STUDY PROTOCOL AND STATISTICAL ANALYSIS PLAN

Official title: EMRSHN 2: Exploring the Modulatory Role of Sex Hormones Along the Neuromechanical Axis in Females

NCT number: NCT03947684

IRB Approved date: 05-08-2020

Form A

IRB	**STU-2018-0192**
Velos	**26783**

PROTOCOL FORM / RESEARCH DESCRIPTION

If an item does not apply to your research project, indicate that the question is "**not applicable**" – do not leave sections blank

Click once on the highlighted entry in each box to provide your response. Click the item number/letter or word, if hyperlinked, for detailed instructions for that question. If your response requires inserting a table, picture, etc, you may need to first delete the box that surrounds the answer and then insert your table or other special document.

1. Purpose and objectives. *List the purpose and objectives:*

OBJECTIVES:

The goal of this project is to test our central hypothesis that changes in sex hormone concentration result in changes to the basic elements of motor control – at multiple levels, from the musculotendinous unit to motor control circuitry. Under Aim 1 we will determine the influence of sex hormone fluctuations on the muscle stretch reflex during active and passive states, and the time lag between hormone concentration changes and the reflex response. We will use a technically simple assessment that could be implemented in the field. Under Aim 2 we will determine the influence of sex hormone fluctuations on spinal motor neuron excitability using H-reflex as a probe and the simultaneous change in the muscle mechanics using muscle twitch response. Aims 1 & 2 will include a focus on the differential role of oral contraceptives. In Aim 3 we will use paired-pulse transcranial magnetic stimulation during active contraction to determine the influence of sex hormone fluctuations on cortical excitability in naturally cycling women. In the basic elements of motor control explained in Aim 2 and Aim 3 is that the sex hormones modulate the synaptic transmission and plasticity of neuron and muscles. The modulation of the synaptic transmission and plasticity of neuron and muscles might be caused by the changes of the neurotransmitter or muscle fiber in the transcriptional level, translational level, or combination of both. In Aim 4, we want to explore these changes through sequencing of whole exome and microRNA in addition to evaluating the expression level of mRNA and proteins related to synaptic transmission and muscle. Our findings have the potential to guide future research in injury prevention strategies, including targeted neuromuscular training. This knowledge will enable clinicians to educate female patients on neuromuscular effects of sex hormones during times of high fluctuations, including puberty, pregnancy, and menopause.

Abbreviations:
nOC = non- users of oral contraceptives
OC = oral contraceptive users

2. Background.

- Describe past experimental and/or clinical findings leading to the formulation of your study.
- For research involving investigational drugs, describe the previously conducted animal and human studies.
- For research that involves FDA approved drugs or devices, describe the FDA approved uses of this drug/device in relation to your protocol.
- Attach a copy of the approved labeling as a product package insert or from the Physician's Desk Reference.

You may reference sponsor's full protocol or grant application (section number and/or title) or if none, ensure background includes references.

Please respond to all components of this item, or clearly indicate which components are not applicable.

a. Background

BACKGROUND:

The National Institutes of Health has called for the study of sex differences in all areas of biomedical research. One of the most striking sex disparities in musculoskeletal medicine is the increased risk of anterior cruciate ligament (ACL) injuries in female athletes. Female athletes are up to 9 times more likely to experience ACL injuries than males competing in the same sports. These injuries cause significant morbidity, including time lost from school or work, psychological distress, and post-traumatic knee osteoarthritis rates (10-15 years after injury) up to 80% higher than in the uninjured knee.

Joint stability depends upon articular congruence, passive stiffness of connective tissues, and reflexive and voluntary muscle contractions. Therefore, changes in muscle mechanics could compromise the force- generating capacity of skeletal muscle and lead to decreased joint stability. Impaired stability increases the risk of musculoskeletal injury, particularly to the ACL. Such injuries are linked to impaired neuromuscular control or abnormal patterns of muscle activation. Specifically, the female pattern of landing with decreased hip and knee flexion, increased knee valgus, and an increased quadriceps-to-hamstring activation ratio is linked to higher rates of injury. Prevention focused on neuromuscular training has reported success, but the sex disparity in ACL injury persists.

