UC San Diego

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Biomedical Protocol for
Interventional Research
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Androgen Effects on the Reproductive Neuroendocrine Axis

Protocol Number: 808679

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Summary of Changes from Previous Version:

Affected Section(s)	Summary of Revisions Made	Rationale
1.2, 1.3, 4.1, 6.1, 7.2	Removed at-home urine collection	Replaced with in-office blood draw for hormones
1.2, 1.3, 6.1	Changed weekly ultrasound to once only during the month of the LH-pulse visits	Protocol revision
1.2, 1.3, 4.1	Renumbered months for all groups	For consistency and clarity
1.2, 1.3	Reinstated ultrasound to initial visit	Protocol revision
3, 6.1, 8.1,9.4.2	Edited endpoints	Consistency, simplification, clarity
All	Grammar and typos	Readability

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1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title: Androgen Effects on the Reproductive Neuroendocrine Axis

Study Description: Testosterone Replacement Therapy (TRT) is the mainstay of gender affirming care for transgender men (TGM) who have male gender identity after female sex assignment at birth [1,2]. TGM receiving TRT, over time exhibit irregular menstrual bleeding, however, the mechanism of menstrual disruption is unknown [3-5]. Therefore, we propose to evaluate the effect of chronic testosterone (T) exposure on ovarian hormones and pituitary gonadotropin release that determines menstrual cyclicity. We will conduct a detailed study of blood reproductive hormone secretion in TGM before and during TRT as well as in untreated cisgender female (CGF) control subjects who report female gender identity congruent with female sex assignment at birth. We will also perform periodic clinical and ultrasonographic evaluations. Furthermore, we will monitor menstrual cycles and reproductive hormones in TGM who make the personal decision to discontinue TRT, whether it is to pursue fertility or for other reasons.

Objectives: *Primary Objective:* To determine if male-level exogenous androgens inhibit menstrual cycles, affect reproductive hormones, and follicle dynamics in TGM receiving TRT. To determine if these effects are reversible in TGM discontinuing TRT.
Secondary Objectives: (1) To determine if exogenous androgens in TGM suppress parameters of pulsatile LH secretion; and (2) To determine if the discontinuation of TRT affects the resumption of cyclicity and LH secretion in TGM.

Endpoints: *Primary Endpoint:* Change in the proportion of ovulatory cycles and menstrual cycle length
Secondary Endpoints: Measurement of pulsatile LH secretion, ovarian folliculogenesis.

Study Population: Transgender men starting or stopping TRT, cisgender female control subjects

Phase: n/a

Description of Sites/Facilities Enrolling Participants: Department of Obstetrics, Gynecology, and Reproductive Sciences, UCSD. Altman Clinical and Translational Research Institute, UCSD.

Description of Study Intervention: Initiation of testosterone replacement therapy and discontinuation of testosterone replacement therapy

Study Duration: 5 years

Participant Duration: 1 to 10 months, dependent on group assignment

1.2 SCHEMA

TGM Initiating TRT

Screening Visit/Study Visit 1[a]:	Informed consent, blood test. If qualified →physical exam, medical history, u/s.
Month 1 (pre-testosterone):	Weekly visits[b] | Daily diary | LH-pulse blood sampling visit[c] | Ultrasound[d]
Month 2 (testosterone):	Weekly visits[b] | Daily diary |
Month 3 (testosterone):	Weekly visits[b] | Daily diary |
Month 4 (testosterone):	Weekly visits[b] | Daily diary | LH-pulse blood sampling visit[c] | Ultrasound[d]
Month 5 (testosterone):	Weekly visits[b] | Daily diary |
Month 6 (testosterone):	Weekly visits[b] | Daily diary |
Month 7 (testosterone):	Weekly visits[b] | Daily diary | LH-pulse blood sampling visit[c] | Ultrasound[d]

TGM Discontinuing TRT

Screening Visit/Study Visit 1[a]:	Informed consent, blood test. If qualified →physical exam, medical history, u/s.
Month 1 (testosterone):	Weekly visits[b] | Daily diary | LH-pulse blood sampling visit[c] | Ultrasound[d]
Month 2 (post-testosterone):	Weekly visits[b] | Daily diary |
Month 3 (post-testosterone):	Weekly visits[b] | Daily diary |
Month 4 (post-testosterone):	Weekly visits[b] | Daily diary | LH-pulse blood sampling visit[c] | Ultrasound[d]
Month 5 (post-testosterone):	Weekly visits[b] | Daily diary |
Month 6 (post-testosterone):	Weekly visits[b] | Daily diary |
Month 7 (post-testosterone):	Weekly visits[b] | Daily diary | LH-pulse blood sampling visit[c] | Ultrasound[d]
Month 8 (post-testosterone):	Weekly visits[b] | Daily diary |
Month 9 (post-testosterone):	Weekly visits[b] | Daily diary |
Month 10 (post-testosterone):	Weekly visits[b] | Daily diary | LH-pulse blood sampling visit[c] | Ultrasound[d]

CGF Control Group

Screening Visit/Study Visit 1[a]:	Informed consent, blood test. If qualified →physical exam, medical history, u/s.
Participation Month:	Weekly visits[b] | Daily diary | LH-blood blood sampling visit[c] | Ultrasound

[a] The Screening Visit and Study Visit 1 may be performed on the same day. The participant will have a choice to think about participation and come back on another day. If the participant is ready to begin and is qualified, they can continue with the study protocol after passing the screening portion.
[b] Weekly visits will include a blood test for progesterone, estradiol, LH, and FSH.
[c] LH-pulse visits will sample blood for 8 hours, with one hour of prep and discharge (total 9 hours)
[d] Ultrasound will measure ovarian volume, all follicles, uterine volume, and endometrial thickness.

1.3 SCHEDULE OF ACTIVITIES (SOA)

TGM INITIATING TRT

Procedures	Screening Visit	Study Visit 1	Month 1 Pre-Testosterone	Testosterone Month 2	Testosterone Month 3	Testosterone Month 4	Testosterone Month 5	Testosterone Month 6	Testosterone Month 7 Final Study Visit
Informed consent	X								
Screening blood test	X								
Demographics	X								
Medical history		X	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly
Randomization	N/A								
Testosterone Administration				Weekly	Weekly	Weekly	Weekly	Weekly	Weekly
Concomitant medication review		X	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly
Physical exam (including height and weight, thyroid, hip and waist measurement)		X	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly
Vital signs		X	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly
Bleeding Log Survey (home)			Daily	Daily	Daily	Daily	Daily	Daily	Daily
Hematology		X	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly
Serum chemistry		X	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly
Pregnancy test		X	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly
Pelvic ultrasound		X	X			X			X
Adverse event review and evaluation		X	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly
LH Pulses visit			X			X			X
Complete Case Report Forms (CRFs)		X	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly

