Evaluation of GnRH antagonist pre-treatment before ovarian stimulation in a delayed-start antagonist protocol in normal ovulatory women undergoing IVF/ICSI: a randomized controlled trial (RCT)

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Research

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Abstract

Background: Synchronization of follicles is key to improving ovulation stimulation with the GnRH antagonist protocol. GnRH antagonist administration in the early follicular phase can quickly decrease gonadotrophin (Gn) levels and achieve downregulation before stimulation, which improves synchronization. A small RCT showed that pre-treatment with a GnRH antagonist for three days before stimulation might increase oocyte retrieval but cannot increase the pregnancy rate. This study investigated whether the "delayed-start" protocol in ovulatory women can increase the synchronization of follicles and pregnancy outcomes compared with the conventional GnRH antagonist protocol.

Methods: This RCT included 136 normal ovulatory women undergoing IVF/ICSI. Both groups were treated with recombinant FSH (r-FSH) and a flexible GnRH antagonist protocol. The women were randomized into two equal groups with or without GnRH antagonist from day 2 of the menstrual cycle. Our primary outcome was the number of oocytes retrieved. Secondary outcomes included the pregnancy and live birth rates.

Results: Both groups had similar baseline characteristics. The number of oocytes retrieved in the study group was comparable to that in the control group (9.5(8.0-13.0) vs. 11.0(7.0-14.8),P=0.469). There was no statistical significance among the follicular size differences. The fertilization rate, number of good-quality embryos, implantation rate, pregnancy rate, ongoing pregnancy rate, live birth rate per ET cycle, and miscarriage rate were all similar between the two groups.

Conclusion: This large RCT analysed the "delayed-start" GnRH antagonist protocol applied to normal ovulatory women performing IVF/ICSI. The number of oocytes retrieved and pregnancy outcomes did not vary significantly. Future trials need to confirm these findings.

Trial registration: Chinese Clinical Trial Registry, ChiCTR1800019730. Registered 26 November 2018,*http://www.chictr.org.cn/showproj.aspx?proj=29969.*

Background

With the development of the gonadotropin-releasing hormone (GnRH) antagonist protocol in theory and application, GnRH antagonists have become widely used tools for controlled ovarian stimulation (COS) cycles. The GnRH antagonist protocol is prevalent because of its advantages, including its shorter duration of stimulation, low Gn dosage, low cost, and low incidence of ovarian hyperstimulation syndrome (OHSS) [1–3]. The GnRH antagonist protocol has been recognized as practical and cost-effective for patients with high or poor ovarian response [4]. We have paid increasing attention to its application in normal responders. Meta-analyses [4] have shown similar live birth rates (LBRs) between the two protocols, so the 2019 European Society of Human Reproduction and Embryology (ESHR) guideline for ovarian stimulation [5] recommends that the GnRH antagonist protocol be used in normal responders. However, the literature used in this guideline includes few RCTs on the LBR, and it remains to be considered whether the outcome of the GnRH antagonist protocol is the same as that of the long

protocol. Additionally, whether the GnRH antagonist protocol is applicable or beneficial to women with normal response in terms of the clinical outcome has been controversial [3, 4].

Because the GnRH antagonist protocol lacks the process of pituitary downregulation [6], endogenous follicle-stimulating hormone (FSH) levels are not inhibited, and the transient increase in endogenous FSH during the luteal-follicular transition recruits early antral follicles, thereby affecting the synchronization of follicles [7, 8]. Addressing synchronization of follicular development is key to improving ovulation induction with the GnRH antagonist protocol. Previous studies have proposed various pre-treatment methods with little success in terms of solving the problem of asynchrony of follicles. Studies have shown that pre-treatment with oestradiol (E2) or progesterone (P) during the luteal phase does not affect the number of oocytes retrieved or the clinical pregnancy rate. There is insufficient evidence to prove that the use of these two pre-treatments can increase the LBR [9, 10]. Some studies have shown that pre-treatment with the oral contraceptive pill (OCP) in the previous menstrual cycle might reduce the ongoing pregnancy rate (OPR) and LBR of the GnRH antagonist protocol [10], so the routine use of oral hormone pre-treatment in an antagonist protocol is not recommended.

