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*Original Article*

# Gonadotropin-releasing hormone agonist for oocyte triggering in endometrial preparation of letrozole stimulation protocols does not affect clinical outcome of frozen-thawed embryo transfer

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**Abstract:** Objective: This study aims to evaluate the effectiveness of GnRH agonist in comparison with hCG for triggering final oocyte maturation in endometrial preparation of letrozole stimulation protocols for frozen-thawed embryo transfer. Methods: The frozen-thawed embryo transfer cycles (FET) that use the letrozole stimulation protocols for endometrial preparation were divided into two groups according the different method of triggering final oocyte maturation. The serum LH and E2 levels, and the endometrial thickness on the day of triggering, the clinical pregnancy rates, the miscarriage rates and live birth rates were compared. Results: There were no significant differences in the age, the endometrial thickness, the number of embryos transferred between the two groups. The clinical pregnancy rate, abortion rate and live birth rates of the group A were similar compared with the group B, $P<0.05$. Conclusion: Using GnRH agonist for oocyte triggering in endometrial preparation of letrozole stimulation protocols for frozen-thawed embryo transfer does not affect the clinical outcome compared with hCG under the same luteal phase support.

**Keywords:** Gonadotropin-releasing hormone agonist, letrozole, frozen-thawed embryo transfer, hCG

## Introduction

In a normal menstrual cycle, a cascade of events drive ovulation, and this cascade is initiated by a surge of luteinizing hormone (LH) from the pituitary, which induces resumption of oocyte meiosis and follicular rupture when received by the follicle. hCG is employed because it possesses the same a subunit and 85% of the amino acid residues of the b subunit of LH, and binds to the same LH/HCG receptors [1]. Unfortunately, given its significantly longer half-life (>24 h versus 60 min for LH), hCG is associated with a high risk of ovarian hyperstimulation syndrome (OHSS) as a result of its sustained luteotrophic effect, characterized by the development of multiple corpora lutea and supraphysiological concentrations of oestradiol and progesterone [2].

In the last decade, GnRH antagonist has been introduced to the market to be used for pituitary desensitization in IVF/ICSI treatment cycles. GnRH antagonist shown to be an effective alternative to the standard long GnRH agonist protocols. Due to the specific mode of action of GnRH antagonist, quick and reversible response, GnRH agonist (GnRHa) as an alternative to hCG-induced ovulation triggering. The administration of a GnRH agonist, which induces an endogenous rise in both LH and FSH concentrations (initial flare effect) [3, 4], has been shown to effectively induce ovulation and has been recommended as an important strategy for reducing the risk of severe OHSS in patients undergoing ovarian stimulation cycles [5, 6].

However, the clinical efficacy of GnRH agonist triggering of ovulation requires confirmation. In a recent review and meta-analysis, final oocyte maturation trigger with GnRH agonist results in very low ongoing pregnancy rate in the GnRH antagonist protocols [7, 8]. Only some studies have reported comparable clinical outcomes