While injury rates, hormones, and some motor control patterns diverge around puberty, it is unclear how such changes increase the risk of ligament injury. Neuromuscular control is influenced by fluctuations in estradiol and progesterone during the menstrual cycle, but the mechanism of this interaction remains unknown. Post-pubertal females have shown variable muscle activation patterns of the gluteus maximus and semitendinosus during a landing task that correlate with cyclic variations in estradiol. Neuromuscular control during hopping also fluctuates with hormone changes, but not in women with more stable levels of estradiol and progesterone induced by monophasic oral contraceptives. To fill the current research gap, we propose to determine the hormonal regulation of different constituents of neuromuscular control.

Our project will increase understanding in four realms. First, we propose a paradigm shift, arguing that increased rates of injury in females is not due to direct changes in cycle specific connective tissue properties, but are secondary to hormone mediated changes in neuromuscular control. Second, we will determine if our muscle stretch reflex protocol can identify hormone-mediated alterations in joint control. If so, this protocol can be employed to determine whether this process causes joint injury. Due to its technical simplicity, the protocol and the associated equipment (hammer/load cell/wireless EMG unit and a tablet) can be used in clinical settings and at athletic training sites, facilitating large-scale examination of the neuromuscular underpinnings of joint injuries. Importantly, our pilot data showed a significant change in the reflexes (on average, up to 2.5 folds reductions) from the luteal to the ovulatory phase, highlighting the robustness of the test in detecting changes in reflex excitability, which may be applicable to multiple clinical settings. Third, our findings should allow clinicians to time neuromuscular retraining programs to certain points during maturation or the menstrual cycle when females might be most receptive to reprogramming neuromuscular control strategies. Similarly, identifying a cortical level locus of sex hormone mediated changes in neuromuscular control would inform the implementation and timing of motor learning specific strategies. Fourth, by including

women taking hormonal contraceptives, we will determine if, and to what extent, endogenous and exogenous estradiol and progesterone have different effects on knee neuromechanics.

It has been suggested that oral contraceptives might prevent musculoskeletal injury in female athletes, but there is a paucity of literature to guide clinicians in this area. As 40-70% of female collegiate basketball and soccer players [more than 10 million women in the United States, and 100 million women worldwide] report taking oral contraceptives, understanding their effects on the neuromusculoskeletal system is critical. Answering these questions has the potential to reduce the risk and rates of ACL injury and mitigate the long- term knee pain in future generations of female athletes. While our focus is on basic neuromuscular changes due to natural hormone fluctuations, we expect that this knowledge will illuminate other effects of sex hormones on the neuromusculoskeletal system, such as in osteoarthritis in perimenopausal women or lumbopelvic control in peripartum females.

Most studies have focused on hormonal modulation of the passive mechanics of connective tissues, measured in humans at the aggregate joint scale. Such studies have yielded conflicting results. Instead, we argue that sex hormones may have a more significant effect on active properties of the neuromuscular system. We suggest the injury sex disparity is likely due to changes in neuromuscular control rather than in aggregate joint stiffness. To the best of our knowledge, this study will be the first to examine, simultaneously, hormonal effects in the musculotendinous unit (Aim 1), motor neuron (Aim 2), and cortical motor neuronal networks (Aim 3), which are key contributors to movement control. This project builds a foundation for the study of sex differences in neurophysiologic properties of cortical networks.

b. Current practice

N/A

3. Study Design.
Describe the study design (e.g., single/double blind, parallel, crossover, etc.) Consider inserting a scheme to visually present the study design.

Same as #4 below.

4. Research Plan / Description of the Research Methods:

4.a. Provide a **comprehensive narrative** describing the **research methods**.

1) Provide the **order in which tests/procedures will be performed**,
2) Provide the **setting** for these events and a description of the **methods used to protect privacy** during the study.
3) Provide the **plan for data analysis** (include as applicable the **sample size calculation)**

Please respond to all components of this item, or clearly indicate which components are not applicable.

