Title: Effect of Progesterone on Testosterone concentrations and Breast development in Transwomen

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The number of individuals with gender incongruence who present to their physician for hormone therapy has increased manifold in the last decade(1). Testosterone therapy in transgender men (also known as female-to-male transgender or transmen) and estrogen therapy in transgender women (also known as male-to-female transgender or transwomen) respectively is the mainstay of hormone regimen. The proportion of individuals that are transwomen is slightly higher than transmen (60% vs 40%). Transwomen, who are genetically men, receive estradiol replacement with the aim of suppressing serum testosterone and achieving serum estradiol concentrations that mimic the serum concentrations of biological women. This leads to an increase in fat mass, breast growth and decreases in lean mass and masculine pattern hair. However, the results of these changes are often less than satisfactory and additional therapy is required.

Breast development is a major concern for transgender females. There is a great deal of variability among individuals, as evidenced during pubertal development (2).Transgender women do not achieve the same level of breast development as cisgender women do after puberty (3). Typically, transgender women plateau at Tanner stage III and half of the transgender women have a AAA cup size or less (4). In transwomen, breast development is generally maximal at 2 years after initiating hormones (5). However, a more recent study of 229 transgender women participating in the European Network for the Investigation of Gender Incongruence cohort found that breast development reached a plateau within the first 6 months of therapy. Half of the transgender women had a AAA cup size or less and only 10% were size A or more after a year of treatment (4). Not surprisingly, at least 60% of transgender women seek surgical breast augmentation (6).

There are transgender females who report an anecdotal improved breast development, mood, or sleep with the use of progestogens(7). Estrogen is important for stromal and early ductal and epithelial proliferation(2). By contrast, although progesterone in cell culture causes proliferation for a few days, it then transforms into its primary, maturational role that includes inhibition of the proliferative effects of E2 (7, 8). Progesterone appears to be relevant for acini/lobuli development and pseudolactational changes. Many women report a feeling of fullness in breasts and their transformation from a conical to more rounded shape (clinical experience of PI). Thus, progesterone may induce optimal breast maturation, if preceded by estradiol therapy in transwomen. However, observational studies with small number of patients do not support a major role for progesterone in enhancing breast size in transwomen (9). There have been no well-designed studies of the role of progestogens in feminizing hormone regimens, so the question is still open. As per Endocrine society transgender guidelines, “current evidence does not indicate that progestogens enhance breast development in transgender females, nor does evidence prove the absence of such an effect. This prevents us from drawing any firm conclusion at this moment and demonstrates the need for further research” (1).

Progesterone has a minor suppressive effect on GnRH production, and this would decrease endogenous testosterone production. In a chart review, transwomen using medroxyprogesterone (in combination with estradiol and spironolactone) had lower testosterone concentrations than those on estradiol and spironolactone alone (79 vs 215 ng/dl)(10). Progesterone also competes for the 5-α reductase enzyme that converts T into dihydrotestosterone (11). These effects would theoretically be desirable in the feminization of transwomen.

We plan to conduct a randomized, placebo controlled double blind study evaluating the effect of adding progesterone for 6 months to transwomen who are being treated with estradiol. We hypothesize

that progesterone will decrease serum testosterone concentrations as compared to placebo and increase breast size. We will also assess its role in mood, sleep, scalp hair and androgenic hair growth.

Study endpoints: -

- Serum testosterone concentrations (*primary endpoint*)
- Serum luteinizing hormone (LH) and follicle stimulating hormone (FSH) concentrations
- Breast size
- Hair growth
- Sleep quality
- Mood

Study plan: The study will be conducted by the division of Endocrinology at Saint Louis University. It is a randomized, placebo controlled double blind study. We expect to recruit patients from the PI's transgender clinic and from advertisements.

Inclusion criteria

1. Transwomen, currently on treatment with estradiol therapy for at least 6 months (as their standard of care gender affirming therapy)
2. Has achieved serum estradiol >100 pg/ml at least once, based on clinical labs in past.
3. Age 18-65 years

Exclusion Criteria

1. Treatment with progesterone in the last 2 months
2. HIV
3. Planning to go for breast enhancement or gender reassignment surgery in the next 6 months
4. Known history of peanut allergy (because the study drug contains peanut oil)
5. Active deep vein thrombosis, pulmonary embolism or history of these conditions
6. Active arterial thromboembolic disease or history of these conditions
7. Active cardiovascular disorders or history of these conditions (e.g. myocardial infarction, uncontrolled hypertension >150/90 mmHg)
8. Known, suspected, or history of breast cancer
9. Known liver dysfunction or disease
10. Known or history of gallbladder disease. This does not apply to subjects who have undergone cholecystectomy
11. Known or history of hypertriglyceridemia (>400 mg/dl)

Screening visit: Informed consent will be obtained. This visit may be conducted during the PI's clinic (if the study volunteer is PI's patient) or at Endocrinology clinical research space at Salus Center. Vital signs (blood pressure, heart rate and weight) will be measured and a general physical examination will be performed.

Subjects who qualify and consent to take part in the study will be assigned a number by a computerized random number generation program and will be randomized (1:1) to receive either progesterone or placebo. An unblinded pharmacist will prepare the study product. Study investigators, staff and the study participants will be blinded.

