

Effect of Flexible Half-dose Gonadotropin-releasing Hormone Antagonist Protocol on in Vitro Fertilization Outcome in Predicted Normal Responder: A Study Protocol for a Multicentered, Randomized, Non-inferiority, Parallel Controlled Trial

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Research Article

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Abstract

Background

Gonadotropin-releasing hormone antagonists (GnRH-ant) are widely used in current *in vitro* fertilization-embryo transfer (IVF-ET), however, whether the lowest daily dose of GnRH-ant is individualized remains unknown. Due to the negative effect of GnRH-ant on endometrial receptivity, lessening the amount of GnRH-antagonists used during controlled ovarian stimulation may be helpful for embryo implantation. As such, a randomized controlled study is essential to validate the feasibility and efficacy of daily GnRH-ant dose reduction to 0.125 mg geared towards providing scientific evidence for guidance in clinical practice.

Methods

In total, 620 infertile women undergoing *in vitro* fertilization will be enrolled in the multicentered, randomized, parallel controlled trial. Based on a computer-generated random list, they will be randomly and equally subdivided into half-dose GnRH-ant group or conventional-dose GnRH-ant group. The primary outcome is ongoing pregnancy i.e., intrauterine pregnancy diagnosed by pelvic ultrasonography at more than 12 weeks of gestation accompanied by normal fetal heartbeats. Secondary outcomes include cycle cancellation, premature luteinizing hormone surge, positive pregnancy, embryo implantation rate, clinical pregnancy, early spontaneous abortion, and live birth. The intention-to-treat and per protocol analyses will be used to initially analyze the difference in ongoing pregnancy rate between the two groups, while the multiple imputation method was used to handle missing values in the data.

Discussion

At present, no randomized controlled trials (RCTs) have been performed on the use of the half-dose GnRH-ant protocol (0.125mg/d) to improve reproductive outcomes of IVF-ET in predicted normal responder, compared to conventional-dose GnRH-ant protocol (0.25mg/d). Half-dose GnRH-ant protocol might provide a suitable clinical solution for predicted normal responder undergoing IVF treatment. Thus, it is critical to conduct a well-designed RCT to evaluate the impact of a half-dose GnRH-ant protocol on the reproductive outcomes of IVF-ET in predicted normal responder.

Trial registration

This study was registered in the Chinese Clinical Trials Registry Platform on August 29, 2020. (chictr.org.cn; identifier: ChiCTR2000037629).

Strengths And Limitations

(1) The first parallel controlled trial on the minimum daily dose of GnRH-ant for individualized flexible use;

- (2) The first trial aims to confirm the feasibility and efficacy of daily GnRH-ant dose reduction to 0.125 mg to provide a scientific basis for improving the success rate of *in vitro* fertilization-embryo transfer;
- (3) The study is a prospective, multicenter, randomized controlled trial with more sufficient clinical evidence;
- (4) Researchers, doctors, and participants cannot be blinded to treatment allocation.

Background

Gonadotropin-releasing hormone antagonists (GnRH-ant) protocols are widely used in present IVF-ET treatment. At the same time, the ESHRE guidelines strongly propose the use of GnRH-ant protocol during controlled ovarian hyperstimulation (COH) in predicted normal responders^[1]. Unlike gonadotropin-releasing hormone agonist (GnRH-a) protocol, GnRH-ant protocol has several advantages, including shortening the treatment time, reducing gonadotropin dosage, and the occurrence of severe ovarian hyperstimulation syndrome (OHSS), as well as preventing pituitary over-suppression and flare-up effects^[2]. Although the GnRH-ant protocol has been reported to be comparable to the GnRH-a protocol in terms of cumulative live birth rates^[3, 4], other studies have shown that the GnRH-ant protocol results in decreased clinical pregnancy rates^[5, 6]. A recent systematic review revealed that frozen-thawed embryo transfer (FET) had no benefit on live birth rate compared to fresh embryo transfer, thus, the “freeze-all” strategy was not recommended for predicted normal responder due to the increased risk of preeclampsia^[7]. Additionally, a number of studies have shown that GnRH antagonists are associated with decreased frequencies of ongoing pregnancy in fresh embryo transfer cycles when compared to GnRH-a long protocol, whereas the detrimental effects of GnRH-ant on endometrial receptivity were the primary reasons for the poor pregnancy rate^[5, 8, 9].