It is assumed that administration of the GnRH antagonist beginning at the early stage of follicle development can quickly reduce the Gn level and achieve immediate- and short-term pituitary downregulation before the start of Gn stimulation. It is conceptually similar to the long agonist protocol but maintains the advantages of the GnRH antagonist protocol. Based on this principle, pre-treatment with a GnRH antagonist in the early follicular phase may improve follicle synchronization [11]. One RCT [11] showed that for normal responders, pre-treatment with a GnRH antagonist for three days before the start of ovarian stimulation could increase the number of mature oocytes and the fertilization rate. Another RCT [12] showed that the number of oocytes after antagonist pre-treatment trended upward, though the increase was not statistically significant. The sample sizes of these two RCTs were both small, and the conclusions were inconsistent. Therefore, whether pre-treatment with a GnRH antagonist before initiation of ovarian stimulation in patients with normal ovarian reserve helps follicular synchronization and improves the pregnancy outcome remains to be studied with a larger sample size.

In this context, we increased the sample size and hypothesized that GnRH antagonist pre-treatment before the start of ovarian stimulation in the antagonist protocol can increase the synchronization of follicles, increase the number of oocytes retrieved and improve pregnancy outcomes. Our study aimed to determine whether three days of GnRH antagonist pre-treatment before Gn ovarian stimulation could increase the number of oocytes retrieved and improve the pregnancy outcomes of ovulatory women undergoing IVF/ICSI treatment.

Methods

Study Design

Between December 2018 and March 2020, we prospectively recruited 136 normal **ovulatory women** under 40 years of age who underwent their first or second IVF/ICSI cycles at the Reproductive Medicine and

Genetics Center of the People's Hospital of Guangxi Zhuang Autonomous Region. On day two of the menstrual cycle, we randomly assigned the eligible patients to either a delayed-start group (study group) or a conventional GnRH antagonist protocol group (control group), at a ratio of 1:1. All study participants used a flexible GnRH antagonist protocol (Figure 1).

Recruitment and Randomization

This was a prospective open randomized controlled trial (RCT). Since a placebo with the same dosage form and shape as a GnRH antagonist was not available, this trial was not suitable as a double-blind, which would include the oocyte retrievers, embryologists, statisticians, and hormone detection personnel involved in the study. With the random block design method, random numbers were generated from the Statistical Package for Social Sciences (SPSS, version 23.0) software and ordered from 1 to 136. The group was divided into 34 block groups, according to every four digits, in which one half was the study group and one half was the control group. We used a computer-generated random list for randomization. We made a series of consecutively numbered and opaque envelopes to seal the grouping details and hide them from the recruiting doctor. These envelopes were opened only when patients met the inclusion criteria.

We enrolled patients who met the group's inclusion criteria according to the sequence of the medical treatment time. All patients who participated signed informed consent forms, and each patient participated in this trial only once. We registered the RCT at the Chinese Clinical Trial Registry, the registration number is ChiCTR1800019730, and the ethics committee approved the trial of the People's Hospital of Guangxi Zhuang Autonomous Region.

Study Population

All consecutive women who underwent their first or second cycle of IVF/ICSI were included, and the first cycles comprised normal responders.

The study inclusion criteria were as follows: age <40 years, anti-Mullerian hormone (AMH) ≥1.2 ng/ml, antral follicle count (AFC) >7, regular regular menstrual cycles over the three months before the study (25-35 days in duration), and a basal serum FSH concentration lower than 12 IU/L.

The exclusion criteria were as follows: endometriosis grade III to IV (American Fertility Society classification of endometriosis [13]); adenomyosis; diagnosis of PCOS [14]; ovarian reserve function decrease (FSH>12 U/L or AFC<8 or AMH<1.1 ng/ml) or poor ovarian response [15], defined as less than four oocytes retrieved in a previous IVF or ICSI cycle; body mass index (BMI) >30 kg/m^2; male severe oligospermia or obstructive azoospermia and use of hormone therapy within the three months before the study.

