I. Hypotheses and Specific Aims:

Hypothesis. *Insufficient FSH pulsatility, as seen in obesity, results in inadequate folliculogenesis and reduced ovarian steroid and protein production.*

AIM: ***To test the hypothesis that insufficient FSH pulsatility, as seen in obesity, results in inadequate folliculogenesis and reduced ovarian steroid and protein production.*** We will determine if exogenous FSH administered in a pulsatile fashion results in a significant increase of ovarian hormones in obese women. Serial inhibin B and E2 levels will be measured in obese and normal weight women undergoing frequent blood sampling studies before and after GnRH antagonist blockade. In addition, we will conduct a pilot study to correlate the fecal microbiome and bacterial metabolism with reproductive hormone levels in normal weight and obese women.

II. Background and Significance:

Maternal obesity is an independent risk factor for decreased reproductive fitness and detrimental impact on offspring. In the US, 32% of women 20 to 39 years old are obese.[1] U.S. women born in the 1980s exhibit a 21 % higher propensity to have a large body mass compared to those born in the 1960s.[2] Thus, a further escalation of the current trends is expected when the 1980s generation reaches its peak of obesity prevalence. Maternal obesity is associated with decreased reproductive fitness: ovulatory and menstrual dysfunction,[3] increased congenital anomalies,[4] iatrogenic[5] and spontaneous[6] preterm birth, recurrent miscarriage.[7] Even in regularly ovulating women, obesity is linked with increased spontaneous pregnancy loss,[8] longer time to pregnancy,[9] and decreased lifetime fertility.[10]

Although weight loss and lifestyle modifications should be the first line therapy for any sequelae of obesity, the evidence for the effectiveness of this approach for impaired reproduction is lacking. A recent Cochrane meta-analysis indicated that there are no randomized trials supporting a benefit of weight loss for pregnancy outcomes.[11] Thus, it is critically important that we improve our understanding of the pathophysiology leading to decreased reproductive fitness in obesity in order to design alternative approaches for intervening. Abnormalities of reproductive hormones and ovarian function have been implicated.[12-15] Our long-term goal is to identify molecular interactions between affected aspects of hypothalamic-pituitary-ovarian (HPO) hormonal changes and pathophysiology of obesity. However, to target molecular interactions we must clarify the mechanisms for altered HPO dynamics in this population. Thus, our short-term goal is to improve our understanding of the mechanisms underlying disrupted reproductive hormone secretion in obese women.

Pituitary follicle-stimulating hormone (FSH) secretion is regulated mostly by hypothalamic Gonadotropinreleasing hormone (GnRH) secretion but also by endocrine input from the ovary. Inhibins, secreted by granulosa cells of ovarian follicles, exert a potent negative feedback effect on FSH. The reproductive aging associated fall in circulating inhibin B (and not a drop in estradiol) is believed to be responsible for the classic, monotropic rise in FSH that accompanies the menopausal transition. FSH pulsatility is challenging to characterize given its long half-life and low signal-to-noise ratio. Our work shows that existing pulsatility methods miss a third or more of FSH pulses. Female obesity is associated with reduced levels of both inhibin B and FSH, providing a unique opportunity to tease apart the secretory dynamics of FSH in the absence of one of its controlling mechanisms. Female adiposity is strongly related to decreased reproductive fitness, subfertility, and harmful metabolic programming of the offspring. Reproductive dysfunction in obesity may be related to abnormal hypothalamic-pituitary dynamics (a central deficit in GnRH, FSH or luteinizing hormone (LH) production), an abnormal ovarian environment (reduced sex steroid or ovarian protein production), or both. Most analyses of FSH have focused on mean levels, leaving us unable to probe if features of pulsatile FSH secretion are related to the reproductive deficits observed in obese women. Thus, a knowledge gap exists because we have not adequately assessed the interplay between pituitary FSH and its feedback in obesity.

There is abundant evidence that the fecal microbiome is altered in obesity and may be a contributing factor to its etiology. To date no studies have correlated measurements of ovarian steroids with microbiome composition and/or bacterial gut metabolism. Evidence in mice suggests that bacterial de-conjugation of estrogen glucuronides may modulate circulating estrogen levels[18,19]. We propose to analyze the fecal microbiome of normal and obese women and correlate bacterial composition and metabolism with host reproductive hormone levels before and after treatment. This will provide preliminary data to test the impact of microbial diversity on obesity related sub-fertility.

