FSH and LH versus FSH alone for ovarian stimulation in non-hormone sensitive onco-fertility patients: a randomized controlled trial

Clinical Study Protocol

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1. List of abbreviations

2PN: two pronuclei
AFC: antral follicle count
AMH: anti-Müllerian hormone
ART: assisted reproductive technologies
COS: controlled ovarian stimulation
E2: estradiol
FET: frozen embryo transfer
FSH: follicle stimulating hormone
GnRH: gonadotropin-releasing hormone
GnRH-a: gonadotropin-releasing hormone agonist
hCG: human chorionic gonadotropin
hMG: human menopausal gonadotropins
ICSI: intracytoplasmic sperm injection
IVF: in-vitro fertilization
LH: luteinizing hormone
MII: metaphase II
OHSS: ovarian hyperstimulation syndrome
PCOS: polycystic ovarian syndrome
r-FSH: recombinant follicle stimulating hormone
r-LH: recombinant luteinizing hormone

2. Background

The National Institute of Health estimates that there will be 1,958,310 new cancer cases in 2023, of which 8.5% will be diagnosed before the age of 45. With increasing cancer cure rates and improvement in long-term survival, fertility preservation is often included as a measure of quality practice [1]. In fact, the American Society of Clinical Oncology recommends that fertility preservation should be part of the counselling for all women diagnosed with cancer in the reproductive age [2]. Many treatments for cancer, including chemotherapy, radiation therapy, and gonadal surgery are gonadotoxic and can irreversibly damage the ovarian oocyte reserve. Therefore, prior to exposure to these oncologic treatments, fertility may be preserved through several methods, one of which is in vitro fertilization (IVF) with embryo or oocyte cryopreservation [3,4]. As cancer treatment is typically urgent and time-sensitive, these patients receive timely fertility consultation and expedited IVF. In IVF, injectable gonadotropins are administered for ovarian stimulation to encourage the development of multiple ovarian follicles prior to retrieval of oocytes for cryopreservation. Gonadotropin injections include follicular stimulating hormone (FSH) with or without the addition of luteinizing hormone (LH) [5]. At our centre, for standard IVF patients, LH is often added to FSH for ovarian stimulation. However, for oncology patients undergoing IVF for cryopreservation, FSH stimulation alone is typically chosen. This difference in treatment is not evidence-based, with decision-making based on patient, financial and convenience factors. The optimal stimulation protocol for these patients has not been comprehensively studied. Accordingly, it has not been determined whether the addition of LH improves outcomes.

Physiologically, FSH is the main trophic hormone that stimulates ovarian follicle development [6]. However, LH also has several roles in follicular development and is present at low levels throughout the early follicular phase of the menstrual cycle during the time of follicular recruitment. According to the two-cell two-gonadotropin theory, LH is responsible for stimulating the ovarian thecal cells to produce androgens. Under the influence of FSH, granulosa cells use these androgens to produce estrogens, which are critical for healthy follicular development [7]. LH is also involved in ovulation induction, completion of meiosis I, and follicular luteinization and progesterone production.

In 2005, Health Canada approved the use of lutropin alfa, a recombinant human luteinizing hormone, for treatment of hypogonadotropic hypogonadism. There is growing evidence that in addition to patients with hypogonadotropic hypogonadism, patients undergoing IVF with a history of low ovarian reserve, poor ovarian response, or advanced maternal age (>35 years) may also benefit from the addition of rLH. A systematic review and meta-analysis of 40 randomized trials totaling 6443 patients undergoing IVF/ICSI using FSH and rLH versus FSH alone suggested that adding rLH to FSH resulted in a 9% increase in clinical pregnancy rates, with a 30% increase in poor responders [8]. Another meta-analysis supported the addition of rLH for patients >35 years, quoting higher pregnancy rate [9].

There has been increased interest in the fertility community of using rLH in oncology patients. In this population, the hypothalamic gonadal axis may be affected given the possible association between malignancy and a catabolic state, increase in stress hormone, and malnutrition [10,11].

Accordingly, some have suggested there may be an impact on ovarian reserve, oocyte quality, and the need for a higher dose of gonadotropins [12,13]. Currently, GnRH antagonist protocol is used as standard of care in this population to avoid delay in their cancer treatment and minimize the risk of ovarian hyperstimulation syndrome [14,15].