TGM DISCONTINUING TRT

Procedures	Screening Visit	Study Visit 1	Month 1 Still on testosterone	Month 2 Post-testosterone	Month 3 Post-testosterone	Month 4 Post-testosterone	Month 5 Post-testosterone	Month 6 Post-testosterone	Month 7 Post-testosterone	Month 8 Post-testosterone	Month 9 Post-testosterone Final Study Visit	Month 10 Post-testosterone Final Study Visit
Informed consent	X											
Screening blood test	X											
Demographics	X											
Medical history		X	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly
Randomization	N/A											
Testosterone Administration			Weekly									
Concomitant medication review		X	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly
Physical exam (including height and weight, thyroid, hip and waist measurement)		X	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly
Vital signs		X	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly
Bleeding Log Survey (home)			Daily	Daily	Daily	Daily	Daily	Daily	Daily	Daily	Weekly	Daily
Hematology		X	Daily	Daily	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly
Serum chemistry		X	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly
Pregnancy test		X	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly
Pelvic ultrasound		X	X			X			X			X
Adverse event review and evaluation		X	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly
LH Pulses visit			X			X			X			X
Complete Case Report Forms (CRFs)		X	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly

CONTROL GROUP

Procedures	Screening Visit	Study Visit 1	Participation Month
Informed consent	X		
Screening blood test	X		
Demographics	X		
Medical history		X	Weekly
Randomization	NA		
Testosterone Administration			
Concomitant medication review		X	Weekly
Physical exam (including height and weight, thyroid, hip and waist measurement)		X	Weekly
Vital signs		X	Weekly
Bleeding Log Survey (home)			Daily
Hematology		X	Weekly
Serum chemistry		X	Weekly
Pregnancy test		X	Weekly
Pelvic ultrasound		X	X
Adverse event review and evaluation		X	Weekly
LH Pulses visit			X
Complete Case Report Forms (CRFs)		X	Weekly

2 INTRODUCTION

2.1 STUDY RATIONALE

In the U.S. alone, ~1.4 million adults self-identify as transgender [7-10] and this prevalence is not only growing[11] but is likely higher than reported due to selection bias and social stigma[9,12-14]. Among transgender (TG) individuals, approximately 1/2 report masculine gender identity after female sex assignment at birth and identify as TGM or gender non-binary people (collectively designated TGM). TRT (provided to typically achieve serum T in the male physiological range) is the mainstay of gender-affirming medical care in TGM [2,15]. In TGM, TRT is used to masculinize physical characteristics and suppress menstrual cycles[4,5,16-19]. Yet, TRT effects on the neuroendocrine brain and pituitary hormone function have been largely overlooked. Importantly, TGM may desire childbearing[20,21] and many engage in vaginal intercourse with the potential to conceive spontaneously[21-27]. Yet, most TGM undergoing TRT become amenorrheic[4,5,17] and cannot spontaneously conceive.

Furthermore, no clinical report to date has prospectively evaluated the time-course or mechanisms of reversibility of TRT-induced effects on the HPO axis, including the time-course and pattern of restoration of normal LH pulse secretion, ovarian measures, and ovulation. Our study will systematically evaluate the sequence of reproductive/endocrine events following discontinuation of TRT in TGM planning to pursue fertility or stopping TRT for other personal reasons.

2.2 BACKGROUND

High anovulation rates were recently reported in TGM undergoing TRT[28], but it is unknown whether this ovulatory dysfunction is due, fully or in part, to altered preovulatory LH surge secretion or how T contributes to such pathophysiology. Aim 1 will clinically study how TRT in TGM impacts pituitary gonadotropin secretion and ovarian function to better understand T effects on HPG axis functioning in healthy individuals with ovaries, an understudied area of investigation.

To date, little is known regarding endocrine/reproductive effects of TRT, and the limited reports have yielded inconsistent results, in large part due to very small sample sizes and variability in TRT doses and durations studied[16,19,29-31]. Some evidence suggests that TGM receiving prolonged T in the typical male range show inhibited LH[16,19,32]. Even less is known regarding effects of TRT on LH pulsatility. Although one study reported no change in LH pulse pattern after short-term (6 weeks) low-dose oral TRT[30], another study by the same group reported a trend for reduced LH pulse frequency after longer TRT (6 mos.; given IM) at a higher T dose considered standard for TGM[16]. While promising, the data in both LH pulse studies was variable, likely due to inadequate sample sizes (n = just 5-6/group), and hence statistical analyses were underpowered. In fact, most prior TGM studies unfortunately had low statistical power owing to very small sample sizes and, hence, even moderate variability precluded definitive conclusions. Our study design will rectify this with larger, statistically appropriate sample sizes and enhanced power.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

Risks of this study include risk of testosterone replacement therapy, discomfort and/or swelling due to blood draws, and the risk of a loss of confidentiality.

1. Testosterone: While testosterone replacement therapy is medically appropriate and considered safe for transgender men, treatment with testosterone carries some risks and side effects. Those undergoing testosterone therapy may notice thicker, oilier skin and larger pores, acne, more masculine facial appearance, growth of facial hair, deepening voice, hair growth on the arms, back and chest, thinning hair on the head, reduction in fat around hips and thighs, an increase in stomach fat, increased muscle in

the arms and legs, more prominent veins, an increase of sweat production, and changes in the odors of sweat and urine.
Reproductive effects include menstrual changes such as lighter or heavier periods or discontinuation of periods as well as difficulty releasing eggs from the ovaries and a reduction in the ability to get pregnant. In case of pregnancy, testosterone may pose risks to the fetus.
Other complications may include abnormal level of lipids (such as cholesterol) salt retention, increased blood pressure, cardiovascular disease, production of too many red blood cells, sleep apnea, and weight gain.
Emotional changes may include emotional shifts or reduction of emotions; when a person receives testosterone for gender dysphoria, they may begin to feel more like themselves and may become more comfortable with their bodies. Sexual changes typically include changes in libido, an enlarged clitoris as well as changes in orgasms, arousal, and sexual interests.
2. Blood draws: Placement of the IV for blood draws and single stick blood drawing can cause pain, discomfort, swelling, bruising, a small risk of getting an infection, and occasionally dizziness and fainting.
3. Loss of Confidentiality: There exists the chance of loss of confidentiality associated with both participants and study data/specimens.
4. Pelvic ultrasound: Either done as transabdominal or transvaginal, there is risk of discomfort during the procedure.

2.3.2 KNOWN POTENTIAL BENEFITS

We plan to enroll three groups of subjects. Each have different potential benefit profiles.

1. TGM subjects who initiate testosterone replacement therapy will be provided medication (testosterone replacement therapy) for the duration of the trial (up to six months of testosterone replacement per subject) at no charge to the subject.
2. Cisgender subjects will have no direct benefit from participation in the study.
3. Transgender subjects discontinuing testosterone replacement therapy will also have no direct benefit from the participation in the study.

Although not all individual subjects will benefit directly from participating in this research study, there is a significant likelihood that this research will help society by advancing knowledge of the effects of testosterone replacement and discontinuation of testosterone replacement on reproductive physiology.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

As stated in above, the risks to subjects in this study are: testosterone replacement therapy for those receiving it, blood draws, loss of confidentiality, and pelvic ultrasound.