III. Preliminary Studies/Progress Report:

Our new preliminary data from Dr. Kumar's lab (co-I) demonstrate that pulsatile FSH secretion dramatically enhances ovarian function in mice (PNAS 2014;111:5735). In this transgenic model, FSH was diverted into the LH secretory pathway that resulted in reduced follicular atresia, prolonged reproductive lifespan and enhanced fertility. The oocytes produced in these FSH transgenic mice were of high quality, developed into viable blastocysts, and resulted in viable pups upon embryo transfer. We aim to translate and expand these novel findings of biological relevance of FSH pulsatility in the current proposal.

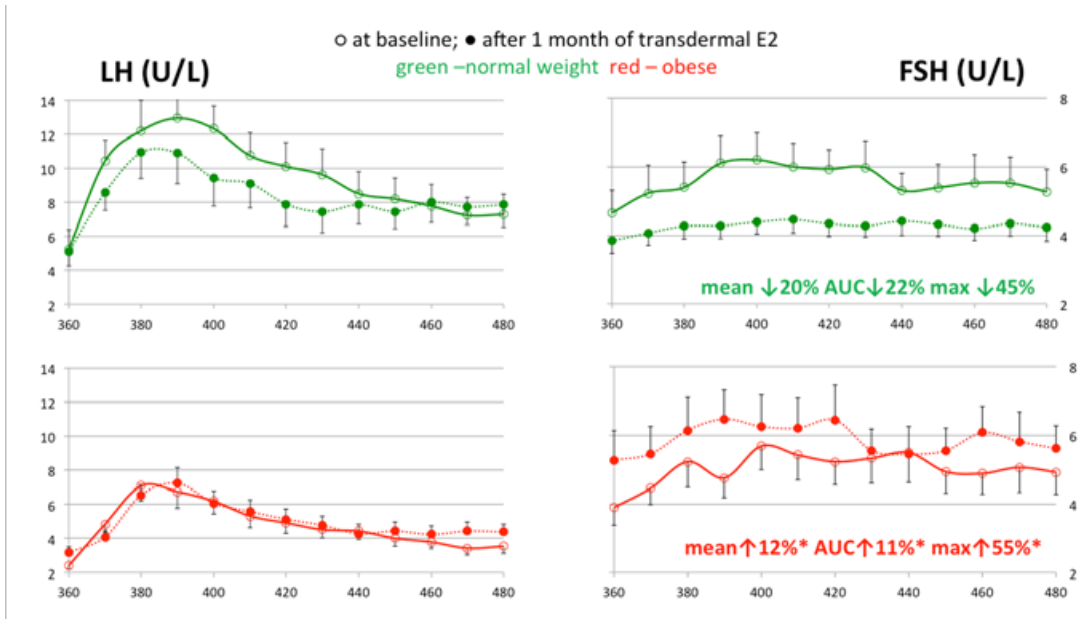
The PI has recently conducted a study recruiting women from the local community for an investigation of pituitary sensitivity to estrogen in obesity (National Clinical Trial (NCT) identifier: 01381016). Significantly lower serum inhibin B level was observed in obese women.

Reduced Early Follicular Inhibin B in Obese Women despite "Un-elevated" Serum FSH

	Normal weight n=10	Obese, n=11	P
Age, years	29.4(1.9)	32.5 (1.8)	0.25
BMI, kg/m$_2$	21.2 (1.5)	36.7 (1.3)	<0.01
AMH, ng/ml	3.2 (2.3)	1.2(1.1)	0.01
Inhibin A, pg/ml	19.3(6.6)	20.2 (6.1)	0.87
Inhibin B, pg/ml	96.6 (36.3)	67.7(38.2)	0.04
Mean FSH, IU/L	6.4 (0.7)	5.8(0.8)	0.57
Mean LH, IU/L	5.2 (0.8)	2.5 (0.2)	<0.01

Notably, antral follicle count is typically NOT reduced in obese women as seen by others and our group. Thus, the reduced inhibin B is unexplained. In this Aim, we will determine whether the pulsatile pattern of FSH secretion is responsible for the reduced circulating levels of inhibin B and E2 in obese women. We and others have shown that the deficit in LH in obesity is greater that the FSH deficit.