To minimize the detrimental effect of GnRH-ant on endometrial receptivity, reproductive doctors have attempted to improve the GnRH-ant protocol by establishing a minimum dose of GnRH-ant. So far, starting on day 6 of ovarian stimulation (OS), 0.25 mg of cetrorelix per day is thought to maintain luteinizing hormone (LH) levels within a safe range, a dose frequently used in clinical practice^[10 - 13]. Nevertheless, a few studies have shown that limiting the daily dosage of GnRH-ant to 0.125-0.2 mg is similarly beneficial.^[14-16] Additionally, Wang and his colleges reported that routine GnRH-ant administration was not necessary during ovarian stimulation, since 87.2 percent of patients did not need any GnRH-ant mostly during COH cycle^[17]. As a result, the minimal effective dosage of GnRH-ant remains controversial.

Hence, we assume that there exists no specific GnRH-ant dose suitable for each participant, therefore, it is absolutely critical that the dosage of GnRH should be tailored to the needs of each patient. Based on the definition of premature LH surge, i.e., serum LH level ≥ 10 IU/L and progesterone (P) level ≥ 1.0 ng/ml, and the less effective GnRH-ant dose described in previous studies^[18-20], we tried a novel and flexible half-dose GnRH-ant protocol in a small number of patients and design this randomized controlled trial in

which the daily GnRH-ant dose will be reduced to 0.125 mg. The daily GnRH-ant dose will be increased to 0.25 mg until the trigger day if the LH level rises above 10 IU/L.

Methods/design

Study design

A multicenter, prospective, randomized parallel controlled trial will be conducted in four reproductive centers in China Mainland. The flow chart for this study is shown in Fig. 1, and the study process schedule is shown in Table 1.

| TABLE 1 Schedule of the study process (SPIRIT Figure). | | | | | | | | |
|--|-----------|---------------|-----------------|------------------|-------------------|-------------------|--------------------|-----------|
| STUDY PERIOD | | | | | | | | |
| | Enrolment | Allocation | Post-allocation | | | | | Close-out |
| TIMEPOINT** | | <i>0 days</i> | <i>1-6 days</i> | <i>6-15 days</i> | <i>16-30 days</i> | <i>6-12 weeks</i> | <i>13-40 weeks</i> | |
| ENROLMENT: | | | | | | | | |
| Inquire medical history | X | | | | | | | |
| Basic information registration (E2, LH, FSH, P, BMI, etc.) | X | | | | | | | |
| Vaginal ultrasound and biochemistry | X | | | | | | | |
| Randomization | | X | | | | | | |
| INTERVENTIONS: | | | | | | | | |
| 0.125 mg of cetorelix | | | | | | | | |
| 0.25 mg of cetorelix | | | | | | | | |
| ASSESSMENTS: | | | | | | | | |
| Cycle cancellation, Premature LH surge | | | | | | | | |
| Positive pregnancy, Embryo implantation rate | | | | | | | | |
| Clinical pregnancy Early Spontaneous Abortion | | | | | | | | |
| Ongoing pregnancy Live birth | | | | | | | | |

Objectives

GnRH-ant is believed to be negatively correlated with the capacity for endometrium receptivity. When using the GnRH-ant protocol, lowering GnRH-ant doses during controlled OS can aid in embryo implantation. Nonetheless, whether the minimum daily dose of GnRH-ant should be individualized

remains unknown. Thus, this study aims to resolve the concerns about the efficacy and safety of administering GnRH-ant a regular dose of 0.125mg.

Participants

Inclusion criteria

Initial IVF/ICSI treatment; Age < 40 years; predicted normal responder, i.e., the number of basal antral follicles count (AFC) was between 5 and 15 [21].

Exclusion criteria

Couples with chromosomal abnormalities; history of oophorectomy; history of pelvic chemoradiotherapy; polycystic ovary (PCO) or polycystic ovary syndrome (PCOS); ovarian endometriotic cysts requiring surgery; untreated hydrosalpinx; history of recurrent implantation failure (i.e. the failure to achieve a viable pregnancy, following a greater than 3 embryo transfers with high-quality embryos) and recurrent pregnancy loss (i.e. couples with two or more consecutive spontaneous miscarriages); embryo transfer cancelled for patient personal reasons; patients requiring egg donation; embryo transfer cancelled for uterine or endometrial abnormalities, such as endometriosis, uterine fibroids, endometrial polyps, or intrauterine adhesions.