Protocols

Baseline ultrasounds and serum sex hormone measurements were performed on menstrual cycle day two and after the completion of GnRH antagonist pre-treatment to note the absence of ovarian cysts or lead follicles >10 mm. In the conventional antagonist protocol (control group), ovarian stimulation with Gn was started on day 2 of the menstrual cycle. In the delayed-start protocol (study group), ovarian stimulation was started after three days of GnRH antagonist pre-treatment (Cetrotide®, 0.25 mg cetrorelix acetate, Serono, Inc.). In both protocols, 150-225 IU recombinant FSH (rFSH) (Gonal - F®, Serono Laboratories Ltd., Geneva, Switzerland) was used for ovarian stimulation. The dose of rFSH could be adjusted according to the patient's reaction conditions after ovarian stimulation for 3-4 days. In both groups, when follicle(s) ≥12 mm or luteinizing hormone (LH) > 10 IU/ml, the GnRH antagonist was given at 0.25 mg/day until the trigger day of human chorionic gonadotropin (HCG). When the diameter of two dominant follicles reached 18 mm or more or the diameter of three dominant follicles reached 17 mm, we triggered ovulation with 250 μg recombinant HCG (r-HCG) (Ovitrelle®, 250 μg/0.5 ml, Merck, Serono, Inc.). **Serum E2, LH**, and P levels were also be considered in the decision to trigger ovulation. Thirty-six to 38 hours after the trigger, specialized physicians retrieved the oocytes. Two embryologists were assigned to perform the oocyte examinations. The embryo was cultured until day three or day five and then transplanted by specialized physicians. The follow-up nurse recorded the results of follow-up and the reasons for losses.

In control group, ovarian stimulation with Gn was started on day 2 of the menstrual cycle. In study group, ovarian stimulation was started after three days of GnRH antagonist pre-treatment.

Outcome Measures

The number of oocytes retrieved was the primary outcome of our study. The secondary outcomes were the HCG positive rate, clinical pregnancy rate (CLR) per embryo transfer (ET) cycle (defined as the presence of one or more gestational sacs on transvaginal ultrasound, including an ectopic pregnancy) [16], OPR per ET cycle (a pregnancy beyond 12 weeks' gestation) and LBR per ET cycle (defined as the delivery of a live-born infant at ≥28 weeks of gestation) [17]. Furthermore, we evaluated the following secondary adverse safety and pregnancy outcomes among the two study groups: incidence of moderate-to-severe OHSS (according to the criteria proposed by Golan and Weissman (2009)) [18] and miscarriage rate defined as foetal loss before the 28th week of gestation [17].

Statistical Considerations

Sample Size Calculation

This was a prospective open RCT. We calculated the sample size using power analysis and sample size (PASS, version 11.0). We estimated the sample size based on the results of oocytes retrieved from one published RCT, the actual number of oocytes retrieved in our centre, a mean number of oocytes retrieved in the control group =7, a mean number of oocytes retrieved in the study group =10, and a standard

deviation= 5. According to the estimation of a sample size of 118 cases, per clinical experience, the estimated depigmentation rate is approximately 15% or 18 cases. (The transplant cancellation rate is approximately 15%.) To achieve 80% power using a 1:1 randomization ratio, each study group would require 68 subjects (136 patients in total).

Statistical Analysis

The data analysis report complies with the 2010 Consolidated Standards of Reporting Trials (CONSORT) clinical trial guidelines [19]. We used the IBM SPSS, version 23.0, software for statistical analysis. Normally distributed data are represented by the mean and standard deviation (SD), and skewed data are described as the median and interquartile range (IQR). We used the chi-square test or Fisher's exact test, where appropriate, to make statistical inferences on qualitative data. In contrast, we used the T-test or the Mann-Whitney test to compare continuous variables as required. A probability P value <0.05 indicated that the difference between the two groups was statistically significant.

Results

1. **Study flow-chart and baseline characteristics**

This trial included 136 infertile normal **ovulatory women** receiving their first of second cycles of IVF/ICSI. After randomization, there were 68 women in each group. The patient demographics and clinical characteristics are presented in Table 1. Baseline data, such as age and AFC, were similar between the two groups. Table 1 presents the ovulation stimulation parameters of both protocols. The stimulation lengths (8.6±1.2 vs. 8.8±1.6 days) and total Gn amounts (1813.6±398.2 vs. 1766.4±415.8 IU) were similar between the control and study groups .