However, FSH deficit in obese women appears to be modifiable. We have observed a significantly improved FSH but not LH response to IV GnRH in obese women after one month of transdermal estrogen.



Exogenous Estradiol Improves FSH Sensitivity in Obese Women. Composite response of mean endogenous LH and FSH (± SEM) for 11 obese and 10 normal weight menstruating women to GnRH bolus of 75 ng/kg given at time =360. Y axis is gonadotropin in IU/ml. X axis is time on minutes.

This suggests that the pituitary is a functionally important locus for mediating the impact of obesity on female reproduction. We posit that abnormal FSH secretion is responsible for ovulatory defects in obesity and is the focus of the current proposal.

IV. Research Methods

A. Outcome Measure(s):

We will examine LH and FSH secretion during frequent blood sampling at 10 min intervals.

Primary: the difference between peak E2 and inhibin B from Day 1 to
day 2. This is defined as the maximum hormone value during the Day2 study minutes after the maximum hormone value during the Day1 study before GnRH antagonist is administered.

Secondary: peak inhibin B and E2 per subject, area under the curve (AUC)
for inhibin B and E2 during stimulation; we will compute LH and FSH pulsatility (pulse frequency, and average pulse amplitude) on day 1 and day 2, along with the means of LH and FSH to assess adequacy of GnRH antagonism and FSH stimulation. LH pulsatility will be assessed to confirm phenotypic concordance of this study population with existing literature.

B. Description of Population to be Enrolled:

Inclusion criteria:

- Age between 21 to 39 years old with regular menstrual cycles every 25-40 days,
- Body mass of 18.5 kg/m2-24.9kg/m2 (normal weight controls) or greater than 30.0 kg/m2 (obese group),
- Prolactin and TSH within normal laboratory ranges at screening, baseline hemoglobin >11 gm/dl.

Exclusion criteria:

- Diagnosis of PCOS, defined by the 2003 Rotterdam criteria as suggested by 2012 NIH Workshop,
- History of chronic disease affecting hormone production, metabolism or clearance or use of thiazolidinediones or metformin (known to interact with reproductive hormones),

- Use of hormones affecting HPO axis (such as hormonal contraceptives) within 3 months of entry,
- Strenuous exercise (>4 hours of intense physical activity per week),
- Pregnancy, breast-feeding or current attempts to conceive, significant recent weight loss or gain,
- Irregularities in uterus or ovaries,
- Serum anti-Mullerian hormone (AMH) equal to or greater than 20 pmol/l.This is based on the data that the specificity and sensitivity of AMH for identifying women with polycystic ovary syndrome were described to be over 97% and 95%, respectively, at this cut-off (Eilertsen et al., Hum Reprod 2012; 27: 2494-2502). Additionally, it has been demonstrated that AMH and antral follicle count (AFC) have an equal level of accuracy in the prediction of an excessive response to ovarian stimulation (Broer et al., Hum Reprod Update 2011;17:46-54) but AFC is subject to inter-observer variability (J. van Disseldorp et al., Hum. Reprod 2010; 25: 221-227),
- Impaired renal, hepatic, or cardiac function,
- Allergy, hypersensitivity, or intolerance to study drug or its excipients
- Presence or sequelae of gastrointestinal, liver, kidney, or other conditions known to interfere with absorption, distribution, metabolism, or excretion of drugs,
- Recent blood or blood product donation.

C. Study Design and Research Methods

Up to 68 subjects will undergo two 10-hour frequent blood sampling studies on consecutive days. However, the PI will first need to identify the dose level that results in a significant pharmacodynamic (PD) effect, namely an increase in serum inhibin B, with a target PD response rate of 80% across patients. Therefore, up to 9 subjects will also be needed for a pilot portion of the study (up to 3 subjects at each level will be needed to identify the level at which the target response rate is reached).
The total maximum number of subjects consented will be 120.

At the date of this protocol (10/11/2016), 6 subjects had already been treated in the pilot portion of this protocol.