1. **Testosterone.** The dose of testosterone proposed in this study follows the most recently published standards for the health care of transgender people [1]. Safety of this TRT has been well-documented [33,34]. Testosterone replacement therapy is well tolerated. This study will enroll subjects who have made the decision along with their medical providers to initiate TRT for gender-affirming care.
2. **Blood Draws** will be performed by qualified/credentialed personnel using aseptic technique to prevent infection. Hemoglobin will be checked in all subjects prior to enrollment to ensure that there is no baseline anemia nor polycythemia; individuals with hemoglobin of less than 11 g/dL or higher than 16g/dL will not be studied. We will test hemoglobin before each LH-pulse visit and disenroll subjects with hemoglobin less than 11 g/dL or higher than 16 g/dL; this timing and frequency of testing is consistent with recommendations to check hemoglobin every three months for people during their first year on testosterone therapy. They will be advised to follow-up with their primary care physician for complete work-up and treatment. The United States Health and Human Services Office for Human Research Protections recommends that in

healthy, nonpregnant individuals who weigh at least 110 pounds. For these subjects, the amount of blood drawn for clinical research purposes may not exceed 550 ml in an 8-week period and collection may not occur more frequently than 2 times per week. The maximum blood draw volume in this protocol will be 240 ml per study day and will be performed no more frequently than every 3 months (for a total of 3 study days in our transgender/non-binary group initiating group and a total of 4 study days in our transgender/non-binary discontinuing group).

3. **Loss of Confidentiality.** Risks associated with a loss of confidentiality could feasibly include risks to employability, insurability, reputation, ability to adopt, or other social risks. These risks will be disclosed during the consenting process in verbal and written form. Risk will be minimized by following good research practices: subjects will be assigned a study number. Most study documents will not contain subjects' names or other identifying information. The Consent Forms will contain name and signature. One database will contain the linkage information to connect study participant identities to study data for cross-referencing. In order to protect subjects' identity and protected information safe, the investigators will keep the list of subjects and study numbers in a locked and protected area and/or in HIPAA-secured databases such as REDCap, and in password-protected servers using password-protected computers. Research records may be seen by the American Society of Reproductive Medicine, the National Institutes of Health (NIH), other governmental agencies, and UCSD Human Research Protections Program. The local resources available ensure this study is conducted in a way that assures protection of the rights and welfare of participants are adequate. Such resources include availability of the research staff and facilities at UCSD; protected research time for the investigational team members to conduct and complete the study; and availability of medical resources that participants may need as a consequence of the research. Procedures are in place for reporting protocol deviations/violations and local unanticipated problems involving risks to participants or others to the UCSD HRPP. The Principal Investigator will be responsible for monitoring and reporting of Adverse Events (AEs) to the IRB.
4. **Pelvic ultrasound:** Either done as transabdominal or transvaginal, there is risk of discomfort during the procedure. These procedures will be done by experienced technicians and/or physicians.

The potential benefits of this study to society and to gender-diverse individuals considering testosterone replacement therapy are huge and outweigh the risks. Scientists, clinicians, and gender-diverse individuals all seek more answers to their questions about the ability to preserve fertility after TRT. This research has a high potential for obtaining important clinically relevant findings on preserving fertility after TRT.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
The primary objective of this study is to evaluate effects of testosterone replacement therapy on reproductive physiology.	The primary endpoints are: Aim 1: Evidence of Luteal Activity (ELA) as defined by serum progesterone level above 3 ng/mL in transgender men initiating testosterone replacement therapy Aim 2: LH pulsatility (amplitude and frequency)	The primary endpoints were selected to provide detailed information regarding effects of testosterone replacement therapy on reproductive physiology and especially on ovulatory function.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Secondary		
The secondary objective is to comprehensively evaluate serum reproductive hormones.	*Aim 1:* Mean serum FSH/LH/Estradiol. Aim 2: Details of LH pulses including total LH secreted in pulses, basal LH secretion rate, and LH half-life Aim 3: Serum gonadotropin levels and sex steroid levels.	Measurements of these endpoints will provide comprehensive evaluation of the secretion of reproductive hormones in the presence and in the absence of testosterone replacement therapy.
Tertiary/Exploratory		
The tertiary objective is to monitor ovarian and uterine appearance via pelvic ultrasonography	Pelvic sonography of ovaries including sizes and number of follicles, presence or absence of corpus luteum and evaluation of uterine endometrium.	These endpoints will provide correlation of reproductive hormones with their effects on the reproductive organs.

4 STUDY DESIGN

4.1 OVERALL DESIGN

Study Design

We will perform a prospective, interventional study of TGM who are initiating or discontinuing gender-affirming testosterone replacement therapy (TRT) as part of their medical care. We will also study a control group of cisgender females who are not receiving testosterone. The interventions in this study will include venipuncture/phlebotomy, questionnaires, and vital sign collection. We will enroll three groups: (1) TGM initiating TRT will be enrolled prior to initiation of TRT; they will undergo one observational month (Month 1) with weekly evaluations and then will undergo weekly testosterone injections and weekly evaluations for 6 months (Months 2-7) while on TRT. (2) CGF controls will be enrolled and undergo weekly evaluations for one menstrual cycle (approximately one month). (3) TGM discontinuing TRT will be enrolled before their final month of TRT; they will undergo one observational month (Month 1) with weekly evaluations while on TRT and then will undergo weekly evaluations for 9 months (Months 2-10) after their final dose of TRT.

Pre-Screening

We will use a pre-screening questionnaire over the phone to see if patients are interested and meet the bare minimums for participating in the research study. Patients will be provided with basic information about the research study and then asked to assent to a pre-screening questionnaire to establish preliminary eligibility. If they meet the inclusion criteria and none of the exclusion criteria, they will be deemed eligible to continue and instructed on how to come in for a screening visit.

Screening

Before or during the first visit, potential participants will make an appointment to be consented. If they agree to the consent, we will do an initial blood draw to check hemoglobin levels. If the hemoglobin is less than 11 g/dL or over 16 g/dL, the subject will not be enrolled in the study.

They will have a history and physical examination performed which will include a urine pregnancy test and thyroid studies.

Subjects will be included in one of three groups: TGM Initiating TRT, TGM Discontinuing TRT, and CGF.

The longest study visits will be conducted at the University of California, San Diego- Altman Clinical and Translational Research Institute or at the _____ [site TBD] and will last approximately 9 hours (8 hours of sampling, plus 1 hour of prep and discharge). All subjects will undergo the following during each 9-hour study visit:

1. Baseline vital signs (Height, weight, waist circumference, hip circumference, urine pregnancy test)
2. An intravenous (IV) line placement in a vein in each subjects' non-dominant forearm. The IV line will be flushed with 1 ml sterile saline after placement.
3. Frequent serum LH sampling- 3 mL blood draw every 10 minutes between 08:30 to 16:20 (8 hours)
    - Prior to each sample collection, 1 ml of "waste" comprised of sterile saline in the line, mixed with a small amount of blood will be removed from the IV line and disposed in a standardized, deidentified manner compliant with hospital protocols.
    - After each sample collection, the IV line will be "flushed" with 1 ml sterile saline.