Recruitment

Detailed history, transvaginal ultrasound, and biochemistry will be required before COH, and if inclusion and exclusion criteria are met, the patient will be contacted by a research assistant, where the study details will be explained to her, then invited to join the study. Each individual may willingly attend the study and will be provided a detailed explanation of the trial. At the same time, their permission will be sought before being recruited into the study. Patients must sign a written informed consent.

Interventions

Cetrorelix acetate can be provided in two groups: some patients will be treated with a regular dose, whereas others will get a low dosage. In the GnRH-ant half-dose group, on day five or six of COH, 0.125mg of GnRH-ant (Cetrorelix acetate, Merck Serono, Switzerland) will be subcutaneously injected daily. Patients will be subjected to serological testing at 1- or 2-days interval, and once LH levels exceed 10 mIU/mL, the daily cetrorelix dose will be adjusted to 0.25mg until the trigger day. In the GnRH-ant conventional-dose group, on day five or six of COH, 0.25mg of GnRH-ant will be subcutaneously injected daily until the trigger day.

In vitro fertilization procedure

OS begins on the second day of menstruation, and all patients will be provided recombinant follicle-stimulating hormone (r-FSH) (75U, Gonal-F, Merck Serono, Switzerland). The initial FSH dose was established by the physician based on age, AFC, basal FSH, and body mass index (BMI), usually ranging

from 150–225 IU/day. This dose is adjusted every 2–3 days, depending on ovarian response, i.e., serum estradiol levels and follicular growth under ultrasound monitoring. Then, all patients will be treated with GnRH-ant (0.25mg, Cetorelix acetate, Merck Serono, Switzerland) from day 5 or 6 of COH until the day before the trigger. Finally, 250µg of recombinant human chorionic gonadotropin (r-hCG, Merck Serono, Switzerland) will be injected when the mean diameter of ≥ 3 follicles reach 17 mm. After 35 to 37 hours of r-hCG injection, oocyte retrieval will be guided by transvaginal ultrasound. Notably, intracytoplasmic sperm injection (ICSI) will be performed only in cases of severe oligozoospermia and asthenozoospermia (i.e., those with sperm density $< 5 \times 10^6/\text{ml}$, $< 30\%$ viable sperm, sperm motility Grade ≤ 2 , or even sperm immotile but confirmed with viable sperm by eosin staining).

In our present study, only fresh embryo(s) will be transferred. On day three after oocyte recovery, embryo transfer will commence. All patients should undergo embryo transfer on the third day after oocyte retrieval, except for the following conditions: (1) serum $E_2 > 5000 \text{ pg/mL}$ on the trigger day; (2) number of oocytes retrieved ≥ 15 ; (3) OHSS; (4) serum $P > 1.75 \text{ ng/mL}$ before the trigger day ^[22]; or (5) patients who violate informed consents and waived fresh embryo transfer. No more than 2 embryos will be transferred at a time.

Luteal support will start on the first day after oocyte retrieval with 8% micronized vaginal progesterone gel 90 mg (Crinone®; Central Pharma Ltd, UK) once daily and dydrogesterone (Duphaston®; Abbott Biologicals, Netherlands) 20 mg orally daily until 10 weeks of gestation.

Primary outcome

Ongoing pregnancy: intrauterine pregnancy confirmed by pelvic ultrasonography at more than 12 weeks of gestation accompanied by normal fetal heartbeats.

Secondary outcomes

- (1) Cycle cancellation: premature ovulation, absence of follicular development, failure to pick up eggs, and personal reasons;
- (2) Premature LH surge: serum LH level $\geq 10 \text{ IU/L}$ or P level $\geq 1.0 \text{ ng/ml}$;
- (3) Positive pregnancy or chemical pregnancy: 14 days after embryo transfer, serum $\beta\text{-hCG} \geq 10 \text{ mIU/mL}$;
- (4) Embryo implantation: the number of gestational sacs identified via pelvic ultrasonography divided by the total number of embryos transferred;
- (5) Clinical pregnancy: intrauterine gestational sac with fetal heartbeat was identified by B-mode ultrasound at 6 gestational weeks;
- (6) Early spontaneous abortion: spontaneous termination of pregnancy occurring in less than 12 weeks of gestation;

(7) Live birth: delivery of a neonate with a gestational age \geq 20 weeks and heartbeat and respiration.

(8) Pregnancy related complications: gestational diabetes, gestational hypertension, preeclampsia, and preterm delivery.