- 3 subjects were treated at 10 IU; none of them showed an increase in inhibin B; adverse events of delayed menses and hot flushes thought be related to GnRH antagonist use were reported.
- 3 subjects were treated at 20 IU; none of them showed an increase in inhibin B; adverse events of delayed menses and hot flushes thought be related to GnRH antagonist use were reported.

With this version of the protocol (10/11/2016), we propose to treat 9 subjects with 30 IU, administered every 60 minutes over 10 hours, for a total of 300 IU.

Preliminary analysis of the completed study visits thus far (09/3/2019) have indicated the obese cohort has lower serum FSH levels than the normal weight cohort in response to the addition of rFSH on Day 2. We want to investigate if the difference we see is because FSH is being excreted in urine. We propose to collect urine at 4 nonspecific time points during day 1 to obtain a baseline value of FSH in urine for each participant and at the following specific time points T=0, 30, 60, 120, 240, 480, 600 minutes during day 2. We understand that asking the participants to provide 3 urine samples in the first hour of day 2 might not be possible for every participant. Therefore, we will ask each participant to give at least 2 samples (0 and 60 min on day 2) and request the 30-minute sample be tried but not required.

(09/18/2019) Due to the pilot phases needing more participants than originally estimated, the fact that we have seen co-morbities in the high BMI cohort which causes more screen fails than the normal BMI cohort and the difficulty scheduling the study visit in the Inpatient CTRC we need to increase the total enrolled number to 90 from 77.

(02/19/2020) We have still been experiencing difficulty in scheduling the study visits at the inpatient CTRC. Specifically, we only can schedule a minimum of 3 months in advance. This has caused some participants to drop due to the long time between the screening visit and the study visit. An accurate prediction of the start of a participant's period is difficult 3 months in advance. We have also seen an increase in loss to follow-up.

Prior to the initiation of the frequent blood sampling, the subject will undergo a screening visit where they will sign the consent, and have a history and physical exam, including a pelvic exam, and a blood draw. Screening labs will include liver function tests and renal function tests. The PI will determine eligibility based on the laboratory values, exam, and medical history. Due to COVID-19 we are adding the option to move part of the screening visit to a Telehealth virtual visit through EPIC or via a Zoom meeting, if necessary. The parts conducted virtually would be the reading of the consent, answering of the participant's questions and the participant's history (after verbal agreement by the participant of consent into the study). We will ask the participant to print and sign the consent and email a scanned copy or a photo taken with their cell phone to us and we will send back a copy with both signatures before the next steps of the screening visit are scheduled. The height and weight, the hip/waist circumference and the screening blood draw will be performed in the outpatient CTRC and will occur after the consent is signed. The participant will come into the outpatient CTRC clinic to complete the aforementioned procedures. The study team will keep their interaction with the subject to a minimum at this visit.

If eligible, the two-day visit will be scheduled to correspond with the subject's early follicular phase (cycle days 3-6). To help predict the date of their study visit we will provide the participants with urine ovulation tests strips to voluntary use. We will ask the participant to start using 8-10 days after the ending date of their last period. We will ask them to use these urine ovulation tests in the morning. Once they observe a positive with the ovulation test strip we will ask them to notify the research coordinator. The study visit will then be scheduled. Prior to the study visit the participant will be asked to provide an optional fecal sample using the Alpco EasySampler® Stool Collection Kit and will be asked to bring it to the study visit in a pre-provided thermal transport bag. Participants will be instructed to freeze the fecal sample after it is collected.