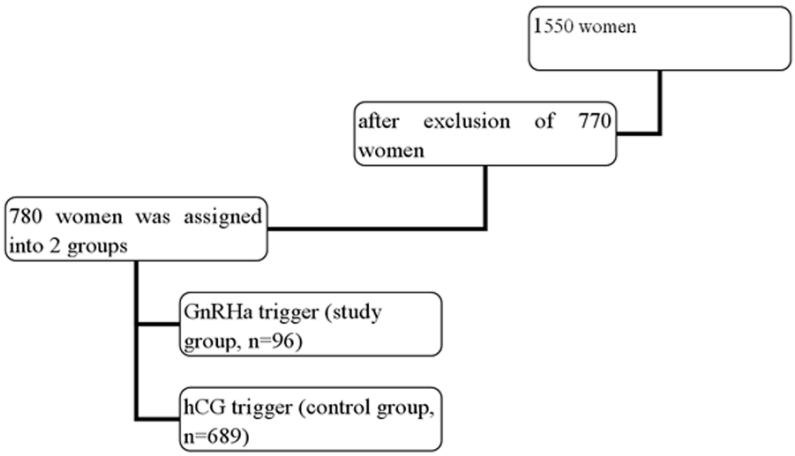


**Figure 1.** Consolidated standards of reporting trial statement flow diagram.

after GnRH agonist and HCG triggering. The theories put forward in order to explain this poor IVF outcome are: GnRH agonist induces endogenous LH and FSH surges which might simulate the natural mid-cycle LH surge. The serum LH and FSH levels rise after 4 and 12 h, respectively, and are elevated for 24-36 h. The amplitude of the surges is similar to those seen in the normal menstrual cycle but, in contrast to the natural cycle, the LH surge consists of two phases. These are a short ascending limb (>4 h) and a long descending limb (>20 h). Thus, final oocyte maturation trigger with GnRH agonist results in corpus luteum deficiency and a defective luteal phase and is associated with very low ongoing pregnancy rate [9].

Despite supplementation with a standard luteal-phase support can not overcome the luteal-phase insufficiency previously reported post GnRH agonist trigger [10, 11]. For this reason, several schemes of intensifying luteal support have been used to increase the chance of pregnancy, include administration of a higher dose of progesterone and oestrogen (oestradiol) or the addition of a small bolus dose of HCG, immediately after oocyte retrieval [12-14]. But there is no agreement yet regarding which is the optimal one.

Common endometrial preparation strategies for FET include ovulation induction cycling, hormone replacement therapy (HRT) cycling, and natural cycling or normal ovulation cycling, while patients with ovulation disorders are administered small doses of human menopausal gonadotropin (HMG), and HMG combined with clomiphene, or HMG combined with letrozole to induce ovulation. However, clomiphene affect estrogen receptors of the endometrium, and might affect the expression of endometrial receptivity markers, therefore affecting endometrial receptivity [14-16]. Letrozole can inhibit the growth of non-dominant follicles, promotes single-follicle development and exhibits no negative effects on endometrial and cervical mucus [17]. In addition, letrozole can enable full endometrial pinopode expression and increase integrin aVb3 expression on the endometrium during implantation, improving endometrial receptivity [18, 19]. Song-jun [20] and Yan-Jun [21] reported that letrozole was an effective drug preparing the endometrium for FET.

However, to our knowledge, no studies have reported the use of GnRH agonist as triggering ovulation in letrozole stimulation endometrial preparation for FET. In the current retrospective study, we have summarized our clinical experience using GnRH agonist as triggering ovulation in the letrozole stimulation endometrial for FET and compared the clinical outcome with HCG triggering. Our aim is to examine whether GnRHa as triggering ovulation affect the pregnancy outcome of FET.

## Materials and methods

### *Subjects and grouping*

All patients enrolled in this study underwent FET at the Center of Reproductive Medicine of Maternal and Child Health Care Hospital of liuzhou, Guangxi province, China between April 2008-December 2012. The required information was retrieved from the electronic database of our centre for this retrospective analysis.

All the patients had following criteria: (1) using Letrozole stimulation in endometrial preparation for FET. (2) aged 22-42 years, (3) had a menstrual cycle of 35-45 days, (4) at least one good embryos freezing.

All patients were required to sign a written informed consent form after they were given information about letrozole or HMG. This study was approved by the ethics committee of Maternal and Child Health Care Hospital of Guangxi liuzhou.

Patients were divided into two groups according the different method of triggering final oocyte maturation. Group A that used GnRH agonists trigger; Group B that used HCG trigger (**Figure 1**).

Exclusion criteria involved: (1) an endometrial thickness of less than 8 mm on the day of triggering final oocyte maturation, (2) patients with diseases like endometrial polyps and intrauterine adhesion or uterine submucosal myomas that might cause endometrial abnormalities, (3) adenomyosis, (4) patients with systemic diseases that would not tolerant of pregnancy, and (5) patients who had serious hydropic fallopian tubes or endometriosis of stage III or higher.