Randomization

When patients volunteering to participate in the study will be joined, stratified block randomization will be used, i.e., stratified by study site. The block length will be set to four. The random number will be generated then block randomization will be performed based on R language version 3.5.1. The proportion of subjects in the two groups is 1:1. The specific grouping details are mastered by the drug management center and the statisticians, and the subjects will be randomly assigned to one of the following two groups: (1) GnRH-ant half-dose group: On the 6th day of COH, 0.125 mg cetorelix was subcutaneously injected daily. Patients underwent serological testing at 1- or 2-days interval, and once LH levels exceed 10 mIU/mL, the daily cetorelix dose will be adjusted to 0.25 mg until the trigger day; (2) GnRH-ant conventional-dose group: On the 6th day of COH, 0.25 mg cetorelix is injected subcutaneously daily until the trigger day.

Blinding

Because of the nature of the trial, neither the researchers nor the patients will be blinded. Nevertheless, the embryologists performing the quality assessment must be blinded to the grouped interventions.

Estimation of Sample Size

PASS software version 11.0 (NCSS, LLC. Kaysville, Utah, USA.) will be used to calculate the sample size of the two groups. It is worth noting that based on the data of our reproductive center at the time of the trial design, the ongoing pregnancy rate of each fresh embryo transfer was about 30%. Moreover, it is calculated that the sample size of the GnRH-ant low dose group (intervention group) and GnRH-ant conventional-dose group (control group) is 279 cases, with a total sample size of 558 cases. The equivalent detection ability of 80% was achieved to provide 80% certainty and to demonstrate non-inferiority with a margin of 10% of the main outcome of ongoing pregnancy between the two groups at bilateral $\alpha = 0.05$. The Calculation hypothesis was the bilateral Z test. This trial sets the dropout rate at 10%. The overall sample size of the two groups is 620, with 310 in each group.

Statistical Analysis

Intention-to-treat and per protocol analyses will be used to analyze the difference of persistent pregnancy rate between the two groups in the main analysis. The multiple interpolation method will be used to deal with the missing values in the data of this study.

Demographic characteristics and baseline data are compared between the two groups. Students' t-test is used to compare continuous variables, while the χ^2 test is used to compare classified variables. Additionally, a subgroup analysis-age \leq 35 years old vs $>$ 35 years old will be conducted to further explore the effect of flexible half-dose GnRH-ant regimen on *in vitro* fertilization outcomes in predicted normal

responders. All the data will be statistically analyzed using SPSS version 26.0, $P < 0.05$ is considered as statistically significant.

Feasibility analysis

Regarding the feasibility of research ideas, this trial considers the population with predicted normal response and a wide range of beneficiary populations. Moreover, a recent meta-analysis including eleven high-quality randomized controlled trials (RCTs) suggested that the whole embryo freezing strategy is not recommended for predicted normal responder due to the increased risk of preeclampsia compared to fresh embryo transfer. The adverse effect of traditional GnRH-ant protocol on the clinical pregnancy rate of fresh embryo transfer is the original intention of this trial. This trial sights to improve the clinical pregnancy rate in this population by reducing the dosage of GnRH-ant to alleviate its adverse effect on the endometrium.

The project applicant, Zhen-Gao Sun, MD, presided over several National Natural Science Foundation of China. Also, the applicant presided over and participated in the implementation of several multicenter RCTs nationwide, with good scientific research literacy; the applicants and researchers had graduate degree or above and a good cooperation relationship. Besides, the members of the research group received systematic training through the National Natural Science Foundation of China and provincial projects, mastered the knowledge of protocol design, reproductive medicine, medical statistics, and other aspects of the study of this project, they also had strong technical strength.

Data Entry and Quality Control

Embryo lab data will be collected on day 3 or 5 post oocyte retrieval. As per the study design, participants' follow-up data will comprise information from the time they enrolled in the study and for the next year. Furthermore, case report forms from patients will be obtained for data input. Investigators will also be needed to determine the data accuracy levels (first and second levels), since both levels will involve data validation and monitoring, which will be performed on a regular basis by the Data Monitoring Committee (DMC) during the study. On a daily basis, data will be backed up to another computer in the same physical area as the server.

Patient and public involvement

Patients and the public are not involved in developing the research questions nor the study design. Moreover, they do not participate in the recruitment exercise of the study. Our results will be disseminated to participants and their families via telephone and patient organization platforms.