STUDY TIMELINE:

	Year 1			Year 2				Year 3				Year 4				Year 5			
month	1-3	4-11	12	1-5	6	7-11	12	1-5	6	7-11	12	1-5	6	7-11	12	1-5	6	7-11	12
Set-up, hiring																			
Recruitment																			
Testing																			
Project Meeting																			
Data Analysis																			
Conferences																			
Manuscripts																			

PROCEDURES INVOLVED:

The sequence of experiments is shown in Fig. 1. During month one, all eumenorrheic subjects (nOCs), will provide blood samples every other day to track their hormones across the menstrual cycle (they will track menstrual cycle for 2-3 months prior). Post hoc analyses will compute subject-specific hormonal fluctuations throughout the month using a nonlinear mixed-effect model. nOCs will use urinary ovulation kits to verify that they have ovulatory cycles and identify the approximate day of ovulation. nOCs and male controls will have a brain MRI to establish consistent coil placement across visits in Aim 3, where TMS will be used to assess cortical excitability.

In month 2, all female subjects, both OCs and nOCs, will undergo every other day testing as described in Aims 1 and 2 (described in detail below) and provide every other day blood samples. nOC subjects will use urinary ovulation kits daily as described above. Hormone concentrations of each nOC subject will be compared to the subject-specific mixed-effect model from month one to determine where she is in her menstrual cycle. This estimation will enable TMS testing at six key points during the cycle (Aim 3). For example, when the subject's daily estradiol concentrations are rising and the model indicates that her peak estradiol should occur the next day, she will be instructed to report to the lab for TMS testing in the next 24 hours. On these days, she will complete the protocols for Aims 1-3. Subjects will be randomized to start testing in the follicular, peri-ovulatory, or luteal phase where Day 1 is defined as the first day of menses. The time required for each experiment, including setup, is approximately 2½ to 3 hours for Aims 1 and 2 combined and 2 - 3 hours for Aim 3. The investigator will be blinded to menstrual cycle phase and contraceptive use. The comparative groups for Aims 1 and 2 will be women on monophasic oral contraceptives containing progestins of similar potency and androgenicity,as justified by our published and pilot data. The control group for all Aims will be age-matched healthy males. Our choice of males as

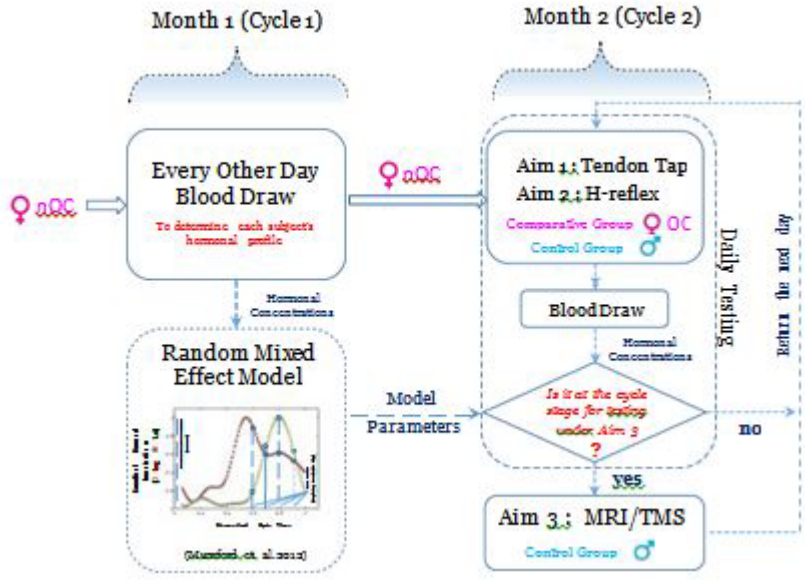

Figure 1. Sequence of experiments. In month one, non-users of oral contraceptives will have their blood drawn to determine their hormonal signature. In month two, subjects will undergo daily blood draws as well as testing for Aims 1 and 2. Subjects will complete tests for Aim 3 on six occasions defined by key hormonal fluctuations.

controls for Aim 3 is motivated by the lack of data to guide interpretation of the effects of exogenous hormones on cortical circuitry.