*Endometrial preparation*

From the 3rd to the 5th day of the cycle [according to cycle length], patients received a daily dose of 3-5 mg letrozole (Laiquzuo, Heng-Rui, Jiangshu, China) for five consecutive days (from day 3 to day 7). Transvaginal sonography was performed from day 10 of the cycle and was repeated when necessary. If the follicular diameter was ≥14 mm on day 10, transvaginal sonography was performed daily and no other ovarian stimulation drugs were needed until follicle maturation and triggering. If the follicular diameter was <14 mm on day 10, daily doses of 75 IU hMG were added to stimulate follicle growth, with dose increments of 37_5 IU every 5-7 days when needed. If there was still no follicle with diameter ≥14 mm after 10-14 days of hMG stimulation, the cycle was cancelled. When endometrial thickness of 8 mm or more was reached and follicle diameter was above 18 mm and serum LH was <20 IU/l, 10 000 IU of hCG (Li-Zhu, Zhuhai, China) or GnRHa 0.1 mg was used to trigger final oocyte maturation between 9 and 10 PM, and the thawed embryos were transferred 5 days later. If serum LH was ≥20 IU/l and endometrial thickness was ≥ 7 mm, the same dose of hCG or GnRHa 0.1 mg was given in the afternoon and FET was performed 4 days later. If serum LH was ≥20 IU/l and endometrial thickness did not reach 8 mm, the cycle was cancelled. Two days before FET, 40 mg progesterone was injected every day.

*Embryo freezing and thawing*

On the second or third day after oocyte retrieval, fresh cleavage-stage embryos generated using IVF or ICSI were evaluated by an embryologist using a grading system based on the following 12: grade I: <10% focal fragments, stage-specific cell size, no vacuoles and multinucleation, four cells on day 2 or 6-9 cells on day 3; grade II: 10-25% fragments, stage-specific cell size in majority of cells, no vacuoles and multinucleation, four cells on day 2 or 6-9 cells on day 3; grade III: >25% focal fragments, cell size not stage specific, evidence of multinucleation or vacuoles, number of cells <4 on day 2 or <6 or >9 on day 3. Only good-quality embryos of grade I or II were cryopreserved according to a protocol described previously, using 1,2-propanediol and sucrose solution in phosphate-buffered saline as cryoprotectants.

13 For synchronization between embryo and endometrial development, 2nd-day embryos were thawed before the day of transfer, and 3rd-day embryos were thawed 2-4 h before transfer. Embryos were thawed in the morning following the manufacturer's instructions (Embryo Thaw Media kit, IrvineScientific, Santa Ana, CA, USA). Briefly, the cryotubes were removed from liquid nitrogen, exposed to room temperature for 40 s and then immersed in a water bath at 30°C for 40 s. The content of the cryotubes was expelled as a droplet in a sterile Petri dish, and the embryos were processed through a series of decreasing concentrations of propanediol and sucrose at room temperature. Then, embryos were transferred to fresh mHTF with 12 mg/ml HAS for 10 min at room temperature and 10 min at 37°C. Finally, the embryos were washed and maintained in fresh culture medium until the time of transfer. After 3 h of in vitro culture, the frozen-thawed embryos were evaluated by an experienced embryologist. Only the embryos that survived (minimum 50% survival rate of the original blastomeres) were used for transfer.

*Luteal phase support*

Luteal phase support was done by administration of 40 mg progesterone every day two days before FET. After pregnancy, progestin dosage was adjusted according to serum progesterone levels, and treatment was administered up until the tenth week of pregnancy.

**Table 1.** Baseline characteristics of patients in the study

| | GnRHa (n=136) | hCG (n=1414) | *P* Value |
|---|---|---|---|
| Age (year) | 31.62±3.9 | 31.56±4.4 | >0.05 |
| Duration of infertility (years) | 4.2±2.8 | 4.5±3.1 | >0.05 |
| No. of past ET failures | 1.45±0.86 | 1.43±0.78 | >0.05 |
| BMI (kg/m$^2$) | 21.36±3.0 | 21.6±2.9 | >0.05 |
| Basal FSH (IU/l) | 5.7±1.6 | 5.9±1.5 | >0.05 |
| Basal LH (IU/l) | 4.1±1.7 | 4.2±2.0 | >0.05 |

**Table 2.** Comparison of FET cycle clinical character between the two groups

| | GnRHa (n=96) | hCG (n=689) | *P* Value |
|---|---|---|---|
| No. of embryos transferred | 2.00±0.48 | 2.01±0.44 | >0.05 |
| Good quality embryo rate | 100% | 100% | >0.05 |
| Endometrial thickness (mm) | 10.77±2.2 | 10.61±2.8 | >0.05 |
| Serum peak oestradiol level | 350.34±52.5 | 290.89±49.89 | >0.05 |