Existing Conditions and Work Basis

Based on the IVF-ET technology platform of the Reproductive Center, Affiliated Hospital of Shandong University of Traditional Chinese Medicine (TCM), the project research team performed IVF-ET for more than 10 years for the earliest TCM unit to start IVF technology. This was approved by the Ministry of Health in China, and implemented IVF/ICSI about 2,000 cycles per year.

The members of the research group had a reasonable echelon, high quality, and a good relationship with many years of cooperation, in completion of many RCTs, and well completed the research work of this topic.

Ethics and Dissemination

Clinicians received written informed consent from each patient participating in the study before the study commences. This study was approved by the Ethics Committee of Reproductive Medicine Ethics Committees of the Affiliated Hospital of Shandong University of TCM (SDTCM-RM-2020.611) in Jinan City, Shandong Province, China. The findings of this study were widely disseminated via conference papers, research reports, and academic publications.

Likewise, data will be electronically inputted, saved, and encrypted in secure computer files available only to the study's research team and investigators. Additionally, the original study forms will be kept and maintained at the study location for three years after the end of the study. Also, the investigators will permit Institutional Review Board/Independent Ethics Committee (IRB/IEC) evaluations, audits, and monitoring of the trial's data sources and required documents. Subsequently, participants will be asked to contact their physician with any study-related concerns.

Trial status

This trial is at version 1.7, 29 August 2020 (ChiTR). The actual study start date was 1 April 2021 and the anticipated study end date is 31 December 2021. The recruitment start date was 15 May 2021; the anticipated recruitment end date is 31 October 2021.

Discussion

Premature LH surge is characterized as an LH level of 10 IU/L and a P level of 1.0ng/ml, and is produced by numerous follicle recruitment and fast estrogen rise.^[23,24] Premature LH surge during ovarian stimulation in IVF is detrimental to egg development, resulting in luteinization and premature ovulation, leading to poor reproductive results and even cycle cancellation^[4]. Although this is still disputed, pregnancy rates with the GnRH-ant protocol have been reported to be lower than those with the GnRH-agonist protocol, with reduced endometrial receptivity being the primary reason for this discrepancy. Reduced GnRH-ant dosages have been used by physicians in reproductive clinics to try to obtain acceptable pregnancy chances. However, the optimal daily dosage of GnRH-ant continues to be hotly debated. At the moment, 0.25 mg of Cetrorelix per day starting on day 5 or 6 of ovarian stimulation is considered the standard GnRH-ant protocol, and the LH level may be controlled within a safe range with this procedure. According to some other studies, limiting the GnRH-ant dosage to 0.125-0.2 mg per day is also beneficial. Daily GnRH-ant dosage requirements for the GnRH-ant regimen, we believe, should be determined on an individualized level. Based on previous studies^[15,25], we tried to reduce the initial daily dose of cetrorelix to 0.125 mg, which was then individually adjusted to 0.25 mg based on LH levels during controlled ovarian stimulation and as a GnRH-ant half-dose group, furthermore individuals

assigned to the GnRH-ant conventional-dose group will receive 0.25 mg cetrorelix daily for the duration of the controlled ovarian stimulation.

A panel of seasoned professionals will perform this randomized controlled study. Based on our experience in conducting RCTs, this study will demonstrate that GnRH-ant half-dose group has a convincing effect on improving reproductive outcomes in patients with predicted normal response, compared to GnRH-ant conventional-dose group.

Abbreviations

GnRH-ant: gonadotropin-releasing hormone antagonists; IVF-ET: in vitro fertilization-embryo transfer; FET: frozen-thawed embryo transfer; RCT: randomized controlled trial; COH: controlled ovarian hyperstimulation; OHSS: ovarian hyperstimulation syndrome; OS: ovarian stimulation; LH: luteinizing hormone; AFC: antral follicle count; PCOS: polycystic ovary syndrome; FSH: follicle-stimulating hormone; BMI: body mass index; r-hCG: recombinant human chorionic gonadotropin; ICSI: intracytoplasmic sperm injection; CRF: case report form; TCM: traditional Chinese medicine.

Declarations

Ethics approval and consent to participate

This study was approved by the Reproductive Medicine Ethics Committee of the Affiliated Hospital of Shandong University of TCM (SDTCM-RM-2020.611) in Jinan, Shandong, China. All participants will be required to sign written informed consent.