A cohort study by Johnson et al. [16] examined the ovarian stimulation response in oncology fertility preservation patients under various IVF stimulation protocols which included the use of rLH in some patients. The study showed no overall difference in baseline measures of ovarian reserve, total dose of gonadotropin used, or mean number of oocytes retrieved in the group of 50 chemotherapy-naïve cancer patients compared to 50 matched healthy controls. Unfortunately, the number of patients receiving rLH and the total dose received by these patients was not specified. Other studies in the literature examining rLH included case reports and case series, with sample sizes insufficient to generate conclusions regarding rLH efficacy in cancer patients [17-19]. A systematic review concluded that research on ovarian stimulation protocols and outcomes in the onco-fertility literature was inconsistent and of low quality [20], calling for higher quality studies to provide clarity on this topic.

Therefore, we intend to conduct an exploratory randomized controlled trial (RCT) comparing FSH and LH versus FSH alone using a GnRH antagonist protocol in non-hormone sensitive cancer patients undergoing fertility preservation. These patients include those diagnosed with malignancy other than breast, uterine and ovarian cancer.

3. Study Question

In non-hormone sensitive cancer patients undergoing IVF fertility preservation, does the use of FSH and LH for ovarian stimulation lead to a greater proportion of mature oocytes available for cryopreservation relative to AFC compared to FSH alone?

We theorize that supplementation with FSH + rLH will increase the yield of mature MII oocytes among patients with non-hormone sensitive cancer undergoing fertility preservation compared to those with FSH alone. In addition, we anticipate the following trends among patients in the FSH + rLH group compared to those supplemented with FSH alone:

- Higher number of total oocytes retrieved per cycle
- Higher "Follicular Output Rate" (FORT), defined as ratio of pre-ovulatory follicles (16-22mm) on day of trigger divided by AFC [22]
- Higher number of cryopreserved embryos (if patient chooses to freeze embryos rather than oocytes)
- No significant difference in incidence of moderate-severe OHSS

5. Rationale

Fertility preservation is performed before the initiation of cancer therapy in an effort to reduce its cytotoxic effects on ovarian function. However, recent literature has demonstrated that ovarian function is reduced in cancer patients even before starting cancer treatment. A case series reported a greater proportion of immature oocytes per cycle and lower maximal levels of estradiol in various cancer patients compared to patients with isolated tubal infertility, suggesting the negative impact

of cancer on oocyte maturation [12]. A matched cohort study reported lower AMH levels in patients with lymphoma before chemotherapy, warranting higher doses of gonadotropins for ovarian stimulation to maximize the number of oocytes retrieved before chemotherapy [13].

Studies have highlighted that administration of exogenous FSH and LH is essential for patients with hypogonadotropic hypogonadism [23], which is consistent with the current approved use of rLH by Health Canada. Evidence also supports the use of rLH in poor responders with advanced maternal age [9]. There is growing concern in the literature that some oncology patients may be poor responders prior to cancer therapy due to hypothalamic gonadal axis dysfunction secondary to their malignancy [2]. With evidence suggesting that the hypothalamic gonadal axis is affected in the oncology population, and that rLH may improve fertility preservation outcomes in a subgroup of patients that mimic hypogonadotropic cases, the efficacy of rLH in oncology patients is plausible.

Given the mixed literature of rLH efficacy in the oncology population and the improved fertility preservation outcomes in various patients supplementing ovarian stimulation with rLH, an exploratory randomized controlled trial is warranted to definitively address the safety and efficacy of rLH in non-hormone sensitive cancer patients.

6. Significance of the study

Managing onco-fertility patients poses a special challenge for the IVF physician. After patients undergo treatment for their underlying cancer with surgery, chemotherapy, and/or radiotherapy, they may demonstrate premature ovarian failure at an early stage of life. Ovarian failure following cancer therapy is particularly significant considering the already compromised fertility of cancer patients. Previous systematic reviews have highlighted the need for higher quality studies evaluating ovarian stimulation in cancer patients [20]. Results of this trial will have immense impact on the future practice of reproductive medicine. In the instance of rLH and FSH demonstrating superiority compared to FSH alone, future patients will have improved fertility preservation outcomes and corresponding greater quality of life knowing the improved success of future biological pregnancy. In the instance of rLH and FSH demonstrating no significant difference compared to FSH alone, IVF physicians will counsel patients regarding the lack of benefit of rLH and accordingly reduce the costs associated with assisted reproductive technology.

7. Study objectives

7.1 Study design

The proposed study is an open-label, exploratory, randomized control trial examining the effect of FSH and rLH (Luveris) versus FSH alone on the number of mature oocytes available for cryopreservation in non-hormone sensitive onco-fertility patients. These patients include those diagnosed with malignancy other than breast, uterine and ovarian cancer.