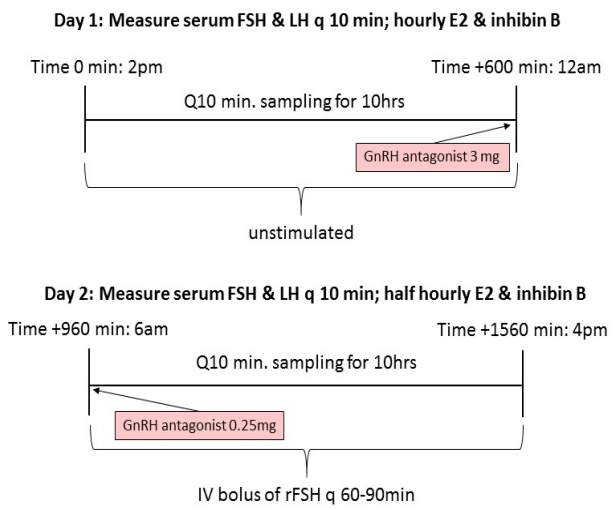
Women will report to the CTRC at 1:30pm on day 1 of the study for placement of an indwelling IV catheter. Prior to initiation of the study drug a serum pregnancy test will be done to rule out the possibility pregnancy. Small volumes (3-4mL) blood samples will be drawn every 10 minutes for 10 hours (2pm – 12am) to assess pituitary gonadotropin dynamics. During the first 10 hours of the study we will collect the participants urine 4 times (non-specific) to asses endogenous FSH elimination in their urine. After the first 10 hours of blood samples have been collected, the GnRH antagonist Cetrorelix will be given subcutaneously (3 mg). The subject will then be allowed to sleep until 6am.

On day 2, at 6am a second, smaller dose of Cetrorelix (0.25mg) will be given at subcutaneously. In addition, beginning at 6 am, small volume blood samples will again be drawn every 10 minutes for 10 hours. Repeated boluses of exogenous recombinant FSH (rFSH) will be given by IV during this 10-hour visit. Urine will also be collected during day 2 at the following specific time points: T=0, 30, 60, 120, 240, 480, 600. If the participant cannot give 3 urine samples in the first hour of Day 2 we will request at least two samples during the first hour instead of 3. Where the 30 minute will be requested, but optional. Final blood samples will be collected after the last dose of FSH. At about 4pm, the visit will conclude, and the subject will be discharged from the clinic. Subjects will be advised to use double barrier contraception until the following menses to prevent conception immediately following the study.

Subjects will also be provided a second Alpco EasySampler® Stool Collection Kit and be asked to return an optional stool sample within 7 days of the study visit.

All subjects who received 30 IU of the rFSH will have safety follow up phone calls the next day, 3-5 days and 7-10 calendar days after discharge from the clinic. Any adverse events identified during that call will be reported and followed until resolution.

If no adverse event is identified, the 7-10 day post discharge phone call concludes the subject's involvement.



Doses & frequencies established in preliminary studies

D. Description, Risks, and Justification of Procedures:

<u>Potential Risks and plan for minimizing:</u>

Phlebotomy. Blood drawing for serum hormone or analyte testing can be associated with bruising or bleeding and phlebotomy involves a transient pricking pain. This is a minimal risk and good laboratory practices will be applied.

Frequent blood sampling studies. This is associated with the risks of bruising and bleeding at the site of the IV line. Anemia can result if amounts of blood withdrawn are excessive. Participants will be screened with hemoglobin. Any participant whose hemoglobin is less than 11 gm/dl will not be enrolled. As with all of the PI's frequent blood sampling studies, a total of 120 blood draws will be done with approximately 0.5 ml – 1 ml of waste. Overall, there will be a total of 60 – 120 ml of waste over a 48 hour period.

GnRH antagonists. One-time administration of long-acting GnRH antagonist, cetrorelix (Cetrotide®; Merck Serono) will be used to suppress endogenous gonadotropin secretion. Reported side effects include: Ovarian Hyperstimulation Syndrome in 3.5% of patients, nausea in 1.3% and headache in 1.1% of subjects. Local site reactions to the subcutaneous injection (redness, erythema, bruising, itching and pruritis) have been reported. These reactions were mild, short-lived and transient. Additional possible risks include delayed menses for up to 1-2 months (occurring in approximately 8-10% of patients) and/or hot flashes. Allergic reactions are possible, but rare and unlikely. Participants will be inpatients at the CTRC bed and will be closely monitored during and after administration of Cetrorelix to assure their safety in the unlikely event of an atopic reaction. GnRH antagonists are Pregnancy Category X drugs and participants who are taking these agents will all be screened with a serum pregnancy test to assure that they are not pregnant while taking the medication.

Recombinant FSH. rFSH will be administered during the frequent blood sampling study to enable follicular production of inhibin B and estradiol. The total daily dose will be 300 IU. Risks of rFSH include ovarian hyperstimulation which is very unlikely with this dosing, but any potential signs or symptoms will be followed with subsequent follow-up. There is no reported antigenicity of exogenous human recombinant FSH. Side effects may include headache, nausea, fatigue, and pelvic pain. There is a possibility of the subject experiencing an allergic reaction.