Table 1 Main subject and cycle characteristics.

Note:*AMH* – anti-Mullerian hormone; *AFC* – antral follicle count; *BMI* – body mass index; *IQR* – inter quartile range; *SD* – standard deviation; *AIH*- artificial insemination with husband's semen

2. **Efficiency outcome measures and pregnancy outcomes**

There was also no statistically significant difference in the numbers of oocytes retrieved (11.0(7.0-14.8) vs. 9.5(8.0-13.0)), follicle output rate (FORT) [20] (80.4%(54.0%-100%) vs. 77.4%(53.5%-100%)), 2PN (pro-nuclei) oocytes retrieved (6.5(4.0-9.0) vs. 6.0(4.0-8.0)), good-quality embryos (2.0(1.0-4.0) vs. 2.0(1.0-4.0)) or frozen embryos (2.0(1.0-4.0) vs. 2.0(0.0-4.0)) between the control and studygroups (Table 2). The numbers of embryos transferred were similar between the two groups (Table 3). Although the implantation rate, HCG positive rate, clinical pregnancy rate, OPR, and live birth rate per ET cycle of the study group were lower than those of the control group, the differences were not statistically significant (Table 3).

Table 2 Stimulation characteristics and embryological data.

Note: Data are presented as median and inter quartile range(IQR).

[a] Control group used as reference .

[b] Follicle output rate determined by the ratio of the preovulatory follicle (14-22 mm) count on the day of HCG trigger× 100/the small antral follicle (3-8 mm) count at baseline.

[c]Good-quality embryos included day-3 and day-5/6 high-quality embryos (according to our centre's quality embryo scoring standards, day-3 embryos considered were 1-2 grade with 7-9 blastomeres and <20% fragmentation [21]; blastocysts considered were at least at expansion stage 3, had an inner cell mass score of A or B and had a trophectoderm score of A or B on day 5).

Table 3 Clinical Outcomes and complications.

Note:*ET*– embryo transfer;*SD* – standard deviation.

3. **Adverse safety and pregnancy outcome measures**

Adverse pregnancy outcomes, specifically biochemical pregnancy loss and clinical miscarriage, did not vary significantly between the two groups (Table 3). Moderate-to-severe OHSS also occurred at similar rates in the in the control and the study groups (1/68, 1.5%, vs 2/68, 2.9%, P=0.559).

4. **Changes in hormones at different points**

The endocrine baselines of the two groups were similar (An additional table file shows this in more detail [see Additional table 1]). Compared with those in the control group, the serum levels of FSH, E2, and P in the study group were significantly lower at the initiation of stimulation (P<0.05). On the day of the HCG trigger, the serum LH (P<0.001) and E2 (P=0.069) levels of the study group were higher than those of the control group, but the serum progesterone levels of the two groups were similar.

5. **Comparison of follicular development characteristics**

As expected, the follicle sizes and variation coefficients (VCs) [22] of the two groups were similar on the cycle starting day and the stimulation starting day (An additional table file shows this in more detail [see Additional table 2]). After starting ovarian stimulation, we observed that the average follicle size of the study group on the day of antagonist addition was significantly lower than that of the control group (P<0.05), but the two groups had a similar follicle variability, average follicle size ≥10 mm, and largest follicle size. On the day of the HCG trigger, the follicle size and VC of the two groups were similar.

Discussion

This study is currently the largest RCT of ovulatory women receiving the antagonist protocol comprising GnRH antagonist pre-treatment for three days before stimulation. The results showed that, compared with the traditional GnRH antagonist protocol, the delayed-start GnRH antagonist pre-treatment before Gn

initiation in the early follicular phase did not improve the synchronization of follicles of ovulatory women. The number of oocytes retrieved in the study group was comparable to that in the control group. There was no significant difference in pregnancy outcome.