**Table 3.** Comparison of FET cycle pregnancy outcomes between the two groups

| | GnRHa (n=96) | hCG (n=689) | *P* Value |
|---|---|---|---|
| Clinical pregnancy rate (%) | 53.12% (51/96) | 47.0% (342/689) | >0.05 |
| Abortion rate (%) | 15.68% (8/51) | 12.34% (40/342) | >0.05 |
| Live birth rates (%) | 43.75% (42/96) | 38.89% (268/689) | >0.05 |

*Outcome variables*

The primary outcomes analyzingd included age, duration of infertility, the serum LH and E2 levels, and the endometrial thickness on the day of trigger, clinical pregnancy rate, spontaneous abortion rate, and live-birth rate. Human chorionic gonadotropin serum levels were measured 14 days after embryo transplantation. HCG levels >25 IU/l were defined as biochemical pregnancies, and ultrasonography was performed on days 28-30 after embryo transplantation. Gestational sacs revealed by ultrasonography were defined as clinical pregnancies. A clinical pregnancy rate was calculated as the presence of fetal cardiac activity confirmed by transvaginal ultrasound per ET. Spontaneous abortion rate was defined as pregnancy loss after sonographic visualization of an intrauterine gestational sac per ET before 12 weeks' gestation. Live-birth rate was calculated as delivery of a viable infant after 27 weeks' gestation per ET.

*Statistical analysis*

Statistical Package for Social Sciences software (SPSS statistics 19.0; IBM, Armonk, NY, USA) was used for data analysis. Descriptive statistical analyses were performed for each variable; quantitative results are presentedas the mean ± SD. Means were compared using one-way ANOVA and two-sample t-tests. Proportions for the two groups were compared using the v2-test. P<0.05 was considered to indicate statistical significance.

## Results

**Table 1** Comparing the baseline characteristics between the GnRHa trigge group and hCG trigger group demonstrating that there were no statistically significant difference regarding mean age, BMI, duration infertility, Basal FSH, Basal LH as well as No. of past ET failures. Women's clinical characteristics including no. of embryos transferred, good quality embryo rate, endometrial thickness, serum peak oestradiol level were similar in both the groups (**Table 2**). **Table 3** shows the Clinical pregnancy rate (53.12% vs. 47.0%); abortion rate (15.68% vs.12.34%); live birth rates (43.75% vs.38.89%) were not significantly different between the GnRHa trigger group and hCG trigger group.

## Discussion

This study was performed to assess and compare the results of triggering ovulation by inducing endogenous LH surge (GnRHa) or (hCG) in Letrozole stimulation protocols for frozen-thawed embryo transfer. The observation showed that the baseline characteristics and clinical characteristics of patients were similar in both the groups. The serum peak oestradiol level of triggering day in the GnRHa triggering group was higher that in the hCG triggering group, that may be incline to choose GnRHa triggering avoiding OHSS (ovarian hyperstimulation syndrome). Regarding abortion rate that in the GnRHa group was higher than in the hCG group. The difference was seen but it was not statistically significant. But clinical pregnancy

rate and live birth rates that in the GnRHa group were higher than in the hCG group. It has not yet found similar research aboat using gonadotropin-releasing hormone agonist for oocyte triggering in endometrial preparation of Letrozole stimulation protocols for frozen-thawed embryo transfer.