The total amount of blood drawn at each study visit will be about 140 - 180 ml over an 8-hour period, with most being <150ml. All blood samples will be processed to collect serum for hormonal testing.

CGF subjects will be studied at:

- one 1-hour initial visit
- 4 weekly visits (3 will be 30 minutes, 1 will last one hour and include ultrasound)
- one 9-hour (8 hours of sampling, plus 1 hour of prep and discharge) LH-pulse monitoring study visit.

Including time spent completing surveys, total study-participation time will be **13 hours.**

TGM Initiating TRT will be studied at:

- one 1-hour initial visit
- 28 weekly visits (25 30-minute visits, 3 will last one hour and include ultrasound)
- three 9-hour LH-pulse monitoring study visits (8 hours of sampling, plus 1 hour of prep and discharge)

Including time spent completing surveys, total study-participation time will be **47 hours**.

LH-pulse visits in TGM Initiating TRT will be scheduled as follows:

- LH-pulse Visit #1 during the mid-follicular phase of a menstrual cycle (cycle day 5-7) prior to initiating T
- LH-pulse Visit #2 timed 3 months ($\pm$ 14 days) after initiation of T
- LH-pulse Visit #3 timed 6 months ($\pm$ 14 days) after initiation of T

TGM Discontinuing TRT will be studied at:

- one 1-hour initial visit
- 40 weekly visits (36 30-minute visits, 4 will last one hour and include ultrasound)
- four 9-hour LH-pulse monitoring study visits (8 hours of sampling, plus 1 hour of prep and discharge)

Including time spent completing surveys, total study-participation time will be **64 hours**.

LH-pulse visits in TGM Discontinuing TRT will be scheduled as follows:

- LH-pulse Visit #1 as soon as possible after enrollment, no later than 7 days after their final dose of T
- LH-pulse Visit #2 timed 3 months (± 14 days) after final dose of T
- LH-pulse Visit #3 timed 6 months (± 14 days) after final dose of T
- LH-pulse Visit #4 timed 9 months (± 14 days) after final dose of T

Research Materials/Data and Specimen Storage

Serum, vital signs, and questionnaire responses collected for this study will be stored using unique study number, without personally identifying information. Research data (demographics, screening data, vital signs, and questionnaire responses) will be stored in REDCap, a secure, web-based, HIPAA-compliant, data collection platform with a user management system allowing project owners to grant and control varying levels of access to data collection instruments and data (e.g. read only, de-identified-only data views) for other users.

De-identified serum specimens will be stored in a freezer in the UCSD Biomedical Sciences Building on a floor with restricted access until they are sent for hormone level analysis at the National Institutes of Health Ligand Assay & Analysis Core of the Center for Research in Reproduction at University of Virginia. No PHI will be disclosed to entities beyond the PI and key personnel.

All research material and data will be obtained specifically for research purposes and no use will be made of existing specimens, records, or data.

Data collection, Data analysis, Significance, and Sample Size

At each 9-hour study visit, each subject will have blood drawn for measurement of serum LH, FSH, testosterone, estradiol, and progesterone levels, immediately followed by initiation of frequent blood sampling at 10-minute intervals for an additional 8 hours beginning at 08:30 and ending at 16:20. During the 8-hour frequent sampling period, blood samples will be used for measurement of LH concentration. Testosterone dose, date of the last T injection and compliance will be assessed and documented.

The data analysis for this study will be very similar to that of previous studies conducted by Drs. Dan Apter and Sam Yen [18]. Analysis of LH pulsatility will be performed using the Cluster 7 computer program of Veldhuis and Johnson [19]. This program uses deconvolution analysis to take into account changes in secretory burst number, amplitude, mass, duration and/or alterations in the hormone half-life. Mean pulse frequency between groups will be compared using ANOVA with post hoc testing.

Based on the findings by Spinder, et al. in 1989, we anticipate LH pulse frequencies of 69 ± 35 minutes before testosterone therapy and every 90 ± 31 minutes after 6 months on therapy, resulting in an effect size of 0.63. Thus, a sample size of 15 subjects per group is estimated to have 90% power to detect an LH pulse frequency effect size of 0.63 between treated TGM and controls when employing Wilcoxon's rank test at a significance level P = 0.05. Assuming a 20% drop out rate, 20 subjects will be

enrolled per group in an effort to complete study of 15 individuals per group. Therefore, a total of 40 individuals will be enrolled (20 per group).

Inclusion of Women and Minorities

This study will include 30 cisgender female and 50 transgender male/non-binary/gender non-conforming subjects. All participants will have had female sex assignment at birth, given that we are interested in disrupted ovulatory signals, which only occur in people with ovaries. The prevalence of gender incongruence in the United States is 0.4-1.2%, and not reported to be significantly affected by race or ethnicity. We suspect that ethnic make-up of our study cohort will reflect the diverse population of San Diego.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

In the U.S. alone, ~1.4 million adults self-identify as transgender [7-10] and this prevalence is not only growing[11] but is likely higher than reported due to selection bias and social stigma[9,12-14]. Among transgender (TG) individuals, approximately 1/2 report masculine gender identity after female sex assignment at birth and identify as TGM or gender non-binary people (collectively designated TGM). TRT (provided to typically achieve serum T the male physiological range) is the mainstay of gender-affirming medical care in TGM [2,15]. In TGM, TRT is used to masculinize physical characteristics and suppress menstrual cycles[4,5,16-19]. Yet, TRT effects on the neuroendocrine brain and pituitary hormone function have been largely overlooked. Importantly, TGM may desire childbearing[20,21] and many engage in vaginal intercourse with the potential to conceive spontaneously[21-27]. Yet, most TGM undergoing TRT become amenorrheic[4,5,17] and cannot spontaneously conceive.

Furthermore, no clinical report to date has prospectively evaluated the time-course or mechanisms of reversibility of TRT-induced effects on the HPO axis, including the time-course and pattern of restoration of normal LH pulse secretion, ovarian measures, and ovulation. Our study will systematically evaluate the sequence of reproductive/endocrine events following discontinuation of TRT in TGM planning to pursue fertility or stopping TRT for other personal reasons.

4.3 JUSTIFICATION FOR DOSE

Testosterone replacement therapy proposed in this study follows the most recently published standards for the health care of transgender people [1]. Safety of this therapy has been well-documented [33,34].

TGM will receive intramuscular or subcutaneous administration of testosterone cypionate or enanthate (TC) 50 mg[4] (**standard dose used clinically**) every 7 days for six months.

TRT proposed in this study follows the most recently published standards for the health care of transgender people [1]. Safety of this TRT has been well-documented [33,34].

Note: This investigation of effects of testosterone on TGM is not intended to be reported to FDA as a well-controlled study in support of a new indication for use. This investigation is not intended to support any other significant change in the labeling of the drug. Testosterone is lawfully marketed as a prescription drug product. This investigation is not intended to support a significant change in the advertising of testosterone. This investigation does not involve a route of administration that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of testosterone. This investigation does not involve a dosage level that significantly increases the risk (or decreases the acceptability of the risks) associated with the use of testosterone. This investigation does not involve use in a patient population that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of testosterone. This investigation does not involve any other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of testosterone.