Consent for publication

Not applicable.

Availability of data and material

Not applicable.

Competing interests

None of the authors have a conflict of interest to declare with regard to this study.

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Authors' contributions

Zhen-Gao Sun and Jing-Yan Song designed and supervised this study. Ying-Jie Ma wrote the manuscript. Meng-Yao Li and Jing-Yan Song modified the manuscript. Ying-Jie Ma made significant contributions to the sample size estimation and performed the statistical analysis.

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Figures

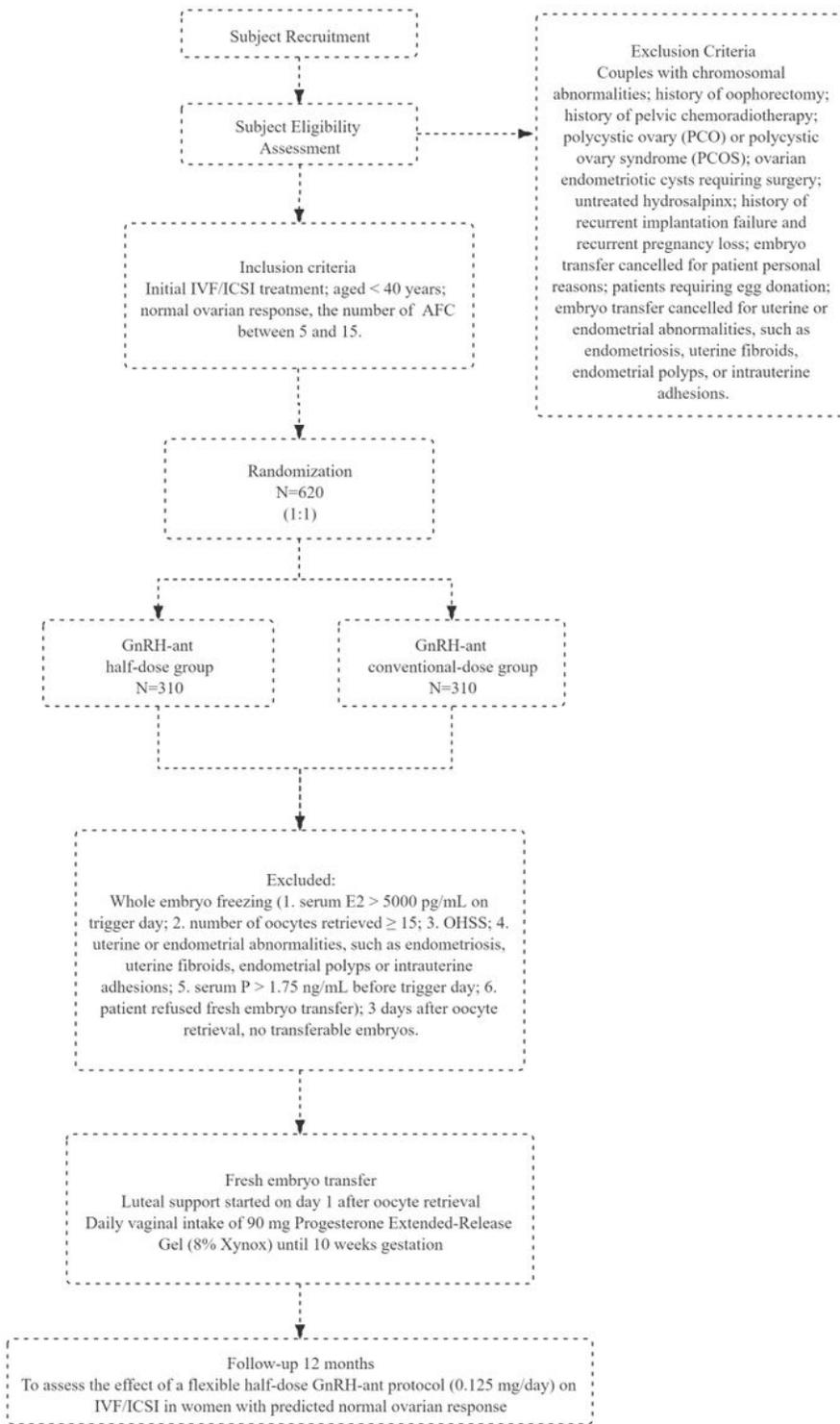


Figure 1

Flowchart showing the study process.

Supplementary Files

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