Other studies on non-IVF cycles found similar pregnancy rates for hCG and GnRH agonist triggering. In a study carried out by Schmidt-Sarosi et al. [22] in which 26 infertile women stimulated by Clomiphene Citrate were randomly divided into 2 groups; 11 women received two doses of nafarelin (400 lg intra nasal) 16 h apart and 15 women received hCG (5000 IU, IM) for triggering of ovulation. Luteal phase support was started at day 6 of the luteal phase by 7 more doses of nafarelin (400 lg intra nasal) every 16 h for the nafarelin group and single dose of 2500 IU of the hCG for the hCG group. They reported that pregnancy rates were 27.27% vs. 13.33% respectively. Another study carried by Romeu et al. [23] in which 364 women were scheduled for IUI, assigned to 345 Hp FSH/GnRHa stimulated cycle and 416 Hp FSH/hCG stimulated cycle. The author reported that pregnancy rate per cycle was 27.25% in the GnRHa group and 17.32% in the hCG group respectively with p=0.007. The higher pregnancy rate observed in the GnRHa group may be attributed to better oocyte quality and endometrial receptivity, as an effect of a more physiologic endogenous surge of FSH and LH as well as steroid balance. Another study carried out by Lee et al. [24] in which 144 women prepared for IUI, GnRHa (0.1 mg SC) or hCG (5000 and 10.000 IU) was used for triggering of ovulation after stimulation by Clomiphene Citrate and hMG. They reported that pregnancy rates were 18.8% vs. 20.9% vs. 13.9% respectively, but the difference was not statistically significant. Badeea S. [25] compared triggering of ovulation by inducing endogenous LH surge (GnRHa) or using hCG in hMG stimulated cycles for intrauterine insemination (IUI). Found that the duration of the luteal phase was similar in both groups. Pregnancy rates per cycle were 17.61% for GnRHa and 13.06% for hCG respectively (P=0.23). In this study Luteal phase support was done by 1500 IU hCG, 12 h after the triggering of ovulation. Shalev E [26] compared gonadotrophin-releasing hormone agonist with human chorionic gonadotrophin for ovulation induction after clomiphene citrate treatment. Found that there were no significant differences in the mean number of pregnancies (12.0 versus 12.6% per cycle) and the abortion rate (18.2 versus 12.5%) between the GnRHa and HCG-treated groups respectively. There were no complications related to treatment in either group. The results show that a relatively low dose of GnRHa can be used in place of HCG to induce ovulation in clomiphene citrate-treated patients.

On the contrary, Rath and Sharma [27] carried out a prospective randomized comparative study in which 167 stimulated cycles in 66 women undergoing IUI were randomly allocated to receive hCG, nafarelin or triptorelin for triggering of ovulation. No luteal phase support was given. They reported comparable rates of ovulation among the three groups nevertheless; in the agonist group, pregnancy rates were slightly lower despite the fact that it was not statistically significant. While others found lower pregnancy rates in GnRHa group [28], but such differences could be related to smaller number of participants in these studies as well as different populations and interventions.

Good luteal phase support after GnRHa triggering is mandatory, as recent Cochrane review [29] on the topic does not recommend the routine use of GnRH agonist alone as a final oocyte trigger due to decreased ongoing pregnancy rates [30]. Many trials demonstrate that the use of standard luteal phase support after GnRHa trigger is inadequate and almost invariably results in lower conception rates. So many modified intensive luteal phase support appear.

But most of these trials aboat the role of GnRHa in triggering ovulation had heterogenous populations and mainly focused on patients receiving GnRH antagonist protocol prior to IVF/ICSI and interventions with variable doses tested. Also, conflicting results have been obtained.

Our studies show that using GnRHa for oocyte triggering in endometrial preparation of Letrozole stimulation protocols for frozen-thawed embryo transfer does not affect the clinical outcome compared with hCG under the same luteal phase support (injection 40 mg progesterone every day). Song-jun reported that it is an effective drug preparing the endometrium for FET. In order to avoid OHSS we

incline to choose GnRHa triggering between April 2008-December 2012. In that time we had not realize that GnRHa triggering result in corpus luteumdeficiency and a defective luteal phase. If GnRHa triggering affecting luteal functionin in the GnRH antagonists protocol is greater than the non-IVF cycles is not clear.

Our study had some limitations. Although it is a retrospective chart review, it provides clinicians with information aboat the routine use of GnRHa trigger protocol.

In our study, it is not clear that the frozen embryo from conventional long protocol or antagonists protocol or micro stimulation protocol. The quality of embryos may be associated with different ovulation induction protocol, that may be affect the judgment of results. Moreover, more studies on larger numbers of patients are needed to confirm the effect of GnRHa on defective luteal phase.

## Disclosure of conflict of interest

None.

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