Below is the specific experimental protocol for each AIM:

AIM 1: Determine the influence of sex hormone fluctuations on muscle stretch reflex at relaxed and active states.

Venipuncture will be performed at UT Southwestern Medical Center or UT Southwestern Lab. Venipuncture will be completed every other day during a full menstrual cycle, for a total of 2 cycles. This equates to 28 to 40 blood draws total (not necessarily consecutively), up to 3.04 teaspoons of blood per draw, for a maximum of 85.12 to 121.6 teaspoons (425.6 ml to 608 ml) over the course of study participation (2 – 3 months, equivalent to 2 menstrual cycles). To minimize the effect of diurnal fluctuations of hormone levels, testing will be done at a consistent time of day for each test session across all Aims. Furthermore, OCs will be asked to ingest their birth control pill at a consistent time each every day of the testing.

At each session, subjects will be seated in an experimental chair with their right hip and knee positioned in 90^{o} of flexion. The ankle joint will be secured in a coupling ring, which will fix the limb to a load cell, via a rigid beam. Pre-amplified surface electromyography will be placed along the lower limb muscles. In an effort to ensure the same electrode placement at subsequent testing sessions, each electrode site will be marked with a permanent marker and subjects will be encouraged not to wash off the markings between sessions. Three maximum voluntary contractions (MVCs) will be recorded with a minimum of 10 seconds of rest between trials. The muscle reflex will be evoked by tapping the patellar tendon with a hammer instrumented with a load cell. The load cell instrumented hammer will allow us to quantify the reflex gain by normalizing the reflex EMG by the tap force. The hammer will be fixed on a pendulum to standardize the tap force and tap rate used to elicit the reflexes. For each pre-activation level (0%, 5%, 10%, and 20% knee extension torque at MVC), up to 40 total taps will be delivered during each testing session. Taps will be randomly spaced (varying from 10-20 seconds between taps) to limit the subjects' ability to anticipate the timing of the next tap. In an attempt to further minimize supraspinal modulation of the observed tap induced muscle activity at rest (0% MVC), subjects will be instructed to relax and maintain a standardized position. Finally, to assess the joint's connective tissue response at varying points in the cycle, anterior knee laxity (AKL) will be measured with the knee arthrometer.

AIM 2: Determine the influence of sex hormone fluctuations on spinal motor neuron excitability

Subjects will be placed in a prone position on an examination table with the right knee in full extension (the targeted side for all participants). An AirCast® boot will secure the ankle joint. The boot will be placed within a coupling ring, which will fix the limb to a six degrees-of-freedom load cell (JR3®). This testing protocol will be employed in an attempt to standardize the testing environment, behavioral state, degree of muscle activation, and ankle joint angle across testing sessions as changes in these factors can influence spinal reflex amplitude and twitch force (i.e., initial muscle length). Two protocols will be employed in order to assess the influence of hormone concentrations on both mono- and poly-synaptic spinal circuits. For the monosynaptic H-reflex testing, a stimulating bar bipolar electrode (Digitimer Ltd, Hertfordshire, England) will be placed over the tibial nerve in the popliteal fossa with the anode positioned distally. The