Given that the care of oncology patients is time sensitive, a random start of the treatment cycle is the usual approach in our clinic, with no prior priming. The patient has a random serum levels of estrogen, progesterone, LH, FSH and HCG levels obtained. A transvaginal ultrasound is performed

as well, and the stage of the cycle is determined. Based on the stage of the cycle, gonadotropin starting time is planned accordingly and the starting dose will be individualized based on AFC as detailed below.

- Early follicular phase: Gonadotropin stimulation started. A GnRH antagonist will be given once the dominant follicle measures >1.4 cm.
- Mid to late follicular phase: patients who have a dominant follicle >1.5 cm with an E2 >300 and a progesterone <5 will be triggered usually with HCG (Ovidrel 250 mcg subcutaneous). After three days gonadotropin stimulation is started.
- Start of luteal phase: start gonadotropin stimulation and once the dominant follicle measure >1.4, a GnRH antagonist will be given.

Patients will be randomly assigned in a 1:1 ratio to either treatment or control group. Treatments in each of the trial arms will be as follows:

a) Treatment arm: Patients will self-administer a subcutaneous injection of Gonal-F (FSH) in addition to Luveris daily until a pre-set criteria to trigger ovulation is reached.
b) Control arm: Patients will self-administer a subcutaneous injection of Gonal-F (FSH) alone daily until a pre-set criteria to trigger ovulation is reached.

Transvaginal ultrasound and bloodwork monitoring is initiated to monitor the ovarian response. Once the patient meets one of the following two criteria

a) A serum estradiol (E2) of greater than 2,000 pmol/L
b) A follicle measuring greater than 14 mm,

A daily, subcutaneous injection of a GnRH antagonist (Cetrotide or Ganirelix 0.25 mg) will be administered by the patient subcutaneously to prevent an endogenous surge in LH. Monitoring of the ovarian response will continue until there are 3 or more follicles visualized by transvaginal ultrasound with a mean diameter of ≥17 mm. Ovulation will be subsequently triggered with a subcutaneous injection of HCG or GnRH agonist depending on the managing IVF physician. If a GnRH agonist trigger is chosen, twelve hours after their trigger medication, patients will return to the clinic for blood work including LH and progesterone as per standard procedure. This is a routine confirmatory blood test to confirm that an endogenous LH surge has occurred. In approximately less than 5% of cases, the GnRH agonist trigger fails to elicit an optimal surge, which may require a "rescue" low-dose hCG trigger to be administered 24 hours following the initial trigger. The decision to administer a "rescue" trigger will be at the discretion of the managing IVF physician. Transvaginal, ultrasound-guided oocyte retrieval will be performed approximately 36 hours following the administration of the initial trigger.

Once the oocytes are retrieved, if the patient desires to cryopreserve oocytes, mature oocytes will be cryopreserved by vitrification process. If embryo cryopreservation is planned, the retrieved oocytes will be fertilized in the lab using traditional IVF or ICSI. Embryos will be grown to day 5 and all "good quality" blastocysts will be cryopreserved using vitrification. At our clinic, day 5/6 embryos are considered "good quality" for cryopreservation if they are at least a grade 2BB (or above) on day 5 and/or a 3CC (or above) on day 6, according to the classification system by Gardner and Schoolcraft, 1999 [25].

7.2 Inclusion criteria

Patients will be included in the study based on the following criteria:

- Between the ages of 18 and 40.
- Undergoing IVF for fertility preservation (freezing all oocytes or embryos)
- Diagnosed with a non-hormone sensitive malignancy (malignancy other than breast, uterine and ovarian cancer)
- GnRH antagonist protocol (standard of care for all fertility preservation patients)

7.3 Exclusion criteria

Patients will be excluded from the study based on the following criteria:

- Any contraindication to treatment with gonadotropins (including medical history or risk factors for TE, hypersensitivity to gonadotropins or to any of the excipients).
- Congenital hypogonadotropic hypogonadism unrelated to the oncological condition.
- Previous adverse or allergic reaction to luteinizing hormone or any of its drug components.
- Had prior radiotherapy to the abdomen or pelvis
- Prior chemotherapy
- Prior history of deep vein thrombosis, or pulmonary embolism
- Patients with a diagnosis of hormone sensitive cancer including ovarian, uterine, or mammary carcinoma
- Patients with uncontrolled thyroid or adrenal failure
- Patients with active, untreated tumors of the hypothalamus and pituitary gland
- Patients who are lactating
- Patients with a known diagnosis of primary ovarian failure
- Previous participant of this study

7.4 Recruitment and informed consent

Patients that are seeking fertility preservation through cryopreservation of oocytes or embryos with a diagnosis of a non-hormone sensitive cancer will be informed about the purpose and aim of the study. If the patient expresses an interest in the study, they will be referred to the clinical research coordinator to discuss the study further and to provide them with additional written information. Patients will be encouraged to ask as many questions as necessary. Communication with the research coordinator may take place in person, over the phone, over Ontario Telemedicine Network, and/or by personal email at the discretion of the patient.

