Luteinising hormone-based protocol versus traditional flexible gonadotropin-releasing hormone antagonist protocol in women with normal ovarian response: study protocol for a non-inferiority trial

Ya-su Lv, Yuan Li, Shan Liu

ABSTRACT

Introduction Many patients demonstrate an insufficient endogenous luteinising hormone (LH) concentration during ovarian stimulation. With traditional fixed or flexible gonadotropin-releasing hormone (GnRH) antagonist protocols, antagonist administration may further reduce LH activity. Previously, we proved that LH can be used as an indicator for the timing and dosage of antagonist. Patients with a persistently low LH concentration during ovarian stimulation may not require antagonists, whereas antagonist administration can affect reproductive outcomes. To further explore this hypothesis, we designed a randomised clinical trial to compare the LH-based flexible GnRH antagonist protocol with traditional flexible GnRH antagonist protocol in women with normal ovarian response.

Methods and analysis This study was a multicentre, parallel, prospective, randomised, non-inferiority study. The primary efficacy endpoint was cumulative ongoing pregnancy rate per cycle. The study aimed to prove the non-inferiority of cumulative ongoing pregnancy rate per cycle with an LH-based flexible GnRH antagonist protocol versus traditional flexible GnRH antagonist protocol. Secondary endpoints were the high-quality embryo rate, clinical pregnancy rate and cancellation rate. Differences in cost-effectiveness and adverse events were evaluated. The cumulative ongoing pregnancy rate per cycle in women with normal ovarian response was 70%. Considering that a non-inferiority threshold should retain 80% of the clinical effect of a control treatment, a minimal clinical difference of 14% (one-sided: α, 2.5%; β, 20%) and a total of 338 patients were needed. Anticipating a 10% drop-out rate, the total number of patients required was 372.

Ethics and dissemination This trial has been approved by the Institutional Ethical Committee of Beijing Chao-Yang Hospital. All participants in the trial will provide written informed consent. The study will be conducted according to the principles outlined in the Declaration of Helsinki and its amendments. Results of this study will be disseminated in peer-reviewed scientific journals.

Trial registration number ChiCTR1800018077.

Strengths and limitations of this study

- This is the first randomised controlled trial to examine the efficacy and safety of a novel luteinising hormone-based protocol in women with normal ovarian response who are undergoing in vitro fertilisation treatment.
- This study was a multicentre, parallel, prospective, randomised, non-inferiority study, all investigators are required to undertake mandatory training in the protocol.
- The individual eligibility criteria used in our study also limit the bias of advanced age, which is associated with a higher risk of adverse outcomes.
- The sample size calculation is based on a difference of 10% in cumulative ongoing pregnancy rate between the two groups, thus, a smaller difference in the ongoing pregnancy rate may not be detected.

INTRODUCTION

There is evidence that a sufficient concentration of luteinising hormone (LH) is necessary for normal follicular growth and oocyte maturation. A low LH concentration during ovarian stimulation can adversely affect follicular morphology, quality and maturation, determining meiotic status and fertilisation ability. Inversely, clinical evidence from multiple LH measurements revealed that low LH levels were associated with increased early pregnancy loss and decreased the CLBRs per oocyte retrieval cycle. Extensive clinical trials have shown that a serum LH concentration of ≥1.2 IU/L is necessary to provide adequate LH support to follicle-stimulating hormone (FSH)-induced follicular development. Some studies have demonstrated that for optimal cyclic follicular development, the serum LH concentration should be between 1.2 IU/L and 5.0 IU/L. Other studies suggest that the indications for use of...
intracytoplasmic sperm injection (ICSI). Patients were randomly assigned to one of two groups: the experimental group (stimulated with an LH-based flexible GnRH antagonist protocol) and the control group (stimulated with a traditional flexible GnRH antagonist protocol) at a 1:1 ratio on the first day of ovarian stimulation. Figure 1 shows a flow chart of the study design. Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) checklist is given online as online supplemental file 1.

Study sites and recruitment procedures
This clinical trial involved eight hospitals in China. All patients undergoing IVF for the first time at the Centre for Reproductive Medicine were included in the study. Physicians will screen patients based on clinical data to assess whether they meet the inclusion criteria. Subjects who are eligible for and who agree to participate in the study are notified and recruited prior to the start of the IVF cycle.

Study population and inclusion/exclusion criteria
The trial enrolled women who were undergoing their first IVF cycle, and a GnRH antagonist regimen was used for ovarian stimulation. In addition, women must have an indication for IVF with or without ICSI treatment, such as tubal disease due to pelvic inflammatory disease and/or adhesions, unexplained infertility, etc. Eligible women met the following inclusion criteria: (1) 23–38 years of age; (2) a spontaneous cycle length of ≥21 days and ≤35 days; (3) a diagnosis of infertility for >1 year; (4) an antral follicle count (AFC) of 8–20 and (5) a body mass index (BMI) of ≥18 kg/m² or ≤28 kg/m².