H-reflex and M-Wave will be recorded from the soleus muscle using surface EMG electrodes (see Aim 1 methodology) placed over the muscle belly at the myotendinous junction. Stimulus intensity will be controlled manually via a digitimer (DS&A constant current stimulator) and waveforms will be visualized on an oscilloscope (Tektronix TDS 2014). One- millisecond stimuli will be delivered at 10-20 second intervals to prevent post-activation depression. The H- reflex and M-wave recruitment curves will be mapped by increasing the stimulus intensity in 1mA increments starting from 2mA, noting the peak of the H-reflex and the plateau of the M-wave. To maximize the accuracy of these measurements, additional measurements using incremental increases in stimulation intensity (0.2mA) will be taken around the peak H-reflex. Once we identify the stimulation intensity necessary to yield the peak H-reflex, we will obtain 5 measurements at 10-20 second intervals, as well as 5 measurements of the maximum M-wave at 10-20 second intervals. To compute the associated changes in the contractile properties of the MT, single electrical pulses (1 ms in duration) at intensity 20% higher than the intensity at the maximal M-wave will be delivered to the soleus muscle. Five supramaximal stimulations will be elicited, and the corresponding M-waves and twitch torques will be stored and later averaged. For polysynaptic reflex testing, stimulation will be applied behind the medial malleolus of the ankle to the distal tibial nerve using a Grass Instruments S48 Stimulator, a Grass SIU5 Stimulus Isolation Unit and a Grass Constant Current Unit (model CCU1) (Grass Instument Company, W. Warwick, RI). The constant current stimulator will allow a constant safe current to be used throughout the experiment. The isolation unit is standard when using the Grass stimulator in human research to ensure maximum safety for the subject. The stimulation consists of a train of eight biphasic rectangular pulses at a frequency of 200 Hz and a pulse duration of 2 ms. Subjects will start with 2 mA stimulation intensity. The current will gradually increase by 2mA until the first visible contraction of the abductor halluces muscle is found. The data collection will occur at double the current of the motor threshold. In the experiment described by Hubli et al (2011) the average stimulation intensity was ~16.0 mA (sd 8.1 mA). Stimulation at this intensity has been shown to be non-noxious and safe in healthy subjects (Dietz et al, 2009). EMG recordings will be taken from the tibialis anterior muscle using self-adhesive surface electrodes. Data collection will include 20 series of 8 train pulses.

AIM 3: Determine the influence of sex hormone fluctuations on cortical excitability using transcranial magnetic stimulation (TMS).

Surface EMG will be recorded from muscles in the right leg. The paired-pulse paradigm isolates any modulatory influence of hormone concentration at cortex. With the subject seated with hip, knee, and ankle flexed at 90 degrees, TMS will be delivered to the leg area of the left motor cortex through using one of our TMS systems (Magstim (Magstim 200 Bistim^2, Whitland, UK), Nexstim (eXimia NBS/T, Nexstim, Helsinki, Finland), or MagVenture (MagPro X100, Denmark)). The TMS coil will be positioned over the intersection of the central sulcus with the mid-saggital plane using the subject's own anatomical MRI using an optical navigation system (Localite GmbH, Brainsight, or Nexstim eXimia). MRI images will be acquired prior to the first TMS laboratory visit at the Shirley Ryan AbilityLab Center or the Center for Translational Imaging, located across the street from the Shirley Ryan AbilityLab. Optimal coil position for producing a motor evoked potential (MEP) in the right leg muscle will be marked on the 3D-rendered cortical surface, ensuring consistent coil placement throughout and across sessions. Active MEP threshold will be determined as the lowest stimulus intensity producing MEPs just distinguishable above background in 5 of 10 consecutive trials during tonic contractions at ~10% of MVC. The conditioning stimulus, first pulse, will be set to 5% less than the active MEP threshold, an intensity below the level required to produce a recordable descending volley in the corticospinal tract. To ensure this stimulus level is sub-threshold, we

will analyze a set of 10 stimuli online for evidence of evoked potentials. Conditioning stimulus strength will be lowered in 1% increments until no evidence is identified. The test stimulation intensity, second pulse, will be set to produce MEPs of 500 - 1500μV in amplitude. While the subject maintains a tonic contraction at ~10% MVC ten to fifteen trials of paired-pulse TMS will be delivered, while an additional 20-25 test pulse trials (without a preceding conditioning pulse) will be randomly interspersed for the control.