Informed, signed consent may be obtained in-person or via email after communication with the research coordinator. Alternatively, patients may wish to defer consent until a later date. If so, they will be able to contact the research coordinator to sign the consent forms prior to the start of the trial. Patients will be advised that participation in the study is voluntary and they have the right to withdraw from the study, at any point, without penalty or prejudice to the medical care provided.

7.5 Data collection

The following demographic information will be collected from included patients:

Demographics

- Age
- Gravida/para status
- BMI
- Type of malignancy
- Stage of malignancy (if known)
- Prior history of fertility treatment and the specific type
- Baseline FSH, LH, progesterone, estradiol levels
- Ovarian reserve: AMH and AFC (3-8mm) based on the FORT criteria [22].
- Any history of infertility, categorized as:
    - Anovulation including PCOS
    - Tubal
    - Male Factor Infertility
    - Endometriosis
    - Unexplained
    - Other
    - None
- Total motile sperm count (if freezing embryos)

Characteristics of treatment cycle

- Type of cycle start (follicular vs. luteal phase)
- Total days of gonadotropin stimulation
- Total dose of FSH (IU)
- Total dose of LH (IU)
- Total number of follicles measuring ≥11 mm on the day of trigger
- Total number of follicles measuring 16-22 mm on the day of trigger, according to FORT criteria [22]
- Type of trigger (hCG vs. GnRH-agonist)
- Need for rescue hCG trigger (ie. failed GnRH agonist trigger)
- Bloodwork on day of trigger ± day after trigger:
    - E2
    - LH
    - Progesterone
- Method of fertilization (standard insemination or ICSI or IVF/ICSI split) if freezing embryos
- Use of a dopamine receptor agonist (Cabergoline) to prevent OHSS

7.6 Outcome measures

The primary outcome measure will be the total number of mature (MII) oocytes adjusted by antral follicle count (AFC) available for cryopreservation.

Secondary outcome measures will include the following:

- Total number of oocytes retrieved per cycle
- Ratio of mature (MII) to immature oocytes
- Total number of mature oocytes (MII) retrieved per IVF/ICSI cycle
- Oocyte yield (defined as the number of metaphase II (MII) oocytes retrieved divided by the number of follicles ≥15 mm on ultrasound on the day of trigger)
- "Follicular Output Rate" (FORT) (defined as ratio of pre-ovulatory follicles (16-22mm) on day of trigger × 100/AFC (3-8mm) [22]
- Incidence of moderate to critical OHSS based on the classification criteria by Golan et al [26].
- If the embryos are frozen:
    - Fertilization rate, which will include two outcomes each defined as follows:
        - Definition 1: number of 2PN zygotes divided by the number of mature oocyte(s) fertilized per IVF/ICSI cycle.
        - Definition 2: number of 2PN zygotes divided by the number of oocytes incubated with at least 10,000 sperm per IVF cycle.
    - Total number of good quality day 5 embryos available for cryopreservation determined by an embryologist using the classification system by Gardner and Schoolcraft, 1999 [25].
- Dose-adjusted evaluation of the safety and tolerability of follitropin alfa + lutropin alfa compared to follitropin alfa as measured by incidence of:
    - Treatment-emergent adverse events (TEAEs)
    - Treatment-related adverse events (AEs)
    - Incidence of thromboembolic events

8. Study methods

8.1 Randomization

Patients that meet inclusion criteria will be randomized in a 1:1 ratio to either treatment or control group prior to beginning of the treatment cycle and stratified by age (lower or equal to 35 versus greater than 35). Allocation concealment will be ensured using a web-based random allocation sequence, which will require trial investigators to input patient information prior to receiving trial arm allocation.

8.2 Drug formulation, dosage, and reimbursement

The dosing and drug formulations are adherent to the care provided to our cancer patients seeking fertility preservation in our clinic.