Patient confidentiality. Patient confidentiality will be respected and guarded by adhering to HIPAA guidelines for personal health information. We will describe this approach to study subjects both verbally and in the informed consent document. They will be told that their information will be made available to appropriate representatives of the National Institutes of Health who may be reviewing the records in accordance with accepted policies and procedures. This approach has been successfully utilized by us previously for numerous clinical research studies at our institution, including the studies directed or co-directed by the PI. No breaches of patient confidentiality or inappropriate disclosure of their medical information have transpired. All participants will be given a study number that will identify their file. Files will be kept in the Clinical and Translational Research Center, under lock and key, or in the PI or research coordinator's office. Identifying information other than the study number will be redacted from participant's study records such that the file cannot be linked to the participant by name. The code linking study numbers to name will be kept by the research coordinator in a separate folder in a separate locked cabinet in a locked office. No identifying information will be released in the course of publication or presentation of study findings.

Samples will be sent to the University of Virginia to run the inhibin B assay as UVA has one of the most sensitive and precise assays for inhibin B. No identifiers will accompany the samples when sent to UVA.

E. Data Analysis Plan:

1) To test whether the difference in maximal inhibin B and E2 response between day 1 and day 2 depends on group (obese vs. normal weight) we will use a two-sample t-test.

2) In a second phase of analysis, we will adjust for potential confounders such as age and AMH level. We will repeat this analysis on the other secondary outcome measures.

3) To understand how inhibin B and E2 respond to FSH stimulation after GnRH antagonist we will compare groups in relation to the maximal change in inhibin B.

4) To understand how changes in inhibin B relate to changes in estradiol, linear regression will be used to assess whether the maximal change in inhibin B between day 1 and day 2 is statistically associated with the change in maximal E2 response between day 1 and day 2 (i.e. $\Delta MaxE2 = \beta_0 + \beta_1 \Delta Inhibin\ B$).

5) To characterize the changes in the microbiome we will analyze bacterial diversity by 16s ribosomal RNA sequencing. We have extensive experience in the analysis of the human microbiome in the environment, nares, vaginal, and intestinal tract and will analyze our data as previously described.[20-22] Microbiome data will be used for exploratory analyses in relation to clinical and other parameters measured among the study participants.

<u>Pilot:</u>

We are planning to assess the change in inhibin B levels following repeated bolus dosing of recombinant FSH (rFHS) following GnRH antagonist blockade. For the purposes of this study, we are interested in identifying the dose level that results in a significant pharmacodynamic (PD) effect, namely an increase in serum inhibin B, with a target PD response rate of 80% across patients. Statistical significance for the PD response at the patient level will be identified as an increase of 2.3 times the baseline standard deviation for inhibin B measurement in normal women. Using the data provided in the above table, showing a standard deviation in serum inhibin B concentration in normal women of 36.3 pg/mL, an increase in serum inhibin B of 83.5 pg/mL following FSH dosing would be considered a significant PD effect. This value provides a one-sided statistical significance at the 0.05 significance level, appropriate as there is an *a priori* anticipation that the response will occur in only one direction.[16] To determine statistical significance of the dose level of 10 IU IV every 60 minutes, three study subjects will

be needed and the dose level will be declared effective if 2 of the 3 subjects demonstrate the significant PD response described above. This will provide 90% power, at the dose level, to detect a true 80% response rate across patients.[16,17] If fewer than 2 subjects demonstrate a significant PD response, dose escalation will be used with three subjects at the second level (20 IU) and nine subjects at the third level (30 IU) to identify the level at which the target response rate is reached. Subjects in the pilot portion of the study will receive the same study procedures as other subjects, except the dose escalation portion of the study.