4.4 END OF STUDY DEFINITION

A participant is considered to have completed the study if they have completed all phases of the study including the last visit or the last scheduled procedure shown in the Schedule of Activities (SoA), Section 1.3 and as follows:

1. TGM subjects initiating TRT will complete the study upon completion of last evaluation at the end of month 7.
2. CGF will complete the study upon completion of one menstrual cycle.
3. TGM discontinuing TRT will complete the study upon the completion of last evaluation at the end of month 10.

The study will be considered complete when full enrollment has been achieved in each study group and all participants have completed their participation.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

All

1. Provision of signed and dated informed consent form
2. Stated willingness to comply with all study procedures and availability for the duration of the study
3. Aged 18-35

Transgender/Non-binary Group - Initiators

4. Plan to initiate testosterone therapy
5. History of regular menstrual cycles (every 24-35 days) at baseline, before beginning TRT

Transgender/Non-binary Group – Discontinuers

4. Plan to discontinue testosterone therapy
5. History of regular menstrual cycles (every 24-35 days) before beginning TRT

Cisgender Female Group

4. Having regular menstrual cycles (every 24-35 days)

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria at baseline evaluation will be excluded from participation in this study:

All

1. Pregnant
2. Incarcerated
3. Known cognitive impairment or institutionalized
4. Hemoglobin <11 g/dL or >16g/dL
5. Weight less than 110 pounds
6. BMI <18 or >35
7. Current endocrine disease- including untreated thyroid abnormalities, pituitary or adrenal disease, polycystic ovary syndrome, or androgen producing tumor
8. Current or recent pregnancy within two months of study enrollment
9. Current or recent breast feeding within two months of study enrollment
10. Diabetes, or renal, liver, or heart disease
11. History of oophorectomy or hysterectomy
12. History of radiation or surgery involving brain structures
13. Currently taking any medications that may affect their reproductive hormones, such as contraceptive medications, androgens, estrogens, progestins, GnRH antagonists, GnRH agonists, insulinomimetics, and metformin.

For TRT Initiation Group and Cisgender Female group:

14. History of prior testosterone therapy

5.3 LIFESTYLE CONSIDERATIONS

Not Applicable

5.4 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently randomly assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE). Individuals who do not meet the criteria for participation in this trial (screen failure) because of hemoglobin less than 11 g/dL or higher than 16 g/dL will not be enrolled.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

Investigators will conduct a prospective, controlled clinical trial of ovarian and menstrual cyclicity in TGM initiating TRT, TGM discontinuing TRT, and cisgender female control subjects who have never used TRT. Enrollment will include **30 TGM initiating, 20 TGM discontinuing and 30 CGF control subjects** who report female gender identity congruent with female sex assignment at birth. All subjects will be at least 18 years old and no older than 35, with female sex assignment at birth, regular menstrual cycles, and body mass index 18-35 kg/m^2. Subjects with history of prior Testosterone use, cancer, chemotherapy, or radiation of the brain, abdomen, or pelvis, current use of hormonal medications (including, but not limited to, metformin, insulin, progestins, or estrogenic medications), current endocrinopathy (including, but not limited to, PCOS, androgen secreting tumor, diabetes, or pituitary, thyroid, or adrenal disease), and renal, hepatic, cardiac, or hematologic disease will be excluded.

TGM subjects in the initiating group will undergo baseline endocrine and menstrual cycle evaluation, followed by intramuscular or subcutaneous administration of testosterone cypionate (TC) 50 mg (standard dose) every 7 d for 24 wks.

TGM in the discontinuing group will undergo baseline evaluation during up to a month before discontinuing TRT.

All subjects will complete a daily uterine bleeding log using REDCap® and undergo weekly measurement of serum FSH, LH, estradiol (E_2), and P_4 (ELISA), and T (LC-MS/MS. Subjects will also undergo interval ultrasound (US) during the month(s) of their LH-pulse visit(s), either transvaginally or transabdominally, using a 4- to 9-MHz probe to obtain 3D pelvic imaging to track corpus luteum (CL) formation, as well as regression analysis of hormone secretion patterns, endometrial thickness, antral follicle count (AFC), and volume of uterus and ovaries. Changes in follicle dynamics will be analyzed via 3D U/S of the mean diameter of each antral follicle in 1-mm increments from 2 to 9 mm[171,172].

A primary endpoint will be Evidence of Luteal Activity (ELA), as defined by a serum progesterone level above 3 ng/mL in transgender men initiating testosterone replacement therapy. Secondary endpoints will include mean serum FSH, LH, and estradiol levels.

UCSD Investigators have already demonstrated successful recruitment and retention of TGM and CF participants in longitudinal studies involving daily bleeding diaries and serial pelvic ultrasound (U/S) in our pilot study and others[171,172]. TGM subjects will be serially evaluated for adverse effects of TRT per Endocrine Society recommendations[1]. Safety monitoring details are in the Human Subjects Section. We calculate that 20 subjects/group will have >95% power to detect a 30% decrease in the proportion of

subjects with ELA at baseline compared to the final 4-wk TC study interval (wks 21-24). Although we anticipate a larger, more clinically meaningful decrease, we utilized a conservative target to maximize the study's power. While we had no dropouts in our TGM pilot study, a 1/3 dropout rate is factored into our enrollment target of 30 subjects/initiating and CGF group and a 1/2 dropout rate is factored into our enrollment target of 20 subjects in the discontinuing group to ensure achieving sufficient power. We aim to recruit **30 TGM initiating (20 complete), 20 TGM discontinuing (10 complete), 30 CGF controls (20 complete)**.

6.1.2 DOSING AND ADMINISTRATION

No enrolled subjects will receive experimental products or doses. The group initiating TRT will receive TRT per standard of care. The form and dose of testosterone used in this study is FDA approved to treat medical conditions associated with low levels of naturally produced testosterone. It is also commonly prescribed off-label to help transgender people transition. The effects of these hormones are not well documented on the transitioning body, so the researchers are interested in learning about the effects of testosterone on participants' reproductive hormones and reproductive organs.

Justification for IND exemption: This investigation of effects of testosterone on TGM is not intended to be reported to FDA as a well-controlled study in support of a new indication for use. This investigation is not intended to support any other significant change in the labeling of the drug. Testosterone is lawfully marketed as a prescription drug product. This investigation is not intended to support a significant change in the advertising of testosterone. This investigation does not involve a route of administration that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of testosterone. This investigation does not involve a dosage level that significantly increases the risk (or decreases the acceptability of the risks) associated with the use of testosterone. This investigation does not involve use in a patient population that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of testosterone. This investigation does not involve any other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of testosterone.[1, 33, 34]

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

Testosterone replacement therapy for the initiating group will be given in the same injection mode and dose as is the standard of care. We will obtain testosterone from the USCD pharmacy, and it will be injected, per standard of care by licensed clinical practitioners. There is no investigational drug being used in this research study. The UCSD pharmacy is experienced in storing, handling, and dispensing TRT.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

There is no investigational drug being used in this research study. We are giving testosterone to the initiating group per standard of care.