Patients will undergo a random start of ovarian stimulation as per the standard of care in our clinic for all onco-fertility patients given that their treatment is time sensitive. Baseline estrogen, progesterone, LH, FSH, HCG levels are measured and a transvaginal ultrasound is performed and the stage of the cycle is determined. Ovarian stimulation will be performed according to results of the transvaginal ultrasound:

- Early follicular phase: FSH and rLH stimulation or FSH alone will be started as per randomization process. A GnRH antagonist will be given once the dominant follicle measures >1.4 cm.
- Mid to late follicular phase: patients who have a dominant follicle >1.5 cm with an E2 >300 and a progesterone <5 will be triggered with human chorionic gonadotropin (hCG, Ovidrel) 250 mcg subcutaneously. After three days, gonadotropin stimulation is started.
- Luteal phase starts: stimulation with FSH and Luveris or FSH alone, and once the dominant follicle measure >1.4 GnRH antagonist will be given.

Dosing:

- Recombinant luteinizing hormone (Luveris): daily dose of 75 IU subcutaneously.
- Follicular stimulating hormone (Gonal-F):
    - If AFC < 15, FSH dose is 300 units
    - If AFC ≥ 15, FSH dose is 200 units
- GnRH antagonist dosing: either cetrorelix 250 mcg subcutaneous or ganirelix 250 mcg subcutaneously.
- Trigger of ovulation: Ovidrel 250 mcg or GnRH agonist (Suprefact 0.5 mg).

FSH dosing is based on a retrospective analysis of data from our centre. A multiple regression analysis found only AFC and AMH to be independently associated with higher number of mature oocytes. AMH is unavailable at the start of the treatment cycle as patients require to start chemotherapy immediately. Accordingly, FSH dosing will be based on AFC. Retrospective review of FSH dosing of our centre showed that when the AFC is less than 15, the majority of patients receive an FSH dose of 300 units to maximize the number of oocytes retrieved. When the AFC is equal to or more than 15, the majority of patients receive an FSH dose of 200 units to minimize the risk of ovarian hyperstimulation. FSH dosing in this trial will correspond to this dosing schedule.

Duration of treatment is guided by measuring serum biochemical markers including estrogen, progesterone, and LH levels. This in addition to monitoring follicular growth by transvaginal ultrasound to assess ovarian response to treatment. By participating in the study, patients will not be subjected to additional investigations or interventions that deviate from the standard of care provided to all our patients undergoing fertility preservation. Patients will not receive compensation for participation in the trial.

8.3 Study timeline

The duration of the trial, from the recruitment stage until completion, will vary depending on the amount of time needed to recruit patients for both arms of the study. Patients typically undergo a random start ovarian stimulation treatment, that ends with oocyte or embryo cryopreservation. Pregnancy rates are not part of the objectives of the study. Therefore, the timeline is expected to be between 2-3 months with each patient. The total duration of the study is expected to be 5 years (rounded up from 4 years 8 months) given 60% recruitment of 50 patients available at our site per year. Should fewer than expected number of patients be recruited at the 3-year mark, the study

period will be extended according to the observed annual recruitment calculated at 3 years for a maximum of 2 additional years.

8.4 Confidentiality

In order to protect the confidentiality of each participant, personal identifiers (such as date of birth and names) will be removed from the database and each participant will be assigned a random unique ID code to track their treatment outcomes. The aforementioned codes will only be accessible to the research coordinator of the study. This information will not be disclosed to any other individual or party without the written consent of the patient.
The information that is collected for the study will be kept in a locked and secure area by the study doctor for 15 years. Only the study team or the people or groups listed below will be allowed to look at your records. Your participation in this study also may be recorded in your medical record at this hospital.

The following people may come to the hospital to look at the study records and at your personal health information to check that the information collected for the study is correct and to make sure the study followed proper laws and guidelines:

- The study sponsor.
- Representatives of the Mount Sinai Hospital Research Ethics Board.

9. Safety

9.1 Discontinuation criteria and safety parameters

All patients undergoing a treatment of IVF at Mount Sinai Fertility are screened to ensure that they are in good health before starting a treatment cycle. All of our patients will be receiving a GnRH antagonist protocol which has lower rates of OHSS [27]. In addition, all patients in this study will have a freeze-all cycle, which will further minimize the risk of developing OHSS. There are pre-established protocols to monitor and minimize the risk of OHSS for any patient undergoing fertility treatment, including cycle cancellation prior to trigger, coasting, and/or administration of a dopamine agonist. All study participants will be extensively counselled about signs and symptoms of severe OHSS and advised to seek medical attention should they experience such signs or symptoms.