The exclusion criteria were as follows: (1) a history of unilateral oophorectomy; (2) recurrent spontaneous abortion; (3) a diagnosis of polycystic ovarian syndrome; (4) uterine abnormalities (e.g., submucosal myoma, adenomyosis, uterine scarring, intrauterine adhesion); (5) a chronic medical disease affecting pregnancy outcomes (e.g., diabetes mellitus, hypertension, heart disease, liver dysfunction, renal disease) and (6) coagulation dysfunction or a history of deep venous thrombosis. All couples were screened by karyotyping, and those with an abnormal karyotype were excluded.

Randomisation
Randomisation was performed by a doctor on the initial day of ovarian stimulation from day 2 of the menstrual cycle. Participants were randomly divided into two groups in a 1:1 ratio and were stratified according to the study site. The randomisation scheme was entered into an online central randomisation database (www.medresman.org). After randomisation, the physicians will be informed about the allocation results by email. Embryologists, data assessors and the patients were all blinded in our study. Patients were randomly assigned to one of two groups: the control group (traditional flexible GnRH antagonist protocol) or the experimental group (LH-based flexible GnRH antagonist protocol).

METHODS AND ANALYSIS

Study design
This study was a multicentre, randomised, controlled, non-inferiority trial that involved patients with normal ovarian responses undergoing in vitro fertilisation (IVF)/intracytoplasmic sperm injection (ICSI). Patients were
**Ovarian stimulation protocol**

All patients underwent baseline transvaginal ultrasound and measurement of serum E2, FSH, LH, progesterone (P) and beta-human chorionic gonadotropin (β-hCG) on days 2–3 of the menstrual cycle. Recombinant FSH (Gonal-F, Merck, Germany) at a dose of 150–300 IU/day was administered according to age, BMI, Anti-Müllerian Hormone (AMH), AFC and basal serum FSH concentration. After 5 days of treatment, an ultrasound examination was performed. The gonadotropin dosage was adjusted according to follicle development and serum E2, P and LH concentrations.

Patients in the control group were administered a GnRH antagonist (cetrorelix acetate, Cetrotide, Merck, Germany) at a dose of 0.25 mg/day, which was initiated when at least one follicle was 14 mm in size or the E2 concentration was ≥300 pg/mL. Treatment was continued until the day of trigger. Blood samples will be collected for LH levels measured to determine the timing and dosage of rLH supplementation.

Patients in the experimental group were administered antagonist, and the dosage was based on the LH concentration from day 6 of ovarian stimulation. No antagonist was administered if the LH concentration was ≤4 IU/L. If the LH concentration was >4 IU/L or ≤6 IU/L, 0.125 mg of cetrorelix acetate was administered daily for 2 days until the next blood test. If the LH concentration was >6 IU/L or ≤10 IU/L, 0.25 mg of cetrorelix acetate was administered daily for 2 days. If the LH concentration was >10 IU/L or ≤15 IU/L, 0.375 mg of cetrorelix acetate was administered daily for 2 days. If the LH concentration was >15 IU/L, 0.5 mg of cetrorelix acetate was administered for 1 day. Whether or not antagonist cotreatment was administered depended on an LH concentration of >4 IU/L until the day of trigger.

**Oocyte retrieval, embryo culture and luteal-phase support**

Previous studies showed that dual trigger could increase the number of oocytes, mature oocytes and number of blastocysts as well as the percentage of top-quality blastocysts compared with triggering with hCG alone. On this basis, the dual trigger has been used in our clinic since 2018 in normal responder women.20–22 Final oocyte maturation is induced when at least three follicles reach ≥18 mm in mean diameter using 2000–3000 IU of hCG and 0.2 mg of triptorelin (Decapeptyl, Ipsen, France). Serum LH, E2 and P concentrations were measured, and transvaginal ultrasound-guided oocyte retrieval was performed 35–36 hours after the trigger injection. Embryo transfer (ET) is usually performed at the cleavage stage, 3 days...
after fertilisation, with two of the highest quality embryos. The remaining embryos are cultured for 2 or 3 more days, and good quality blastocysts are vitrified. Fresh ET was cancelled if patients were at risk of ovarian hyperstimulation syndrome (OHSS), had an unfavourable endometrium (endometrial thickness of \( \geq 6 \) mm or \( \geq 16 \) mm, cavity fluid or endometrial polyps), had a P concentration of \( \geq 1.5 \) ng/mL on the day of hCG trigger, or if no embryo was present.