Strength-dexterity (SD) test

The SD test requires that participants use their thumb and index fingers to compress a spring as far as possible before buckling. This test involves dynamic control of finger motions and force generation. The SD test consists of a custom spring (Century Springs Corp., Los Angeles, CA, USA) with two compression load cells (ELB4–10, Measurement Specialties, Hampton, VA, USA). The load cells are connected to a signal-conditioning box and USB-DAQ (National Instruments, Austin, TX, USA), sampled at 2000 Hz using custom Matlab (The Mathworks, Natick, MA, USA) software. Participants will be asked to compress the spring at their own pace to the point of maximal instability they can sustain (i.e., beyond which they feel it will slip out of their hand), and maintain that compression at a steady level for at least 5 s. After familiarization, 10 trials will be performed by the dominant hand and the compression force, defined as the mean of the three maximal trials, will be used as the outcome measure. Participants will be allowed as many practice trials as needed to obtain steady state compression for the minimum required compression time of 5 s.

AIM 4: The modulation of the synaptic transmission and plasticity of neuron and muscles.

Small portions of the blood samples taken in AIM 1 will be used for analysis in AIM 4.

The modulation of the synaptic transmission and plasticity of neuron and muscles might be caused by the changes of the neurotransmitter or muscle fiber in the transcriptional level, translational level, or combination of both. We want to explore these changes through sequencing of whole exome and microRNA in addition to evaluating the expression level of mRNA and proteins related to synaptic transmission and muscle.

This research study includes genetic testing. Human blood contains genes that determine many of a person's physical characteristics, such as the color of eyes and hair. In some cases, genetic testing of blood can be used to indicate a risk for the development of certain diseases. Genetic information is unique to each individual and could potentially be used to discover possible changes in a person's future health status or life expectancy, or that of his/her children and family members.

Releasing this information to the subject could cause psychological distress, anxiety or family problems. Releasing this information to others, such as including it in their medical record, may pose a possible risk of discrimination, or increase difficulty in obtaining or maintaining disability, long-term care, or life insurance.

These risks would occur if the subject's information is released by mistake. The measures being taken to protect the subject's privacy are discussed below and make this possibility unlikely.

Even though the results of genetic testing may not be linked to the subject, it is possible that people of their ethnic background may be found to be at more risk for certain diseases based on future genetic research and this information might harm the subject in the future as a member of the group. Also, there may be unknown risks of genetic testing in the future.

DATA AND SPECIMEN BANKING AND MANAGEMENT:

Paper questionnaires and signed consents will be kept in a locked cabinet at UT Southwestern Medical Center or UT Southwestern Lab (CS6.102A and H9.104, respectively), during data collection and analysis. Only listed key personnel specifically designated and authorized by the Principal Investigators shall have access to these documents. All personnel will be properly trained and supervised regarding the management and handling of confidential materials. All data (paper and electronic) will be destroyed within 5 years of data analysis completion. Serum samples will be collected UT Southwestern Medical Center or UT Southwestern Lab, which will then be analyzed at UT Southwestern Medical Center or UT Southwestern Lab. Samples will be labeled with subject number and date and time of testing. They will be held in temporary storage at UT Southwestern Medical Center or UT Southwestern Lab. Once the data collection is complete, the de-identified frozen samples at UT Southwestern Medical Center/Lab will be shipped to Dr. Ellen Casey to be analyzed for additional hormones, such as relaxin and cortisol, and their relationship to estradiol and progesterone. All blood samples will be destroyed 5 years after the completion of the study. Only members of the study team and those individuals responsible for the analysis will have access to the specimen samples.

All necessary data pertaining to the study from SRAlab will be transferred to UT Southwestern Medical Center. Data (except for blood samples) will be maintained for future use, for indefinite amount of time. All data would be de-identified and stored in a protected database. Researchers only with IRB approval and approval from authorized personnel of this study, would have access to the controlled database of data, to be used to answer questions related specifically to their research study.

SAMPLE SIZE JUSTIFICATION

AIMS 1 and 2: We wish to rule out an effect of hormonal state on motor neuronal excitability. To do this, specified the minimum effect size that we considered meaningful along with high statistical power that minimized the chances of a Type-I error. We conducted statistical simulations to evaluate this scenario. Pooling N = 30 OCs and N = 30 nOCs that provide daily H-refelxes from soleus muscle (Aim 1) and MSR from 3 quadricepts muscles (VM, VL, RF) over one menstrual cycle provided power = .95 to detect effects as small as an average within-individual correlation of r = -0.075 (R2 = .005) between a single hormone and reflex responses across a range of situations. Smaller differences are considered as pragmatically meaningless. This degree of power holds even if menstrual cycles vary from 22 to 32 days, and participants miss an average of 25% (see above) of their lab visits (and assumes the data are missing at random).