9.2 OHSS questionnaire

All study participants will be contacted by phone two weeks after completion of treatment using a standardized questionnaire to identify whether they experienced moderate-severe OHSS. The following script will be used:

I am going to ask you a few questions to determine whether you have experienced ovarian hyperstimulation syndrome, which is a medical condition caused by your body's excessive response to the hormones you received during your fertility treatment.

1. Have you experienced abdominal pain during your fertility treatment?

If yes, please describe the pain from a scale of 1 (no pain) to 10 (worst pain in your life): ___

2. Have you experienced abdominal distension during your fertility treatment?

3. Have you experienced abnormal nausea or vomiting?

4. Some patients may experience symptoms related to abnormal fluid collection in their abdomen. Did you have an ultrasound performed during your fertility treatment that revealed abnormal fluid collection in your abdomen?

5. Did you have an ultrasound performed during your fertility treatment that revealed abnormal fluid collection in your chest?

6. Did you experience any blood clots during your fertility treatment?

10. Adverse events

Patients will be counselled extensively about the potential symptoms of potential side effects of taking Luveris. The most common side effects experienced by around 2% of patients during clinical trials of the drug include but are not limited to the following: headache, pelvic and abdominal pain, nausea, breast pain, ovarian cysts, flatulence, injection site reactions, general pain, constipation, fatigue, painful menstruation, diarrhea and upper respiratory tract infections.

The most serious reported side effect is OHSS. Patients will be counselled extensively about the signs and symptoms associated with OHSS including but not limited to: nausea, vomiting, abdominal pain, abdominal discomfort, increased abdominal girth, bloating, decreased urine output, chest pain, shortness of breath, leg swelling, and/or pain. Subjects will be encouraged to contact the clinic if they have any concerns and seek immediate medical attention at the nearest hospital if they develop any of the aforementioned signs/symptoms.

Although extremely rare, potential serious adverse events related developing OHSS include:

- Development of ascites and/or pleural effusion
- Prerenal acute kidney injury
- A thromboembolic event such as a deep-vein thrombosis and/or pulmonary embolism
- Death

Any serious adverse event, directly attributed to the study medication, will be documented, managed and reported to the Mount Sinai Research Ethics Board immediately. In case of a rare event whereby a patient develops severe OHSS secondary to ovarian stimulation, further management may include:

- Hospitalization and/or extended hospital stay.
- Additional interventions such as a culdocentesis, paracentesis and, in rare circumstances, thoracentesis.
- Administration of therapeutic or prophylactic doses of anti-coagulation medication.

11. Risk-benefit estimate

There is evidence supporting the benefits of adding rLH to FSH in patients with hypogonadotropic hypogonadism, and those who are poor responders and are of advanced maternal age. Several studies reported the concern about a possible lower ovarian reserve and lower ovarian response in a subgroup of cancer patients seeking IVF treatment. This may be partly explained by the theoretical concern that the hypothalamic-pituitary-gonadal axis is impacted by the underlying disease. Studies show conflicting outcomes in terms of ovarian stimulation outcomes in this study group. The potential benefit of rLH supplementation is that it will result in a relatively improved ovarian stimulation cycle, thus yielding more mature oocytes and embryos to be cryopreserved.

In our study, only patients with a diagnosis of a non-hormone sensitive cancer will be part of the study. Patients with a diagnosis of breast, uterine or ovarian cancer will be excluded from the study regardless of their receptor status.

11.1 Sponsor

The cost of the intervention, Luveris (rLH), will be covered by a research grant from the drug producing company EMD Serono. The grant will also cover the cost of additional supplies including syringes and needles. In addition, the study grant will cover the cost of AMH test, which estimated to cost 45 CAD per patient. The patient will not incur any additional costs by choosing to participate in this study.

12. Statistical analysis

12.1 Sample Size

A sample size calculation was performed according to the primary outcome of the total number of mature oocytes available for cryopreservation [28]. We assume the control group will have a mean of 8 cryopreserved oocytes with a standard deviation of 4 according to data from a previous meta-analysis of randomized trials evaluating FSH and LH versus FSH alone using a GnRH antagonist protocol [29]. We will use an alpha level of 5%, power of 80%, enrolment ratio of 1, and assume a 20% improvement in FSH and LSH versus FSH alone. This calculation will yield a sample size of 126 patients (n=63 per group). Accounting for 10% loss to follow-up, the total sample size will be 140 patients (n=70 per group). Reported p-values will be recorded as two-tailed. The following formula was used for sample size calculation, which was doubled to account for two trial arms and multiplied by 1.1 to account for 10% loss to follow-up:

$$n_1 = \frac{(\sigma_1^2 + \sigma_2^2/K)(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta})^2}{|u_2 - u_1|^2}$$

$$n_1 = \frac{(4^2 + 4^2/1)(1.96 + 0.84)^2}{|10 - 8|^2}$$

$$n = 63 * 2 * 1.1$$

$$n = 140$$

Where n_1 is the sample size for the FSH and LH group; σ_1^2 is the variance for FSH and LH group; σ_2^2 is the variance for the FSH only group; $Z_{1-\frac{\alpha}{2}}$ is the Z-score corresponding to an alpha level of 0.05; $Z_{1-\beta}$ is the Z-score corresponding to a power of 0.80; u_1 is the projected mean number of cryopreserved oocytes in the FSH and LH group; u_2 is the projected mean number of cryopreserved oocytes in the FSH only group; n is the total sample size after accounting for projected loss to follow-up

12.2 Planned analysis

The analysis will be performed on an intention-to-treat principle. Continuous variables will be examined for normality using a normal probability plot. Normally distributed continuous variables will be compared using Welch's t-test. Continuous variables not normally distributed will be compared using Mann-Whitney U test. Categorical data will be reported as counts with percentage, and compared with chi-square test or Fisher's exact test whenever appropriate. Additional sensitivity analysis will be performed taking into account potential confounding factors (i.e. Age, AMH, AFC). Regression analysis with age and FSH dose as factors will also be performed. Statistical analysis will be performed using R statistical software (R Foundation for Statistical Computing).

13. Trial registration

The clinical trial will be registered and available online at www.clinicaltrials.gov once the protocol is approved by the Mount Sinai Hospital Research Ethics Board.

14. Knowledge translation

The lack of consensus on the safety and efficacy of rLH in addition to FSH for ovarian stimulation calls for high quality randomized trials. Once the trial is completed and results are available, knowledge translation is of critical importance and involves the translation of findings to policy and practice [30]. Several procedures will be implemented to share the results of this trial internationally given the increasing number of young patients diagnosed with cancer. The study results will be firstly presented at international fertility conferences. The results will also be published in a high impact fertility journal, such as *Human Reproduction*. Experts in the field will be contacted to ensure they are aware of trial results.

15. Role of principal investigator

Dr. Ellen Greenblatt is a reproductive endocrinologist at Mount Sinai Fertility as well as a Professor in the Department of Obstetrics and Gynecology at Mount Sinai Hospital. She will be supervising all aspects of the trial.