For fresh ET, luteal-phase support with vaginal progesterone gel (Crinone, Merck Serono) at a dose of 90 mg daily and oral dydrogesterone (Duphaston, Abbott) at a dose of 10 mg two times per day was started after oocyte retrieval and was continued until the day of hCG testing. For frozen ET, the endometrium was prepared using either a natural cycle regimen or an artificial cycle regimen based on the physician of decision. For the natural cycle regimen, luteal phase support is started from the ovulation day with oral dydrogesterone 10 mg two times daily; for the hormone replacement cycle regimen, the endometrium is prepared with oral estradiol valerate at a dose of 6–8 mg daily which started on day 3–5 of the menstrual cycle. Vaginal progesterone gel 90 mg daily and oral dydrogesterone 10 mg two times per day are added for endometrium translation. If pregnancy is achieved, luteal phase support will be continued until 10 weeks’ gestation.

Outcome measurement
The primary outcome measure was cumulative ongoing pregnancy rate per cycle. Ongoing pregnancy was defined as a gestational period of more than 12 weeks and fetal heart activity on ultrasound.

Secondary outcomes were high-quality embryo rate, clinical pregnancy rate and cancellation rate. Moreover, differences in cost-effectiveness and adverse events were evaluated. High-quality embryos were defined by two criteria: the number of cells in the embryo and their appearance under a high-power microscope. Typically, high-quality embryos on day 3 contained 7–9 cells with moderate or no fragmentation. Clinical pregnancy rate was defined as the presence of a gestational sac at 6–7 weeks of gestation when visualised by transvaginal ultrasound. Cancellation rate was defined as the number of cycles with no embryo for transfer divided by the number of ovum pick-up cycles. Moderate OHSS was diagnosed when ovarian enlargement of \( >5 \) cm and \( <12 \) cm was observed and when ultrasonographic ascites was present with or without nausea, vomiting and/or diarrhoea. Severe OHSS was diagnosed when ovarian enlargement of \( \geq 12 \) cm was observed and when there was clinical evidence of ascites and/or hydrothorax or breathing difficulties with or without haemocoagulation, severe hypoproteinaemia, abnormal liver function, coagulation abnormalities or diminished renal function.

Study assessment
Screening and baseline assessments included an evaluation of the inclusion and exclusion criteria, study recruitment and informed consent processes. All patients were evaluated on the day of ovarian stimulation from day 2 of the menstrual cycle. Treatment phase assessments included blood and ultrasound monitoring of follicular development during ovarian stimulation. Hormone analyses were performed during ovarian stimulation as follows: (1) on the day of stimulation; (2) 4–5 days after stimulation initiation; (3) on the day of trigger and (4) 2 days after egg retrieval.

A pregnancy test was performed 12–14 days after ET to confirm pregnancy. In the case of biochemical pregnancy, vaginal ultrasound was performed 6–7 weeks after transplantation to confirm clinical pregnancy. Pregnancy that proceeded beyond 12 weeks of gestation was defined as an ongoing pregnancy. Pregnancy complications (eg, ectopic pregnancy, OHSS, miscarriage, gestational trophoblastic disease) will be evaluated by inspecting medical records.

Patient and public involvement
All aspects of this study (development of the research question, study design and conduct of the trial, interpretation of results and editing of the final manuscript for publication) are taking place independently of patients and public involvement. The results will be disseminated to participants by their physicians.

Data analysis
Sample size calculation
The sample size calculation was based on the cumulative ongoing pregnancy rate. The cumulative ongoing pregnancy rate per cycle in women with normal ovarian responses was approximately 70% in our retrospective clinical database. Considering that a non-inferiority threshold should retain 80% of the clinical effect of a control treatment, a minimum clinical difference of 14% (one-sided: \( \alpha = 2.5\% ; \beta = 20\% \)) and a total of 338 patients were needed. Anticipating a 10% drop-out rate, the total number of patients required was 372.

Data management
A clinical trial electronic case report form (http://www.clinicaltrials.gov) was used to record and deposit patient data to manage the data, monitor the process and promote research transparency. The study protocol (7 December 2018, V.1.0), operational and procedural manuals, case report forms, informational brochures, and informed consent forms were reviewed and approved by the eight participating hospitals.

