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Administration of follicle-stimulating hormone (FSH) and low dose human chorionic gonadotropin (hCG) for oocyte maturity while decreasing hCG exposure in In Vitro fertilization (IVF) cycles

NCT02310919

Study Protocol

Specific Aims

Primary Aim:

1) To determine if concomitant low dose hCG (1500 IU) and 450 IU FSH for oocyte maturation trigger is non-inferior to the standard hCG alone in total fertilization proportion (i.e. total competent proportion), according to the primary outcomes involving maturation and fertilization:

Total fertilization proportion (i.e. total competent proportion), defined as the number of fertilized oocytes (2PN) divided by the total number of oocytes retrieved, will be compared between the two treatment groups.

2) To further assess the outcomes of oocyte recovery and developmental competence of the mature oocytes the following will be compared between the two treatment groups:

- a) Oocyte recovery
- b) Oocyte maturity
- c) Fertilization rate
- d) Embryo quality
- e) Pregnancy rates

3) To compare serum and follicular post-trigger steroid hormones levels of estradiol, progesterone, hCG, FSH LH, and vascular endothelial growth factor (VEGF) between the two treatment groups at different times points:

- a) 12 hours after trigger (T+1), at oocyte retrieval (T+2), and 5 days after trigger (T+5) to assess serum levels of, as appropriate, based on the study visit day.
- b) Follicular fluid from the first aspirate of a dominant follicle on each ovary at retrieval

Secondary Aim: To determine if symptoms of OHSS are decreased with low dose hCG (1500 IU) and 450 IU FSH compared to standard hCG dose alone.

Compare symptoms related to OHSS using the following outcomes:

- 1) The change in bloating scores prior to trigger and after trigger
- 2) The change in abdominal circumference prior to trigger and after trigger
- 3) The change in body weight prior to trigger and after trigger

Experimental Design and Methods:

Study population:

The target population includes female patients undergoing IVF. All eligible female patients will be asked to join the study. Patients receiving any type of stimulation protocol for IVF will be offered participation in the study. Study participants will be recruited from the Reproductive Endocrinology Clinic at University of California at San Francisco Center for Reproductive Health.

Inclusion/Exclusion Criteria:

Inclusions: Only patients undergoing IVF will be offered participation in the study.

Exclusions:

- Age >41 years old
- Antral Follicle Count (AFC; 2-10 mm) < 8
- Body Mass Index > 30 kg/m²
- History of ≥ 2 prior canceled IVF cycles secondary to poor response
- Diagnosis of cancer
- Any significant concurrent disease, illness, or psychiatric disorder that would compromise patient safety or compliance, interfere with consent, study participation, followup, or interpretation of study results
- Undergoing embryo co-culture
- Use of any of the following medications: Growth Hormone, Sildenafil, or Aspirin (except if being used for hypercoagulable state)
- Severe male factor infertility diagnosis. Male factor infertility diagnosis should be cleared for eligibility by the PI based on previous patient history of fertilization outcomes and/or expected fertilization outcomes of the cause of male factor infertility based on known scientific data.
- Ovulation trigger less than or greater than 36 hours to oocyte retrieval
- Serum estradiol level >5,000 pg/ml on the day of expected ovulation trigger

Recruitment Plan: The physician will identify patients eligible for the study at the consultation visit. A research coordinator will follow up and meet with eligible participants at the saline sonography visit or the patients next clinical visit prior to start of IVF treatment. Informed consent will be performed by the research coordinator.

Design: Double –Blinded Randomized trial

Randomization:

The patients will be randomized on the day of ovulation trigger for assignment to receive either standard dose of hCG (5,000 IU or 10,000 IU) alone or low dose hCG (1,500 IU) + FSH (450 IU) for oocyte maturation trigger by the research coordinator. All participants will be assigned a unique subject ID, and only the research coordinator and unblinded study physician will be aware of what treatment arm each patient is in. All other physicians, nurses, embryologists, clinic staff, and patients will be blinded.

Blinding process for the medication administration:

The hCG and FSH trigger shots for each patient will be prepared by the unblinded study physician the same day the patient will administer the medications. Each medication will be reconstituted in their own separate syringe so the patient will be presented with two syringes for administration that will be labeled with only “hCG” and “other”. The hCG sterile powder will be reconstituted with bacteriostatic water, and the designated dose suspended in a total volume of 1 ml in a syringe by the unblinded study physician or nurse. It will only be labeled with “hCG” and excluding the dose. The FSH sterile powder will also be reconstituted with bacteriostatic water and suspended in a total volume of 1 ml in a syringe, then labeled “other”.

The patients that were not randomized to receive the FSH co-trigger will receive a similar syringe with only 1 ml of bacteriostatic water and no FSH, also labeled "other". Immediately after reconstitution and suspension, the trigger medication will be given to the patient. Each designated syringe will be placed in an insulated cooler travel container for the patient to take to administer that day based on assigned trigger time. Since the unblinded study physician will be preparing the trigger shots for each patient they will be aware of what treatment arm each patient is in.