Different FSH thresholds of the follicles are the main reason for the asynchrony of follicles [8]. GnRH antagonist use in the early follicular phase can reduce FSH and may prevent the early recruitment of follicles. This study used the maximum diameter, average diameter, and coefficient of variation of the follicles at each stage during COS to reflect follicle synchronization. The results showed that the diameter and variability of the antral follicles were the same. The maximum diameter, average diameter, and variability of the follicles on the day of antagonist addition and the day of HCG triggering were not different between the two groups. Clearly, GnRH antagonist pre-treatment in the early follicular phase did not achieve follicle synchronization, which is inconsistent with previous research results. One previous study [23] used antagonist pre-treatment in the luteal phase to narrow the difference in follicle size. Nevertheless, the effect of antagonist pre-treatment in the follicular phase on follicle size was not reported. In addition, we used the indicator of FORT to reflect the effect of follicle synchronization. The formula for calculating the FORT in this study is the number of follicles with a 14-22-mm diameter on the HCG trigger day divided by the number of follicles with a 3-8-mm diameter on the cycle entry day. The results showed that the FORT of the study group was slightly lower than that of the control group (77.4% (53.5%-100%) vs. 80.4% (54.0%-100%)), but the difference was not statistically significant, further indicating that the antagonist did not increase the synchronization of follicles after pre-treatment. One of the main indicators used to measure the synchronization of follicles is the number of oocytes retrieved. In this study, the number of oocytes retrieved and the number of mature oocytes in the study group were slightly lower than those in the control group (9.5(8.0–13.0)vs. 11.0(7.0-14.8)) (7.0(6.0–11.0) vs. 9 (5.3–12.0)), but the difference was not statistically significant. There was no statistically significant difference in the number of available embryos, frozen embryos, or good-quality embryos. This is consistent with previous research results. One RCT [12] by Blockeel et al. recruited 69 women with normal ovarian response. The number of oocytes retrieved after pre-treatment with GnRH antagonist for three days on the first 3 days of menstruation showed an increasing trend (12.8 ± 7.8 vs. 9.9 ± 4.9). There was also no significant difference. Veronique et al.'s case-control study [24] of women < 35 years old with normal ovarian response showed that the delayed start of pre-treatment with GnRH antagonist to three days after menstrual days 2–4 did not affect the number of oocytes retrieved.

The timing and length of the use of antagonists are inconsistent, so the studies' conclusions may be incompatible. The RCT [11] by Younis JS et al. of women under 39 years old with normal ovarian response showed that pre-treatment with an antagonist (0.25 mg GnRH antagonist per day for three consecutive days) on the first day of menstruation can increase the number of mature oocytes and normal fertilization rate. Nevertheless, there was no comparison of pregnancy outcomes in their study. The reason may be that FSH starts to rise on the first day of menstruation, and antagonists can prevent the early recruitment of follicles caused by the FSH rise. In our study, pre-treatment with an antagonist was used on the second day of menstruation. At this time, the follicles may have already been recruited. Even when using the antagonist, the follicle recruitment cannot be reversed, and the asynchrony of

follicles cannot be changed. Because most of our patients were far away and could not see the doctor on the first day of menstruation, the antagonist could not be used on the first day.

The timing of antagonist addition may also affect the outcome. The antagonist protocol has a fixed and flexible protocol. Our study used a flexible antagonist protocol. The maximum follicular diameters and E2 levels on the day of adding the antagonist in the two groups were 13.2 ± 1.9 vs. 13.8 ± 2.0 and 671.0(474.5–1000.0) vs. 781.0(530.8-1025.5) ng/L, respectively, and the differences between the two groups were not statistically significant (Additional Tables 1, 2), consistent with the method reported in the literature [25].

The patient's ovarian response to stimulation also affects the results of antagonist pre-treatment [26]. A meta-analysis of the delayed initiation of antagonist pre-treatment for low-response patients showed that this measure reduces the Gn dose and increases the clinical pregnancy rate. Studies have also found that pre-treatment with antagonists for PCOS patients can increase the biochemical pregnancy rate and significantly reduce the incidence of OHSS [27]. PCOS and poor responders were excluded from the patients enrolled in this study. The influence of different ovarian reactions on the results can be excluded, making the research more reliable.