Week 0 (baseline): Subjects will come fasting to the Salus Center. Breast exam and testicular exam will be conducted. They will complete the study questionnaires. Blood sample will be taken for total testosterone, total estradiol, sex hormone binding globulin (SHBG), LH, FSH, complete blood count (CBC), comprehensive metabolic panel (CMP) and research labs. For subjects who are on intramuscular estradiol, this visit will be performed 3-4 days after their last injection. Subjects will be randomized to receive generic micronized progesterone 200 mg or placebo tablets, to be taken at bedtime daily. They will be given a 3-month supply of the study drug. Subjects will be asked to bring the pill bottles back with them at the next visit to assess compliance.

Week 4: Subjects will be called by the study coordinator to counsel on drug compliance and to ask for side-effects.

Week 12: Subjects will come fasting to the Salus Center. For subjects who are on intramuscular estradiol, this visit will be performed 3-4 days after their last injection. Vital signs (blood pressure, heart rate and weight) will be measured and a general physical examination will be performed. Blood sample will be taken to measure total testosterone, total estradiol, SHBG, LH, FSH and research labs. They will complete the study questionnaires. They will be given a 3-month supply of the study drug. Subjects will be asked to bring the pill bottles back with them at the next visit.

Week 24 (end of study): Subjects will come fasting to the Salus Center. For subjects who are on intramuscular estradiol, this visit will be performed 3-4 days after their last injection. Blood sample will be taken to measure CBC, CMP, total testosterone, total estradiol, SHBG, LH, FSH and research labs. Vital signs (blood pressure, heart rate and weight) will be measured and a general physical examination will be performed. Breast exam and testicular exam will be conducted. They will complete the study questionnaires. They will then be discharged from the study.

Study Procedures

Clinical laboratory assays: All measurements will be carried out by Quest diagnostics as part of a standardized contract. We have previously published studies in which measurements of testosterone and estradiol on serum samples of our study subjects were done by Quest Diagnostics (12, 13). Testosterone and estradiol will be measured by current “gold standard” assay: liquid chromatography tandem mass spectrometry. SHBG, FSH and LH concentrations will be measured by a solid-phase, chemiluminescent immunometric assay (Siemens, IMMULITE 2500). Free testosterone and estradiol will be calculated using concentrations of total testosterone, total estradiol, SHBG and albumin by the formula of Sodergard *et al* and Vermuelen *et al* (14, 15).

Breast size: The primary measure of breast size will be the difference between breast circumference (measured in centimeters with a tape placed horizontally around the thorax over the fullest part of the bare breasts) and chest circumference (measured with a flexible tape horizontally placed around the thorax in the inframammary fold) (4). Maximal breast hemi-circumference (measured with a flexible tape crossing horizontally over the nipple) (9) and areolar diameter will be measured. Investigator will note Tanner stage of breast (5). Patients will be asked about feeling of fullness in breast (none, same as baseline, more than baseline) and their bra cup size.

Testicular exam: Both testes will be palpated while the subject is standing. Size of each testis will be compared against a standard orchidometer.

Hirsutism/hair growth: The degree of androgenic hair growth will be assessed by investigators according to the modified Ferriman and Gallwey (FG) scoring system (16, 17). Any shaving, depilation or waxing by the patient will be considered in the assessment. Shaving frequency will be recorded as times per week. Patients will be asked to rate their scalp hair growth (no change, better than before, worse than before) and scalp hair quality (no change, finer than before, coarser than before).

Mood: Subjects will complete two psychometric tests (18):-
a) Body-image concern questions of Body-uneasiness test (BUT-BIC)(19): This questionnaire has 9 questions and is meant to capture worries related to physical appearance. Responses are recorded on a 6 point Likert scale. The mean of all 9 questions is presented as the global severity index.
b) Symptom-Checklist-K-9 (SCL-K-9) to measure psychological distress level(20): This questionnaire has 9 questions and is meant to assess overall psychological distress. Responses are recorded on a 5 point Likert scale. The mean of all 9 questions is presented as the global severity index.

Sleep quality: We will use single-item sleep quality scale (SQS) to assess change in sleep quality during the study.
FG score calculator and study questionnaires are attached in the IRB application.

Research labs: 10 ml of blood will be obtained for research. Serum will be stored for future analysis based on study results. For example, if there is a decrease in FG score, we will check dihydrotestosterone levels.

Side effects: Progesterone may cause fluid retention, mood swings, breast tenderness, depression, transient dizziness and drowsiness, headache, abdominal pain or bloating, hot flashes in women. Long term use is associated with possibly increased risk of heart disease and stroke in women after menopause. Short term and long term risks in transwomen are not known.
If a significant or serious adverse event occurs, the subject will cease participation in the study. Discovery of two or more patients experiencing a significant or serious adverse event will lead to stopping of this study altogether.
Statistical analysis: The primary end-point of the study is change in total testosterone concentrations in transwomen after progesterone treatment at 24 weeks as compared to placebo. Transformations of the

data to meet statistical assumptions may be considered. *t*-test will be used for comparison. We estimate that testosterone concentrations will decrease by 20%. A sample size of 20 subjects per group (assuming a drop-out rate of 15%) will provide adequate power ($\beta = 0.8$) to detect a significant difference ($\alpha = 0.05$), provided the pooled standard deviation is not greater than the mean difference. Hence, we will recruit 40 transwomen in this study.

Secondary end-points: Change at weeks 12 and 24 in a) LH and FSH concentrations, b) breast size, breast hemi-circumference, areolar diameter, Tanner stage, feeling of fullness in breast, bra cup size, c) FG score, shaving frequency, scalp hair growth, scalp hair quality, d) global severity index scores on BUT-BIC and SCL-K-9 scales, e) SQS score.

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