16. References

1. Anazodo A, Ataman-Millhouse L, Jayasinghe Y, Woodruff TK. Oncofertility–an emerging discipline rather than a special consideration. *Pediatr Blood Cancer* 2018;65:e27297.
2. Lee SJ, Schover LR, Partridge AH, Patrizio P, Wallace WH, Hagerty K, Beck LN, Brennan LV, Oktay K. American Society of Clinical Oncology recommendations on fertility preservation in cancer patients. *J Clin Oncol* 2006;24:2917-31.
3. Sonmezer M, Oktay K. Fertility preservation in female patients. *Hum Reprod Update* 2004;10:251-66.
4. Rienzi L, Romano S, Albricci L, et al. Embryo development of fresh 'versus' vitrified metaphase II oocytes after ICSI: a prospective randomized sibling-oocyte study. *Hum Reprod* 2010;25:66-73.
5. Shoham Z. The clinical therapeutic window for luteinizing hormone in controlled ovarian stimulation. *Fertil Steril* 2002;77:1170-7.
6. Raju GA, Chavan R, Deenadayal M, et al. Luteinizing hormone and follicle stimulating hormone synergy: A review of role in controlled ovarian hyper-stimulation. *J Hum Reprod Sci* 2013;6:227.
7. Hsueh AJ, Adashi EY, Jones PB, Welsh Jr TH. Hormonal regulation of the differentiation of cultured ovarian granulosa cells. *Endocr Rev* 1984;5:76-127.
8. Lehert P, Kolibianakis EM, Venetis CA, et al. Recombinant human follicle-stimulating hormone (r-hFSH) plus recombinant luteinizing hormone versus r-hFSH alone for ovarian stimulation during assisted reproductive technology: systematic review and meta-analysis. *Reprod Biol Endocrinol* 2014;12:17.
9. Hill MJ, Levens ED, Levy G, et al. The use of recombinant luteinizing hormone in patients undergoing assisted reproductive techniques with advanced reproductive age: a systematic review and meta-analysis. *Fertil Steril* 2012;97:1108-14.
10. Fritz MA, Speroff L. Clinical gynecologic endocrinology and infertility. Lippincott Williams & Wilkins; 2012.
11. Schenker JG, Meirow D, Schenker E. Stress and human reproduction. *Eur J Obstet Gynecol Reprod Biol* 1992;45:1-8.
12. Pal L, Leykin L, Schifren JL, et al. Malignancy may adversely influence the quality and behaviour of oocytes. *Hum Reprod* 1998;13:1837-40.
13. Lawrenz B, Fehm T, von Wolff M, et al. Reduced pretreatment ovarian reserve in premenopausal female patients with Hodgkin lymphoma or non-Hodgkin-lymphoma–evaluation by using antimüllerian hormone and retrieved oocytes. *Fertil Steril* 2012;98:141-4.
14. von Wolff M, Thaler CJ, Frambach T, et al. Ovarian stimulation to cryopreserve fertilized oocytes in cancer patients can be started in the luteal phase. *Fertil Steril* 2009;92:1360-5.
15. Acevedo B, Gomez-Palomares JL, Ricciarelli E, Hernández ER. Triggering ovulation with gonadotropin-releasing hormone agonists does not compromise embryo implantation rates. *Fertil Steril* 2006;86:1682-7.
16. Johnson LN, Dillon KE, Sammel MD, et al. Response to ovarian stimulation in patients facing gonadotoxic therapy. *Reprod Biomed Online* 2013;26:337-44.
17. Mahany EB, Hershman DL, Sauer MV, Choi JM. Pregnancy despite ovarian insufficiency in a patient with breast cancer. *Reprod Med Biol* 2013;12:35-8.
18. Bedoschi GM, de Albuquerque FO, Ferriani RA, Navarro PA. Ovarian stimulation during the luteal phase for fertility preservation of cancer patients: case reports and review of the literature. *J Assist Reprod Genet* 2010;27:491-4.

19. Oktay K, Bedoschi G. Oocyte cryopreservation for fertility preservation in postpubertal female children at risk for premature ovarian failure due to accelerated follicle loss in Turner syndrome or cancer treatments. *J Pediatr Adolesc Gynecol* 2014;27:342-6.
20. Friedler S, Koc O, Gidoni Y, Raziel A, Ron-El R. Ovarian response to stimulation for fertility preservation in women with malignant disease: a systematic review and meta-analysis. *Fertil Steril* 2012;97:125-33.
21. Alviggi C, Conforti A, Esteves SC, et al. Understanding ovarian hypo-response to exogenous gonadotropin in ovarian stimulation and its new proposed marker–the Follicle-To-Oocyte (FOI) Index. *Front Endocrinol* 2018;9:589.
22. Grynberg M, Labrosse J. Understanding follicular output rate (FORT) and its implications for POSEIDON criteria. *Front Endocrinol* 2019;10:246.
23. Shoham Z. The clinical therapeutic window for luteinizing hormone in controlled ovarian stimulation. *Fertil Steril* 2002;77:1170-7.
24. Agarwal A, Said TM. Implications of systemic malignancies on human fertility. *Reprod Biomed Online* 2004;9:673-9.
25. Gardner DK, Schoolcraft WB. In vitro culture of human blastocyst. In: Jansen R, Mortimer D (eds). Towards Reproductive Certainty: Fertility and Genetics Beyond 1999. UK: Parthenon Publishing Carnforth, 1999:378-388.
26. Golan A, Ron-El R, Herman A, Soffer Y. Ovarian hyperstimulation syndrome: an update review. *Obstet Gynecol Surv* 1989;44:430-40.
27. Lambalk CB, Banga FR, Huirne JA, et al. GnRH antagonist versus long agonist protocols in IVF: a systematic review and meta-analysis accounting for patient type. *Hum Reprod Update* 2017;23:560-79.
28. Rosner B. Fundamentals of biostatistics. Nelson Education; 2015.
29. Baruffi RL, Mauri AL, Petersen CG, et al. Recombinant LH supplementation to recombinant FSH during induced ovarian stimulation in the GnRH-antagonist protocol: a meta-analysis. *Reprod Biomed Online* 2007;14:14-25.
30. McKibbon KA, Wilczynski NL, Haynes RB. What do evidence-based secondary journals tell us about the publication of clinically important articles in primary healthcare journals? *BMC Med* 2004;2:33.