TESTOSTERONE CYPIONATE INJECTION

Injection: 200 mg/mL in a single-dose vial

Testosterone Cypionate Injection, USP for intramuscular or subcutaneous injection, contains testosterone cypionate which is the oil-soluble 17 (beta)-cyclopentylpropionate ester of the androgenic hormone testosterone.

Testosterone cypionate is a white or creamy white crystalline powder, odorless or nearly so and stable in air. It is insoluble in water, freely soluble in alcohol, chloroform, dioxane, ether, and soluble in vegetable oils.

The chemical name for testosterone cypionate is androst-4-en-3-one, 17-(3-cyclopentyl-1- oxopropoxy)-, (17ß)-. Its molecular formula is C27H40O3, and the molecular weight 412.61.

Testosterone Cypionate Injection, USP is provided as sterile, clear colorless to pale yellow solution

containing 200 mg/mL testosterone cypionate in vials.
Each mL of solution contains:

Testosterone cypionate:	200 mg
Benzyl alcohol:	20 mg
Benzyl benzoate:	0.2 mL
Cottonseed oil:	542 mg

6.2.3 PRODUCT STORAGE AND STABILITY

Testosterone Cypionate Injection is supplied as a sterile, clear colorless to pale yellow solution in single-dose vials as 200 mg/mL testosterone cypionate.
NDC Number Package Size 71225-127-01 1 mL vials
Store at 15°C to 25°C (59°F to 77°F); excursions permitted to 2°C to 30°C (36°F to 86°F). Store product in carton to protect contents from light.

6.2.4 PREPARATION

There is no special preparation required. TRT will be prepared as per standard clinical care.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

We have no plans for randomization or blinding.

6.4 STUDY INTERVENTION COMPLIANCE

Subjects will be expected to come in for weekly evaluations and, if in the TRT initiator group, for weekly testosterone injections.
Study staff will send appointment reminders and follow-up calls for no-shows. Study staff will help participants to minimize barriers to study completion. Study staff will closely monitor each participant for compliance with the protocol.

6.5 CONCOMITANT THERAPY

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the Case Report Form (CRF) are concomitant prescription medications, over-the-counter medications and supplements.

Prohibited therapies, medications, or supplements include:

- **FOR ALL GROUPS:**
  - Contraceptive medications, androgens, estrogens, progestins, GnRH antagonists, GnRH agonists, insulinomimetics, and metformin.
    - Justification: these medications may affect their reproductive hormones.
  - Medications for Diabetes, or renal, liver, or heart disease.
    - Justification: We are excluding subjects with significant systemic diseases. If they start medications for these diseases during the study, they will be withdrawn from the study.

6.5.1 RESCUE MEDICINE

No applicable rescue medications.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

Discontinuation from testosterone, if in the initiating group, should be done in consultation with a participant's personal physician. If a participant in the initiating group discontinues testosterone voluntarily, they will be disenrolled from the study. There will be no useful knowledge gained from completing remaining study procedures. If a clinically significant finding is identified (including, but not

limited to changes from baseline) after enrollment, the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an adverse event (AE).

Participants in all groups are welcome to withdraw from the study at any time.

The data to be collected at the time of study intervention discontinuation will include the following:

- Reason(s) for discontinuing testosterone
- Reason for discontinuing study.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request.
An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Pregnancy
- If the patient starts a hormonal birth control method
- Significant study intervention non-compliance, e.g., subject does not complete daily diaries, subject misses weekly visits.
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- A new disease or condition that requires treatment with medications that are contraindicated to this study or disease progression which requires discontinuation of any study tasks including, if applicable, taking testosterone.
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- If the patient misses 2 weekly visits and/or is unable to receive testosterone for two weeks.

The reason for participant discontinuation or withdrawal from the study will be recorded on the Case Report Form (CRF). Research participants who sign the informed consent form but do not complete tasks, ask to withdraw, or are withdrawn or discontinued from the study may be replaced.

7.3 LOST TO FOLLOW-UP

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within 2 weeks of a planned visit and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.
- A participant will be considered lost to follow-up if they fail to return for two weekly scheduled visits and is unable to be contacted by the study site staff.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 EFFICACY ASSESSMENTS

Study procedures and evaluations to be done as part of this study to support the determination of efficacy therein are outlined and described in **Section 1.3 Schedule of Activities**.

- A pre-screening telephone call will be conducted prior to any interventions. Subjects will have time to consider their options before coming to a screening visit.
- During the screening visit, the study investigators will discuss the protocol in detail and the medical aspects including potential risks and benefits.
- Once the patient has all their questions answered satisfactorily and has agreed to participate in the study, the participant will be asked to sign the informed consent in English.
- The participant will be given a copy of the consent form and other applicable forms. Research personnel who may obtain consent include the principal and co-investigators, research coordinators, medical students, and UCSD resident physicians in the Department of Obstetrics, Gynecology, and Reproductive Sciences. All research personnel associated with this study will have completed proper training and certification and will be named in the IRB Kuali module.
- The Investigators will retain the signed consent form in either REDCap or as a paper pen-and-ink signature (control subjects may sign electronically whereas subjects on Testosterone must sign in a method that is FDA Part 11 compliant, either Part 11-compliant Docusign or Paper). Other associated forms will be stored in the HIPAA-compliant REDCap system.
- After signing the consent forms, the subject may undergo screening. Screening may be conducted within a week or more after signing the consent forms.
- Actual study participation may begin between 1 and 5 weeks thereafter, depending on timing of menstrual cycle, timing of TRT initiation, and/or timing of TRT discontinuation. These waiting periods will allow potential subjects sufficient time to consider whether to participate and minimize the possibility of coercion or undue influence.

<u>All procedures and exams will be conducted by licensed and experienced personnel. All instruments used will be calibrated and routinely maintenanced.</u>

- **Physical examination by physician:** height, weight, waist circumference, hip circumference
- **Radiographic or other imaging assessments by qualified personnel:** Pelvic ultrasound, either transabdominal or transvaginal, according to participant's preference.
- **Biological specimen collection and laboratory evaluations**: Blood test for hemoglobin, urine pregnancy test. Blood tests for LH, FSH, estrone (E_1) conjugates, and pregnanediol glucuronide (PdG). Serum tests for FSH, LH, estradiol (E_2), and P4 (ELISA), and T (LC-MS/MS) for confirmation of hormone dynamics.
- **Frequent serum LH sampling:** 3 mL blood draw every 10 minutes for 8 hours.
    - An intravenous (IV) line will be placed in a vein in each participant's non-dominant forearm.
    - Prior to each sample collection, 1 ml of "waste" comprised of sterile saline in the line, mixed with a small amount of blood will be removed from the IV line and disposed in a standardized, deidentified manner compliant with hospital protocols.
    - After each sample collection, the IV line will be "flushed" with 1 ml sterile saline.
- **Daily bleeding diaries:** surveys will be delivered via text message every morning and participants will complete these electronically.
- **Procedures that will be completed during the study as part of regular standard of clinical care:** weekly testosterone injections for TGM participants initiating TRT

Evaluations of weight, blood tests evaluating reproductive hormones, and pelvic ultrasounds are expected to provide information relevant to understanding the effects of testosterone on reproductive physiology. Participants will be notified of any irregularities that could affect their health and/or safety. All tests and procedures will be done for and paid by this study.