Treatment Plan:

The subjects will undergo ovarian stimulation with IVF protocol stimulation treatment chosen by their primary doctor. Baseline antral follicle count (AFC) will be recorded.

Standard of care guidelines for ovarian stimulation to be used as a reference (not part of the experimental protocol):

Type of gonadotropins: FSH, (i.e. Gonal-F) + hMG, (ie. Menopur)

Starting dose of gonadotropins is 150-450 IU per day of FSH and hMG, which are standard starting doses in our clinical practice. Initial dose will be determined by the patient's primary doctor based on AFC and body weight. As per standard practice, the minimal dose for the desired response will be used. The primary doctor will manage the entire ovarian stimulation based on their clinical knowledge, expertise, and standard of care practices.

Standard Guidelines:

Gonadotropin medications will be started as designated by the patient's primary doctor and baseline ultrasound is performed. Ultrasounds will be performed and serum estradiol (E2) will be checked as determined by the patient's primary doctor. Gonadotropin dose will be adjusted based on ultrasound findings and serum E2 level as needed to be determined by the patient's primary doctor. Gonadotropin injections will be continued until ovulation trigger.

The day of administration of ovulation trigger will be determined by the patient's primary doctor.

Guidelines for the experimental protocol:

Study participants will receive a syringe on the day of ovulation trigger containing either the standard dose of hCG or low dose hCG + FSH according to the randomization. The subjects will be triggered with the following:

- a) Standard dose of hCG (10,000 IU or 5,000 IU) or
- b) Low dose of hCG 1,500 IU SQ + FSH 450 IU SQ

For the control arm, the standard hCG dose will be given based on the E2 level on the day of ovulation trigger. If the E2 level is less than 3,500 pg/ml, then the trigger will be 10,000 IU of hCG. If the E2 level is more than 3,500 pg/ml but less than 5,000 pg/ml, then the trigger will be 5,000 IU.

On the day of ovulation trigger, the subject's body weight and abdominal circumference will be measured. The subject will also complete a questionnaire regarding her current symptoms. In

addition to the standard of care estrogen lab, residual blood from that draw will be retained by the study for an additional lab to assess additional hormone levels. Subjects will return 12 hours after trigger (T+1), at oocyte retrieval (T+2), and 5 days after trigger (T+5) to assess serum levels of estradiol, progesterone, hCG, FSH, LH, and vascular endothelial growth factor (VEGF), as appropriate, based on the study visit day.

The oocyte retrieval will be performed 36 hours after the ovulation trigger. All egg retrievals will be performed using transvaginal ultrasound and the associated needle guide in the standard fashion. On the day of the oocyte retrieval, the patient will have a blood draw to assess serum hormone levels. When possible, efforts will be made to use pre-existing venipuncture, such as an intravenous line, to minimize needle sticks. In these instances, approximately 3-5 mL of blood will be drawn and discarded as waste to clear the line of possible heparin and/or saline flush, and have been factored into the total blood draw volumes required by the study.

Both the physician performing the oocyte retrieval and the laboratory personnel will be blinded to the study. All visible follicles will be aspirated. The first follicle from each ovary on every patient will be aspirated and the system (needle and tubing) will be flushed into a separate tube prior to aspirating the remaining follicles. The follicular fluid from the first aspirate from each ovary will be collected to assess follicular hormone levels. Oocyte stripping will be performed under the standard protocol. The status of oocyte maturation at the time of stripping (approximately 38-40 hours after hCG administration) and at the end of the ICSI procedure (approximately 40-42 hours after hCG administration) will be recorded. Fertilization will be assessed 16-19 hours after insemination. The embryos will be transferred to growth media and cultured with standard protocols.

Subjects will return five days after ovulation trigger for blood draw to assess serum hormone levels. The subject's body weight and abdominal circumference will be measured. The subject will complete a questionnaire regarding her current symptoms and bloating score.

Interventions to prevent OHSS in high risk patients:

Any patients in either arm with an E2 serum level >5,000 pg/ml on the day of expected trigger administration will be excluded from the study. These patients will be individually assessed by their primary physician. Their primary physician will decide the safest trigger option based on the patient's risk of OHSS. From our own clinical experience, we expect that <5% of subjects will have an E2 serum level of >5,000 pg/ml and be excluded.

Interventions to avoid ovulation trigger failure:

In order to avoid failure of ovulation trigger, the post trigger day serum hCG will be determined and communicated to the unblinded clinician. If the serum hCG level is less than 18 IU/L, then the subject will be excluded from the study and assessed by their primary doctor for possible re-trigger. If the ovulation trigger fails then administration of a second trigger medication is the standard of care.