Data analysis plan
Intergroup differences in demographic variables and baseline information were compared before the beginning of the study. Continuous data were analysed using a two-sample t-test or Wilcoxon’s rank-sum test, while categorical data were analysed using the \( \chi^2 \) test or Fisher’s exact test. The primary analysis used an intention-to-treat analysis approach to examine differences in the cumulative ongoing pregnancy rate between the two groups using the \( \chi^2 \) test. The relative risk and 95% CI were calculated. The \( \chi^2 \) test or Fisher’s exact test was used to analyse secondary
efficacy parameters and safety parameters (eg, clinical pregnancy rate, OHSS rate, cancellation rate).

An independent-samples t-test was used for continuous variables with a normal distribution, and the Mann-Whitney U test was used for data with a non-normal distribution. A p value of <0.05 (two-sided) was considered statistically significant.

**Missing data and dropouts**

Patients who dropped out of the study for whatever reason could still undergo IVF treatment without adversely affecting their cycle. Those who cannot use dual triggering will drop out of the study on account of an unexpected ovarian high response and a high risk of OHSS. Other outcome variables may have missing data due to missed patient visits.

The flow chart of this study is presented in figure 1, and the Standard Protocol Items: Recommendations for Interventional Trials checklist is included as figure 2.

**DISCUSSION**

This study aimed to evaluate the efficacy of an LH-based flexible GnRH antagonist protocol in women with a normal ovarian response. Moreover, we aimed to further determine whether LH could be used as an indicator for the timing and dose of antagonist administration with the GnRH antagonist protocol. We planned to enrol 372 subjects from eight academic IVF centres in China. Patient enrolment began on 29 August 2018. The results of this multicentre randomised trial will provide strong evidence for an LH-based flexible GnRH antagonist protocol during ovarian stimulation in patients with normal ovarian responses.

This study is a multicentre, randomised, prospective, parallel, non-inferiority study. A threshold concentration of 4 IU/L of serum LH was used as the cut-off value to determine whether a GnRH antagonist should be administered. This threshold is based on our previous study of frequent LH measurements during ovarian stimulation.19 Previously, we found that most patients with a low LH concentration (<4 IU/L) throughout ovarian stimulation had no LH surge. Considering that administration of a GnRH antagonist would further reduce the LH concentration, we decided to stimulate patients with LH at a concentration of <4 IU/L without antagonist cotreatment. If our hypothesis proved to be true, a new protocol could be established to control the LH concentration more effectively and potentially improve the effectiveness of IVF treatment, especially in patients with low LH concentrations during ovarian stimulation.

The majority of patients had sufficient endogenous LH to successfully maintain follicular development and oocyte maturation. However, a significant proportion of patients...
had an insufficient endogenous LH concentration. Poor pregnancy outcomes were observed in patients who had a continuously low LH concentration and in patients who experienced a sharp decrease in LH concentration during follicular development from baseline. A relative reduction in LH concentration in the mid-follicle during GnRH agonist cycles results in a lower live birth rate. Studies have shown that when the serum LH concentration on trigger day is lower than one-third of the baseline concentration, pregnancy and implantation rates are significantly reduced. For patients with endogenous LH deficiency, a regimen without antagonists would be more beneficial. Therefore, we implemented this randomised controlled trial to illustrate that LH can be used as an indicator for antagonist administration with a GnRH antagonist protocol among women undergoing ovarian stimulation for IVF/ICSI. This scheme can be applied to all patients, but is more beneficial for patients with insufficient endogenous LH concentrations. We hope to complete data collection and analysis in order to provide recommendations for the choice of protocol. The data will provide us with a new perspective on the administration of antagonist with a GnRH antagonist protocol.

**Trial status**

The protocol version number and date: V.1.0, 7 December 2018. The study was conceived and designed in 2017. Enrolment began in 2018 and is expected to end in December 2020. At the time of manuscript preparation, more than 200 subjects had been enrolled. Enrolment in this study was ongoing at the time of manuscript submission.

**Acknowledgements** We thank all subjects for their voluntary participation in this study and physicians at all recruiting sites.

**Contributors** YL was involved in the drafting of the manuscript. YL was involved in the study concept and design. SL was involved in the study concept and design and in the revision of the manuscript. All authors read and approved the final manuscript.

**Funding** This study was supported by 1351 Talent training Programme of Beijing Chao-Yang Hospital (CYXX-2017-20, CYMV-2017-21); Capital Health Development Scientific Research Project (Independent Innovation , 2020-1-2093); Beijing Health Promotion Foundation (2019-09-05); 2018 Fertility Research Programme of Young and Middle-aged Physicians-China Health Promotion Foundation. 2020 Fertility Research Programme of Young and Middle-aged Physicians-China Health Promotion Foundation; Beijing Hospitals Authority Youth Programme (GML202000301).

**Disclaimer** The funding bodies had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

**Competing interests** None declared.

**Patient consent for publication** Obtained.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Supplemental material** This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.


