 1*																															
Menstrual Cup Flow 2*																															

Record 0 = none, 1 = minimal, 2 = moderate, 3 = moderately intense, 4 = very intense

Amount Flow																															
Cramps																															
Breast Sore: Front																															
Breast Sore: Side																															
Fluid Retention																															
Mucus secretions																															
Constipation																															
Headache																															
Sleep Problems																															
Feeling Frustrated																															
Feeling Depressed																															
Feeling Anxious																															

Record M = much less, L = a little less, U = usual, Y = a little increased, Z = much increased

Appetite																															
Breast Size																															
Interest In Sex																															
Feeling Of Energy																															
Feeling Of Self-Worth																															
Outside Stresses																															
Cyclic Progesterone (300mg at bedtime) take 3/day for 14 days. Record # of capsules taken																															
Spironolactone (200 mg at bedtime) take 2 daily. Record # of tablets taken																															
Comments (feeling sick, poor sleep, etc.)																															

***Menstrual Cup Flow: Please record flow (ml) whenever you empty your cup. Use the scoring outlined below (ie. flow between 7.5 and 15ml = C)**

Flow Scoring Choices

0	A	B	C	D	E	F
0ml	0 – 7.5ml	7.5ml	7.5 – 15ml	15ml	15 – 30ml	30ml

Phase II 6-month Cyclic Progesterone/Spironolactone Pilot Therapy Trial in Polycystic Ovary Syndrome - pre-post one-arm feasibility study

Menstrual Cycle Diary - Cycle 7

Participant ID: _____ Month: _____ Year: _____

Cycle Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Date																															
Tampons +/- pads (normal size-soaked/day																															
Menstrual Cup Flow 1*																															
Menstrual Cup Flow 2*																															

Record 0 = none, 1 = minimal, 2 = moderate, 3 = moderately intense, 4 = very intense

Amount Flow																															
Cramps																															
Breast Sore: Front																															
Breast Sore: Side																															
Fluid Retention																															
Mucus secretions																															
Constipation																															
Headache																															
Sleep Problems																															
Feeling Frustrated																															
Feeling Depressed																															
Feeling Anxious																															

Record M = much less, L = a little less, U = usual, Y = a little increased, Z = much increased

Appetite																															
Breast Size																															
Interest In Sex																															
Feeling Of Energy																															
Feeling Of Self-Worth																															
Outside Stresses																															
Saliva Sample (Add ✓ on 3 days/first week)	☐	☐	☐	☐	☐	☐	☐																								
Lab Blood work (Add ✓on day blood drawn during first week)	☐	☐	☐	☐	☐	☐	☐																								
Comments (feeling sick, poor sleep, etc.)																															

***Menstrual Cup Flow: Please record flow (ml) whenever you empty your cup. Use the scoring outlined below (ie. flow between 7.5 and 15ml = C)**

Flow Scoring Choices

0	A	B	C	D	E	F
0ml	0 – 7.5ml	7.5ml	7.5 – 15ml	15ml	15 – 30ml	30ml