AIM 3: To test hypothesis 3, that estradiol will produce a targeted facilitatory influence on lower limb muscles, we used a GEE model that views the mean conditioned MEPs at each ISI as correlated outcomes before separate post-hoc tests for each inter-pulse interval (ISI = 7, 10, 15, 20, 25, 30 ms) and each subject between peak estradiol and baseline hormone concentration (Fig. 7). This synthesis establishes a within-

subject control. Based on preliminary data of four subjects, at each of the 6 ISIs >7 ms (see Fig. 6), a sample size of 20 subjects achieved over 90% power to detect a main effect for a GEE model that pools the ISIs as correlated outcomes. Additionally, we have sufficient power (.80) to follow up a main effect, for each of the six ISIs, to detect differences of 40% or larger from the null hypothesis, with an estimated standard deviation of 37% and with a significance level (alpha) of a Bonferroni-corrected 0.05/6 = 0.008. To allow for attrition we will aim to enroll 30 subjects in each female cohort. To ensure that our within-subject changes can be attributable to changes in the hormonal milieu, we will enroll N = 15 males and test them at multiple occasions.

4.b. List of the study intervention(s) being tested or evaluated under this protocol

☑ **N/A -** this study does not test or evaluate an intervention. Skip to item 4.d.

#	Study intervention(s) being tested or evaluated under the protocol *Add or delete rows as needed*	Affiliate Place a check next to institution(s) where the intervention will be performed	Local Standard Practice? Indicate whether the intervention is considered acceptable practice locally for applicable institutions
1	Insert study intervention 1 here	☐ UTSW	☐ Yes
		☐ PHHS	☐ Yes
		☐CMC	☐ Yes
		☐THR	☐ Yes
		☐TSRH	☐ Yes
		☐ Other: _____	☐ Yes
2	Insert study intervention 2 here	☐ UTSW	☐ Yes
		☐ PHHS	☐ Yes
		☐CMC	☐ Yes
		☐THR	☐ Yes
		☐TSRH	☐ Yes
		☐ Other: _____	☐ Yes

4.c. Risk:Benefit Analysis of study interventions being tested or evaluated under this protocol

For each study intervention identified in section 6b above, complete a risk:benefit analysis table.

(Two tables are provided, copy & paste additional tables as needed or delete both tables if this study does not test an intervention)

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4.c.
Study Intervention #1
Insert name used in 4.b.

List each group exposed to this intervention on a separate line. (e.g., experimental, control, Arm A, Arm B, etc) **Or** state All Groups/Subjects	For each group, list the **benefits** of this intervention. (Benefits can be directly from the intervention or from a monitoring procedure likely to contribute to the subject's well being). If there are no benefits, state "none".

If you are requesting a Waiver of Informed Consent, complete the table below.

If you have a consent form, **list the reasonably foreseeable risks in the consent form (and do not complete this section).**

List the risks according to the probability (likely, less likely or rare) and magnitude (serious or not serious).
(include: 1) expected adverse events; 2) rare and serious adverse events; 3) all other psychological, social, legal harms)
Do not delete frequency. Frequency must be estimated because it will assist you with determining which adverse events will require prompt reporting.

	Not serious	**Serious**
Likely These risks are expected to occur in more than **20** out of **100** subjects.	•	•
	Not serious	**Serious**
Less likely These risks are expected to occur in **5-20** subjects or less out of **100** subjects.	•	•
		Serious
Rare These risks are expected to occur in less than **5** subjects out of **100**		•

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4.c.
Study Intervention #1
Insert name used in 4.b.

List each group exposed to this intervention on a separate line. (e.g., experimental, control, Arm A, Arm B, etc) **Or** state All Groups/Subjects	For each group, list the **benefits** of this intervention. (Benefits can be directly from the intervention or from a monitoring procedure likely to contribute to the subject's well being). If there are no benefits, state "none".