Assessment of OHSS related symptoms:

To assess the incidence of OHSS related symptoms, patients will be evaluated objectively by a change in the abdominal circumference as well as subjectively by clinical symptomatology based on patient's bloating symptoms using a questionnaire.

Prior to starting the stimulation, the abdominal circumference (C) and body weight (BW) will be measured in centimeters at the baseline ultrasound visit (C_{baseline}, BW_{baseline}). Also, the abdominal circumference (C) and body weight (BW) will be measured in centimeters on the day of ovulation trigger. Five days after the oocyte retrieval the abdominal circumference and body weight will again be measured in centimeters (C_{stimulated}, BW_{stimulated}). The difference in the abdominal circumference and body weight will be calculated.

The patient's clinical symptoms will be evaluated based on a bloating score reported by each patient on the day of ovulation trigger and then 5 days after the oocyte retrieval will be determined. A venipuncture will also be obtained for hormone assays 5 days after oocyte retrieval.

The bloating score will range from 0-5, and will be determined as follows:

0 - No bloating

1 - Mild bloating. Able to continue daily activities without discomfort.

2 - Mild to moderate bloating. Able to continue daily activities but with mild discomfort.

3 - Moderate bloating. Able to continue daily activities but with moderate discomfort.

4 - Moderate to severe bloating. Difficulty performing daily activities.

5 - Severe bloating. Abdomen feels very tense and unable to perform daily activities.

The difference in the bloating score will be calculated.

Sample Collection:

The serum samples will be collected and stored in a freezer (-80°C). The follicular fluid samples will be labeled and identified with the unique subject ID. The follicular fluid samples will be spun down immediately after the oocyte retrieval and the supernatant then aliquoted into 2ml cryo-vials and stored at -80°C for later analysis. The serum and follicular samples will be batched and the serum assays will be run by the Center for Reproductive Health Endocrinology laboratory at UCSF. The following hormone levels will be measured with commercially available electrochemiluminescence immunoassays on a Cobas E 411 Analyzer (Roche Diagnostics International Ltd): (estradiol, progesterone, hCG, FSH and LH). The analysis of VEGF serum and follicular fluid concentrations will be performed by an outside lab at the Zuckerberg San Francisco General Hospital laboratory. Serum and follicular fluid assays were calibrated to known standards and validated by serial dilution.

Sample size and power analysis:

Primary outcome:

The sample size was calculated for the primary outcome of fertilization proportion (i.e. total competent proportion) which was defined as the number of fertilized eggs (2 Pro-nuclei (PN) divided by the total number of oocytes. The average fertilization rate in our clinic is 0.7 and the

average number of mature oocytes from a cohort of oocytes is 0.7. Therefore, the average fertilization proportion (i.e. total competent proportion) is approximately $0.7 \times 0.7 = 0.49$ or about 0.5. The alternative trigger would be regarded as non-inferior if it is at least 80% effective in promoting oocyte competence compared to the standard trigger, for a non-inferiority margin of 20%. Considering a cluster size of 15 oocytes (the average number of oocytes retrieved per patient in our clinic) and an estimated intra-cluster correlation of 0.1, we calculated that approximately 50 participants were needed in each study arm when the non-inferiority difference is -0.1 (i.e. 20% of 0.5 total competent proportion) with a power of 0.8 and a one-sided alpha of 0.05.

Data collection:

All data will be recorded in a secured database at the Reproductive Endocrinology Clinic at University California San Francisco, Center of Reproductive Health. Each subject will be deidentified and assigned a unique personal ID.

Statistical analysis:

Analyses will be performed based on intention-to-treat (ITT). Using the standard trigger as reference, the relative risk and associated 95% confidence interval (CI) for the probability of laboratory outcomes was calculated using log-binomial regression models. Generalized estimating equations (GEE) with an exchangeable correlation structure was applied to account for clustered data (i.e. oocytes and embryos originating from the same patient). Continuous laboratory outcomes were compared using generalized linear modeling with log link function. For pregnancy outcomes, relative risk and 95% CI were calculated to estimate effect size. Hormone concentrations were compared using Wilcoxon rank sum test. For the non-inferiority hypothesis testing of the primary outcome, a one-sided 95% CI was constructed for the relative risk. The alternative trigger will be considered non-inferior to the standard trigger if the lower bound of the one-sided CI of the relative risk is not less than 0.8 (i.e. risk reduction limit of 20%). For secondary outcomes, the statistical significance was based on a two-sided alpha of 0.05. Data were analyzed using SPSS version 28.0.1.0 (Statistical Package for Social Sciences; Chicago, IL).

For the secondary aim evaluating OHSS related symptoms, the abdominal circumference, body weight, and bloating score will be analyzed using t-tests to determine if the change is significantly different between the trigger groups. For these secondary aim outcomes, we do not expect to have sufficient numbers of events to conduct a formal analysis.