The base dosage for rFSH was 10 IU q 60 mins. Based on the calculation of achieving a statistically significant pharmacodynamic effect at 2.3 times the baseline standard deviation for serum inhibin B levels in normal women, the initial dose given to three patients (10 IU every 60 minutes x 10 for a total daily dose of 100 IU) provided increases of only 9%, 13% and a decrease in serum inhibin B; this was considered to be ineffective with regard to the desired pharmacodynamic effect and the next cohort received a dose that was doubled from the previous group (20 IU every 60 minutes x 10 for a total daily dose of 200 IU). The results from this group revealed that two patients had increased inhibin B levels of 38.5% and 13.2% with the third experiencing a decrease in serum inhibin B levels. With the pharmacodynamic target of 80% increase at the patient level in 2 out of three subjects, this second dose should again be considered ineffective. For this reason, and due to the safety profile of the study drug, it would be prudent to double the dose in an attempt to reach the desired PD effect in at least one patient, at which time increases in dose can be modified to follow the originally proposed modified Fibonacii sequence. Therefore, the dose for the next patient cohort is proposed to be 40 IU every 60 minutes x 10 for a total daily dose of 400 IU. Based on recommendations form FDA's clinical review team, the next patient cohort will consist of 9 subjects treated with 30 IU of rFSH every 60 minutes x 10 for a total daily dose of 300 IU. As requested by the FDA, safety data (adverse events) will be submitted to FDA for review after 9 subjects have received 30 IU Gonal-f IV every 60 minutes x10, with a total daily dose of 300 IU in the pilot study, prior to proceeding with additional study participants.

F. Summarize Knowledge to be Gained:

The knowledge to be gained from this study will help elucidate the underlying mechanism for reproductive hormonal alterations in female obesity. While recent years have brought an increased understanding of the link between obesity and reproductive impairment, including risk for the offspring, there is still a tremendous gap in our understanding of how and why specific reproductive hormones are changed the way they are in obese individuals and not in other women. In addition, it will lay the ground work for future studies that could potentially identify targets for therapeutic regimens. The findings of this study will also contribute to the general body of knowledge about the endocrine regulation of pituitary and hypothalamic secretion by the ovary. Thus, the potential knowledge gained is significant.

G. References:

1. **Flegal KM, Carroll MD, Kit BK, Ogden CL** 2012 Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. JAMA 307:491-497
2. **Robinson WR, Keyes KM, Utz RL, Martin CL, Yang Y** 2012 Birth cohort effects among US-born adults born in the 1980s: foreshadowing future trends in US obesity prevalence. Int J Obes (Lond)
3. **Lake JK, Power C, Cole TJ** 1997 Women's reproductive health: the role of body mass index in early and adult life. International Journal of Obesity & Related Metabolic Disorders: Journal of the International Association for the Study of Obesity 21:432-438
4. **Stothard KJ, Tennant PWG, Bell R, Rankin J** 2009 Maternal Overweight and Obesity and the Risk of Congenital Anomalies. JAMA: The Journal of the American Medical Association 301:636-650
5. **Smith GC, Shah I, Pell JP, Crossley JA, Dobbie R** 2007 Maternal obesity in early pregnancy and risk of spontaneous and elective preterm deliveries: a retrospective cohort study. Am J Public Health 97:157-162
6. **Cnattingius S, Villamor E, Johansson S, Edstedt Bonamy AK, Persson M, Wikstrom AK, Granath F** 2013 Maternal obesity and risk of preterm delivery. JAMA 309:2362-2370
7. **Metwally M, Saravelos SH, Ledger WL, Li TC 2010** Body mass index and risk of miscarriage in women with recurrent miscarriage. Fertility and Sterility 94:290-295