If you are requesting a Waiver of Informed Consent, complete the table below.

If you have a consent form, **list the reasonably foreseeable risks in the consent form (and do not complete this section).**

List the risks according to the probability (likely, less likely or rare) and magnitude (serious or not serious).
(include: 1) expected adverse events; 2) rare and serious adverse events; 3) all other psychological, social, legal harms)
Do not delete frequency. Frequency must be estimated because it will assist you with determining which adverse events will require prompt reporting.

	Not serious	**Serious**
Likely These risks are expected to occur in more than **20** out of **100** subjects.	•	•
	Not serious	**Serious**
Less likely These risks are expected to occur in **5-20** subjects or less out of **100** subjects.	•	•
		Serious
Rare These risks are expected to occur in less than **5** subjects out of **100**		•

4.d. List ALL other research procedures or components not listed in table 4.b.
The combination of Tables 4b and 4d should account for all of the research procedures that will take place during this study.

Consider grouping similar procedures under a single component (e.g., blood work, CT = safety assessments)

#	Research component • **individual procedures** *example:* **Eligibility Assessments** • History and physical • Questionnaire • Laboratory tests *Add or delete rows as needed*	**Column A** **Local Standard Practice** Indicate the number of times each procedure will be performed as stipulated in the research plan **that would be performed if the participant were not participating in the study.**	**Column B** **Research Only** Indicate the number of times each procedure will be performed solely for research purposes *(meaning that the participant would not undergo the same number of procedures or would not undergo the procedure(s) at the same frequency if they were not participating in the study)*	**Column D** **Risks** **If you are requesting a Waiver of Informed Consent, complete the table below.** List the reasonably expected risks for each procedure or group of procedures under the following categories as appropriate: • Serious and likely; • Serious and less likely; • Serious and rare; • Not serious and likely; • Not serious and less likely
1	**Aim 1**	N/A	Every other day, for a menstrual cycle	N/A
	Blood draw for Estradiol, Progesterone, andTestosterone			
	Reflex testing			
	Anterior Knee Laxity Measurement			
2	**Aim 2**	N/A	Every other day, for a menstrual cycle	N/A
	Blood draw for Estradiol, Progesterone, and Testosterone			
	EMG/Nerve conduction Testing			
3	**Aim 3**			
	TMS	N/A	5 – 6 times in a menstrual cycle	N/A
	MRI	N/A	once	N/A
	Strength-dexterity test	N/A	5 – 6 times	N/A
4	**Aim 4**	N/A	Every day for a menstrual cycle, until ovulation	N/A
	Blood analysis on microRNA,	N/A	2	N/A
	Blood analysis for DNA sequencing	N/A	2	N/A
	Blood analysis on mRNA	N/A	2	N/A
5	**Ovulation testing**			
	Urine Ovulation Kit		1.	

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5. Safety Precautions. *(Describe safeguards to address the serious risks listed above.)*

a. Describe the procedures for protecting against or minimizing any potential risks for each of the more than minimal risk research procedures listed above.

1. Skin irritation – cleaning the skin with alcohol prior to placing surface electrodes and tape will help minimize the skin irritation associated with the use of surface electrodes
2. Muscle twitching and discomfort – taking breaks during sessions will lessen the discomfort; subjects also have the option to stop the testing
3. MRI – there are no known serious risks associated with MRI for subjects without contraindications to the test. However, some may become claustrophobic, anxious, or fatigued. Subjects will be in constant communication with the study staff/technician throughout the procedure and they have the option to stop the MRI at any time;
4. Noise (from the TMS/MRI procedures) – noise-cancelling ear buds will be provided

b. Where appropriate, discuss provisions for ensuring necessary medical or professional intervention in the event of adverse events, or unanticipated problems involving subjects.

The laboratory at H9.104 is adjacent to the James W. Aston Ambulatory Care Center, which is fully equipped to handle the very rare probability of adverse events or unanticipated problems involving subjects.

c. Will the safeguards be different between/among groups?

☐ Yes ☑ No