Serum, medical history data, vital signs, and questionnaire responses collected for this study will be stored using unique study numbers, without personally identifiable information. De-identified serum specimens will be stored in a freezer in the UCSD Biomedical Sciences Building or in the UCSD_____ (TBD office building) on a floor with restricted access until they are sent for hormone level analysis at the National Institutes of Health Ligand Assay & Analysis Core of the Center for Research in Reproduction at University of Virginia. No PHI will be disclosed to entities beyond the PI and key personnel.

Research data (demographics, screening data, vital signs, and questionnaire responses) will be stored in REDCap, a secure, web-based, HIPAA-compliant, data collection platform with a user management system allowing project owners to grant and control varying levels of access to data collection instruments and data (e.g. read only, de-identified-only data views) for other users.
All research material and data will be obtained specifically for research purposes and no use will be made of existing specimens, records, or data.

A primary endpoint will be Evidence of Luteal Activity (ELA), as defined by serum progesterone level above 3 ng/mL. Secondary endpoints will include mean serum FSH, LH, and estradiol levels

8.2 SAFETY AND OTHER ASSESSMENTS

Subjects are encouraged to ask any questions concerning the research study both before agreeing to participate and at any time during the course of the study.

Subjects will be seen weekly and will be encouraged to report any unexpected or undesired side-effects. We will closely monitor all subjects during the study and will address any discomforts or side effects as warranted. The most common testosterone-related effects include acne, hair thinning, and mood changes. If a participant reports displeasure from unwanted side effects greater than the subjective benefits of testosterone treatment, they can elect to disenroll from the study. They will be advised to consult with their personal physician/treatment team. The testosterone used in this study will be the same dose for all participants; if they choose to disenroll, they can modify dose of testosterone with their primary care physician if desired.

The specific timing of procedures/evaluations to be done at each study visit are captured in Section 1.3, Schedule of Activities (SoA). All procedures/evaluations will be performed by qualified personnel.

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS (AE)

This study does not include the use of experimental drugs or devices. TRT is being studied per the same dose and mode of administration per standard of care. The FDA definition of an Adverse event is any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or

convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.3.3 CLASSIFICATION OF AN ADVERSE EVENT

8.3.3.1 SEVERITY OF EVENT

For adverse events (AEs) not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".]

8.3.3.2 RELATIONSHIP TO STUDY INTERVENTION

All adverse events (AEs) must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Related** – The AE is known to occur due to the study procedures/exams/sample collections/testosterone, there is a reasonable possibility that something related to being in the study caused the AE, or there is a temporal relationship between the study and the event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study and the AE.
- **Not Related** – There is not a reasonable possibility that participation in the study caused the event, there is no temporal relationship between the study and event onset, or an alternate etiology has been established.

OR

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study participation and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal from the study should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after study participation, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response.
- **Potentially Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of testosterone or participating in a blood draw). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.
- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study participation makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after study participation) and in which other drugs

or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).

- **Not Related** – The AE is completely independent of study participation, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

8.3.3.3 EXPECTEDNESS

Principal investigator, any co-investigators, and key personnel will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study events.

8.3.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to testosterone or study procedures (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on the study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

Study staff will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

8.3.5 ADVERSE EVENT REPORTING

Adverse events will be identified during weekly visits with subjects and if the events reach the threshold of an unanticipated problem will be reported to IRB in accordance with the requirements.

8.3.6 SERIOUS ADVERSE EVENT REPORTING

The study clinician(s) will immediately report to the sponsor any serious adverse event, whether or not considered study intervention related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that testosterone or any of the study procedures caused the event. Study endpoints that are serious adverse events (e.g., all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the study procedures and the event. In that case, the investigator must immediately report the event to the sponsor.

All serious adverse events (SAEs) will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the participant is stable. Other supporting documentation of the event may be requested by the Data Coordinating Center (DCC)/study sponsor and should be provided as soon as possible.

8.3.7 REPORTING EVENTS TO PARTICIPANTS

In the event of identifying adverse findings (for example abnormal blood test results), subjects will be contacted, and the findings will be discussed.

8.3.8 EVENTS OF SPECIAL INTEREST

Not applicable

8.3.9 REPORTING OF PREGNANCY

In the event of pregnancy, subjects will be withdrawn from the study and the possible effects of testosterone on pregnancy will be discussed.

8.4 UNANTICIPATED PROBLEMS

8.4.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.4.2 UNANTICIPATED PROBLEM REPORTING

The investigator will report unanticipated problems (UPs) to the reviewing Institutional Review Board (IRB) and to the Data Coordinating Center (DCC)/lead principal investigator (PI). The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are serious adverse events (SAEs) will be reported to the IRB and to the DCC/study sponsor within **7 calendar days** –per NIH policy—of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB and to the DCC/study sponsor within **14 calendar days** of the investigator becoming aware of the problem.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and the Office for Human Research Protections (OHRP) within **30 days** of the IRB's receipt of the report of the problem from the investigator.

8.4.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

In the event of unanticipated problems, participants will be notified of any that affect their safety or confidentiality.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

Our **overall hypothesis** is that in otherwise healthy (non-polycystic ovary syndrome) females, male-level exogenous androgens inhibit the reproductive neuroendocrine axis through androgen-receptor (AR) signaling in hypothalamic *Kiss1* neurons to alter luteinizing hormone (LH) pulse secretion and LH surges and inhibit neural circuits controlling these modes of LH secretion. This work will greatly improve understanding of the impact of high androgens on female reproductive physiology and be of high clinical importance to the rapidly growing, yet currently understudied, TGN/TGM population.

9.2 SAMPLE SIZE DETERMINATION

Primary Efficacy Endpoint(s):

Aim 1, comparing TRT initiators to CGF: We calculate that 20 subjects/group will have >95% power to detect a 30% decrease in the proportion of subjects with evidence of luteal activity at baseline compared to the final month of the study. We plan to enroll 30 subjects/these groups (30 TRT Initiators and 30 CGF) assuming a loss of 1/3 of the subjects in course of the study.

Aim 2, TRT Initiators: We anticipate that 20 subjects will provide >95% statistical power to detect a 30% decrease in LH pulse frequency among testosterone treated transgender men. Assuming a 1/3 drop-out, 30 subjects in the TRT Initiators group will be enrolled to ensure a final n=20.

Aim 3, TRT Discontinuers: Based on a reported ovulatory rate of 4.5% in patients with a median 11 months on testosterone replacement, we calculate that 10 subjects will provide >95% power to detect an increase in the proportion of subjects with ELA to 60% in the final month interval after discontinuation of testosterone replacement therapy. Assuming a 1/2 drop-out, 20 subjects in the TRT discontinuation group will be enrolled to ensure a final n=10. We assume the drop-out rate for this group might be higher because subjects in this group may be very eager to discontinue testosterone and may withdraw at a higher rate during the first month.