In this study, the Gn stimulation days of the two groups were similar. Although the LH level of the study group was higher than that of the control group on the day of antagonist addition, the LH level increased (13.2% (9/68) vs. 7.4% (5/68)). The increase in P (1.5% (1/68) vs. 0) between the two groups was not statistically significant, which is consistent with the literature [28]. The E2 level (2529.0 (1642.0-3246.5) vs. 2230.0 (1432.0-3134.0) ng/L, $P = 0.069$) and LH level (3.5(2.5–6.1) vs. 2.1(1.1–3.5) IU/L, $P < 0.001$) on the HCG trigger day were higher in the treatment group than in the control group. The reason may be that even if pre-treatment with GnRH antagonist for three days did not achieve the purpose of inhibiting follicular recruitment, the follicles began to develop after delayed initiation of stimulation. At the time of the HCG trigger, the cycle is in the late follicular phase, and LH receptors are exclusively expressed on granulosa cells; higher LH levels might cause more oestrogen production [29]. However, the deleterious effect of high E2 levels on endometrial receptivity has been controversial [30]. There was no statistically significant difference in the clinical pregnancy rate and OPR of the two groups in our study, which is consistent with previous research showing similar findings [12] [31].

The ultimate goal of assisted reproductive technology (ART) is a live birth, namely, a healthy baby with one ovulation induction. Therefore, the LBR as a research indicator can better demonstrate the efficacy of ART. There is no analysis of LBR in previous studies. Therefore, we calculated the LBR in this study. The results showed that the difference in LBR between the two groups was not statistically significant. We can see that the pre-treatment did not affect the LBR. Additionally, we compared the incidence of moderate-to-severe OHSS between, and the results showed that the incidence of OHSS after pre-treatment did not increase.

In summary, the delayed start after pre-treatment with antagonists has no significant impact on the outcome of assisted pregnancy and does not increase the risk of OHSS. This approach can be used as a

flexible protocol.

Strengths And Limitations

Advantages

First, our research sample size is large. This study's sample size was calculated based on the number of oocytes retrieved in the previous literature and our actual average number of oocytes retrieved. After our calculation, the sample size was 132. The sample size of a similar previous study in 2011 was only 69. Our sample size significantly higher, with sufficient testing power, and the results are more convincing. Second, we examined additional indicators, such as the follicle diameter at each node, the follicle variation coefficient, and the follicular output rate, to reflect the synchronization of follicles from multiple angles, which is more objective than using only the number of oocytes retrieved. Third, we increased the LBR per transplant cycle to quantify ART outcomes, making the research more complete.

Limitations

First, because our patients could not see the doctor on the first day of menstruation, they could use GnRH antagonist pre-treatment only on the second day of menstruation. This is a limitation of our study. Second, the study group patients used the GnRH antagonist for three more days, but it neither increased the number of oocytes retrieved nor improved the pregnancy outcome. Instead, it increased the number of visits and treatment costs for these patients.

Due to the limited sample size and research time of our medical centre, this study's conclusions still have certain limitations. We look forward to a larger sample from a multicentre randomized controlled prospective trial to confirm this conclusion in the future. Alternatively, whether pre-treatment with a GnRH antagonist throughout ovarian stimulation improves the outcomes of patients with unsynchronized follicle development remains unclear.

Conclusion

For normal ovulatory women, pre-treatment with GnRH antagonist for the first three days before the start of ovarian stimulation in the early stage of follicle development neither increases follicular synchronization nor improves the clinical outcomes of IVF-ET.

Declarations

Ethics approval and consent to participate

The trial was approved by the ethics committee of the People's Hospital of Guangxi Zhuang Autonomous Region((Youth Fund of the Hospital)2018-01). We registered the trial with http://www.chictr.org.cn/showproj.aspx?proj=29969 and the registration number is ChiCTR1800019730.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors state no conflicts of competing interests in the study.

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Authors' contributions

Weihong Tan and Yisheng Zhang discovered the study concept and designed the study. Jie Qin and Hongyi Huang took part in patient enrolment, management and follow-up. Lintao Xue and Shikai Wang involved in embryological experiments. Yisheng Zhang collected the data for the analysis and performed the first draft of the manuscript. Liling Liu and Weihong Tan contributed to interpreting the data and provided critical revision for important content. All authors approved the final version published and agree on the role of the work.