8. **Metwally M, Ong KJ, Ledger WL, Li TC** 2008 Does high body mass index increase the risk of miscarriage after spontaneous and assisted conception? A meta-analysis of the evidence. Fertil Steril 90:714-726
9. **Gesink Law DC, Maclehose RF, Longnecker MP** 2007 Obesity and time to pregnancy. Human Reproduction 22:414-420
10. **Polotsky AJ, Hailpern SM, Skurnick JH, Lo JC, Sternfeld B, Santoro N 2010** Association of adolescent obesity and lifetime nulliparity—The Study of Women's Health Across the Nation (SWAN). Fertility and sterility 93:2004-2011
11. **Anderson K, Norman RJ, Middleton P** 2010 Preconception lifestyle advice for people with subfertility. Cochrane Database Syst Rev:CD008189
12. **Jungheim ES, Moley KH** 2010 Current knowledge of obesity's effects in the pre- and periconceptional periods and avenues for future research. American Journal of Obstetrics and Gynecology 203:525-530
13. **Robker RL** 2008 Evidence that obesity alters the quality of oocytes and embryos. Pathophysiology 15:115-121
14. **Igosheva N, Abramov AY, Poston L, Eckert JJ, Fleming TP, Duchen MR, McConnell J** 2010 Maternal diet-induced obesity alters mitochondrial activity and redox status in mouse oocytes and zygotes. PLoS One 5:e10074
15. **Jain A, Polotsky AJ, Rochester D, Berga SL, Loucks T, Zeitlian G, Gibbs K, Polotsky HN, Feng S, Isaac B, Santoro N** 2007 Pulsatile Luteinizing Hormone Amplitude and Progesterone Metabolite Excretion Are Reduced in Obese Women. Journal of Clinical Endocrinology & Metabolism 92:2468- 2473
16. **Rubinstein L.V., Steinberg S.M., Kummar S, Kinders R, Parchment R.E., Murgo A.J., Tomaszewski J.E., Doroshow J.H.** The statistics of phase 0 trials. Statistics in Medicine (2010) 29: 1072-1076
17. **Murgo J.M., Kummar S, Rubinstein L, Gutierrez M, Collins J, Kinders R, Parchment R.E., Ji J, Steinberg S.M., Yang S.X., Hollingshead M, Chen A, Helman L, Wiltrout R, Tomaszewski J.E., Doroshow J.H.** Designing Phase 0 Cancer Clinical Trials. Clinical Cancer Research (2008) 14(12):3675-3682.
18. **Baker J.M., Al-Nakkash l., Herbest-Kralovetz M.M.** Estrogen-gut microbiome axis: Physiological and clinical implications. Maturitas (2017) 103: 45-43.
19. **Kwa M., Plottel C.S., Blaser M.J., Adams S.** The Intestinal Microbiome and Estrogen Receptor-Postivie Female Breast Cancer. JNCI Journal National Cancer Institute (2016) 108(8): djw029.
20. Markle JG, Frank DN, Mortin-Toth S, et al. Sex Differences in the Gut Microbiome Drive Hormone-Dependent Regulation of Autoimmunity. *Science.* 2013;339(6132):1084-1088.
21. Soderborg TK, Clark SE, Mulligan CE, et al. The gut microbiota in infants of obese mothers increases inflammation and susceptibility to NAFLD. *Nature communications.* 2018;9(1):4462.
22. Friedman JE, Dobrinskikh E, Alfonso-Garcia A, et al. Pyrroloquinoline quinone prevents developmental programming of microbial dysbiosis and macrophage polarization to attenuate liver fibrosis in offspring of obese mice. *Hepatol Commun.* 2018;2(3):313-328.

Appendix: Study Visits and Procedures Flow Chart

Visit #

1

Screening Visit:

- Consent signed
- H&P
- Blood Draw
- Pelvic Exam

→ *Serum Labs run at Clinical or Core Lab*

- Hematocrit
- TSH
- Prolactin

2

10-hour Sampling Day 1 (Inpatient CTRC) (2pm-12am)

- Frequent Blood Draw (q10 mins)
  - 60 red tops (10 ml filled with 3 ml each)
- Urine Collection during day 1
- GnRH antagonist, Cetrorelix (3 mg subcutaneously at 12am)
- Return of Fecal Sample

→ Serum aliquots stored in PI's Lab for FSH, LH, inhibin B and E2

3

10-hour Sampling Day 2 (Inpatient CTRC) (6am-4pm)

- GnRH antagonist, Cetrorelix (0.25 mg subcutaneously at 6am)
- Frequent Blood Draw (q10 mins)
  - 60 red tops (10 ml filled with 3 ml each)
- IV bolus of rFSH q 60-90 min (dose and frequency tba prior to visit)
- Urine Collection during day 2
- Fecal sample to be returned post visit

→ Serum aliquots stored in PI's Lab for FSH, LH, inhibin B and E2

SAFETY Follow Up phone calls (24 hours, 3-5 days and 7-10 post-discharge)

** All blood draws conducted by CTRC Inpatient or Outpatient staff