9.3 POPULATIONS FOR ANALYSES

Group 1: Transgender men who are initiating gender-affirming testosterone replacement therapy
Group 2: Transgender men who are discontinuing gender-affirming testosterone replacement therapy
Group 3: Cisgender women who are not and have never been on testosterone replacement therapy

We intend to analyze the data from all subjects who successfully complete the study per protocol.

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

Data will be analyzed with the aid of statistical packages such as JMP. Continuous data will be summarized as means, standard deviations and ranges. Categorical data will be presented as percentages.

P-values will be considered significant at 0.05 threshold.

9.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

Aim 1: primary endpoint (Evidence of Luteal Activity) will be analyzed with the aid of Chi-Square test (for comparison of proportion of ovulatory cycles in cis-gender subjects with transgender subjects) and by McNemar test (for paired comparison of proportion of ovulatory cycles before and during testosterone treatment in subjects undergoing testosterone replacement therapy).

Aim 2: primary endpoints (LH pulse amplitude and frequency) will be analyzed with the aid of t-test (comparing cisgender subjects with transgender subjects) or by paired t-test comparing transgender subjects before and during testosterone replacement therapy). Normality of distribution will be assessed by the Shapiro-Wilk W test. In the absence of normality and/or unequal variance, data were appropriately transformed and/or non-parametric testing will be performed.

Aim 3: primary endpoint (Evidence of Luteal Activity) will be analyzed with the aid of McNemar test (for paired comparison of proportion of ovulatory cycles before and after discontinuation of testosterone treatment in subjects undergoing testosterone replacement therapy).

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

Secondary endpoints will be analyzed in identical fashion to primary endpoints whereby categorical variables will be tested using Chi-Square test or McNemar tests, as appropriate, while continuous variables will be evaluated using t-test or paired t-test, as appropriate. In the absence of normal distribution, nonparametric tests will be used.

9.4.4 SAFETY ANALYSES

Not applicable

9.4.5 BASELINE DESCRIPTIVE STATISTICS

Cisgender group and transgender group (Aims 1 and 2) will be compared with regard to demographics and laboratory measurements using identical tests to tests described in 9.4.3.

9.4.6 PLANNED INTERIM ANALYSES

Not applicable

9.4.7 SUB-GROUP ANALYSES

Not applicable

9.4.8 TABULATION OF INDIVIDUAL PARTICIPANT DATA

Individual participant data will be listed by measure and time point.

9.4.9 EXPLORATORY ANALYSES

Not applicable

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigators, staff, and the funding agency. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or Food and Drug Administration (FDA).]

10.1.2 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participants. Therefore, the study

protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

To further protect the privacy of study participants, a Certificate of Confidentiality will be issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.1.3 FUTURE USE OF STORED SPECIMENS AND DATA

Data collected for this study will be analyzed and stored at UCSD. De-identified data pertinent to Aim 2 will be shared with our collaborator, Dr. Chris McCartney (University of Virginia).
With the participant's approval and as approved by local Institutional Review Boards (IRBs), de-identified biological samples will be stored at the ACTRI Biorepository with the same goal as the sharing of data with the University of Virginia Biorepository. The ACTRI Biorepository will also be provided with a code-link that will allow linking the biological specimens with the phenotypic data from each participant, maintaining the blinding of the identity of the participant.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to biosample storage may not be possible after the study is completed.

When the study is completed, access to study data and/or samples will be provided through the ACTRI Biorepository.

10.1.4 KEY ROLES AND STUDY GOVERNANCE

Provide the name and contact information of the Principal Investigator and the Medical Monitor.

Principal Investigator	**Medical Monitor**
Antoni J Duleba, MD, Professor	N/A
University of California, San Diego	
9500 Gilman Drive, 0633 La Jolla, CA 92093-0633	
203-804-7123	
Antoni.duleba@health.ucsd.edu	

10.1.5 SAFETY OVERSIGHT

Adverse events will be monitored by the investigators.

10.1.6 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with International Conference on Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s).

- No external monitoring is anticipated and the study will comply with all UCSD requirements.
- Independent audits will not be conducted by the study sponsor to ensure monitoring practices because this is the only site.

10.1.7 DATA HANDLING AND RECORD KEEPING

10.1.7.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into RedCAP, a 21 CFR Part 11-compliant data capture system provided by the ACTRI. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

10.1.7.2 STUDY RECORDS RETENTION

Study documents should be retained according to UCSD and NIH requirements.

10.1.8 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly. These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations in accordance with IRB requirements. All deviations must be addressed in study source documents. Protocol deviations must be sent to the reviewing Institutional Review Board (IRB) per their policies. The investigator is responsible for knowing and adhering to the reviewing IRB requirements.

10.2 ADDITIONAL CONSIDERATIONS

Not applicable

10.3 ABBREVIATIONS

The list below includes abbreviations utilized in this template. However, this list should be customized for each protocol (i.e., abbreviations not used should be removed and new abbreviations used should be added to this list).

AE	Adverse Event
AFC	Antral Follicle Count
ANCOVA	Analysis of Covariance
AR	Androgen Receptor
AUC	Atypical Urothelial Cells
CGF	Cisgender Female
CFR	Code of Federal Regulations
CL	Corpus Luteum
CRF	Case Report Form
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
DRE	Disease-Related Event
E_1	Estrone
E_2	Estradiol
EC	Ethics Committee
eCRF	Electronic Case Report Forms
ELA	Evidence of Luteal Activity
ELISA	Enzyme-Linked Immunosorbent Assay
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FFR	Federal Financial Report
FSH	Follicle-Stimulating Hormone
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GWAS	Genome-Wide Association Studies
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ISO	International Organization for Standardization
ITT	Intention-To-Treat
IV	Intravenous
LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry
LH	Luteinizing Hormone
LSMEANS	Least-squares Means
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
MSDS	Material Safety Data Sheet
NCT	National Clinical Trial

NIH	National Institutes of Health
NIH IC	NIH Institute or Center
OHRP	Office for Human Research Protections
PCOS	Polycystic Ovary Syndrome
PdG	Pregnanediol Glucuronide
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMC	Safety Monitoring Committee
SoA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
T	Testosterone
TC	Testosterone Cypionate or Enanthate
TG	Transgender
TGM	Transgender Male
TGN	Transgender Non-Binary
TRT	Testosterone Replacement Therapy
UP	Unanticipated Problem
US	United States
U/S	Ultrasound

10.4 PROTOCOL AMENDMENT HISTORY

The table below is intended to capture changes of IRB-approved versions of the protocol, including a description of the change and rationale. A Summary of Changes table for the current amendment is located in the Protocol Title Page.

Version	Date	Description of Change	Brief Rationale
1.0	November 1, 2023		
1.1	May 7, 2024	Revisions in response to IRB pre-review	To clarify protocol.
1.3	June 29, 2024	Changed consent form protocol to paper collection.	To comply with FDA Part 11 regulations.
1.4	July 1, 2024	Added option for subcutaneous TRT injections	Similar bioavailability, may be more palatable to some participants.
1.5	August 6, 2024	Revisions in response to IRB conditional approval.	To clarify protocol.

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