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Abbreviations

A list of abbreviations shows this in more detail (see Additional file 3).

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Tables

Tables 1-3 are available as downloads in the Supplementary Files section.

Figures

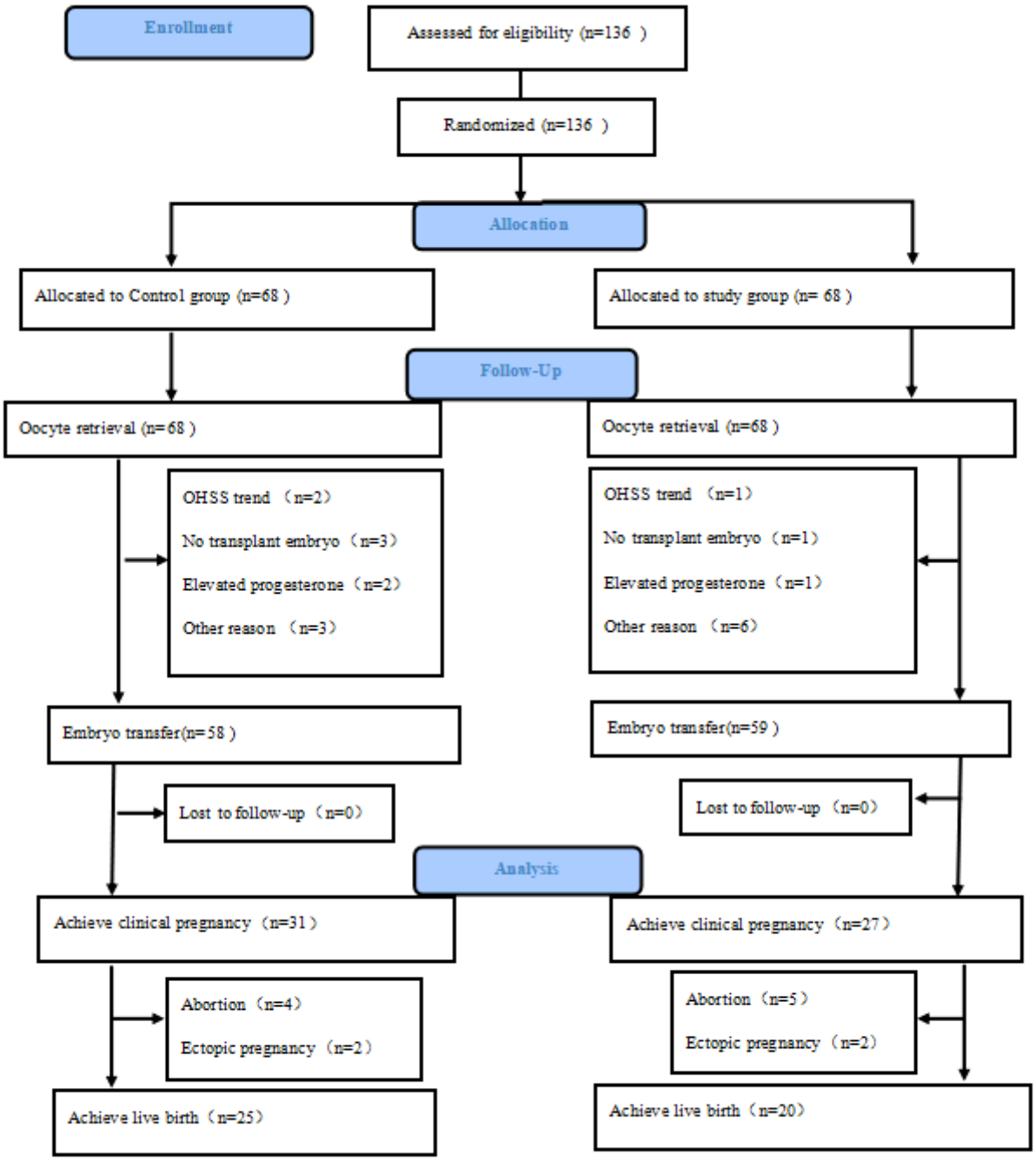

Figure 1

Flow diagram

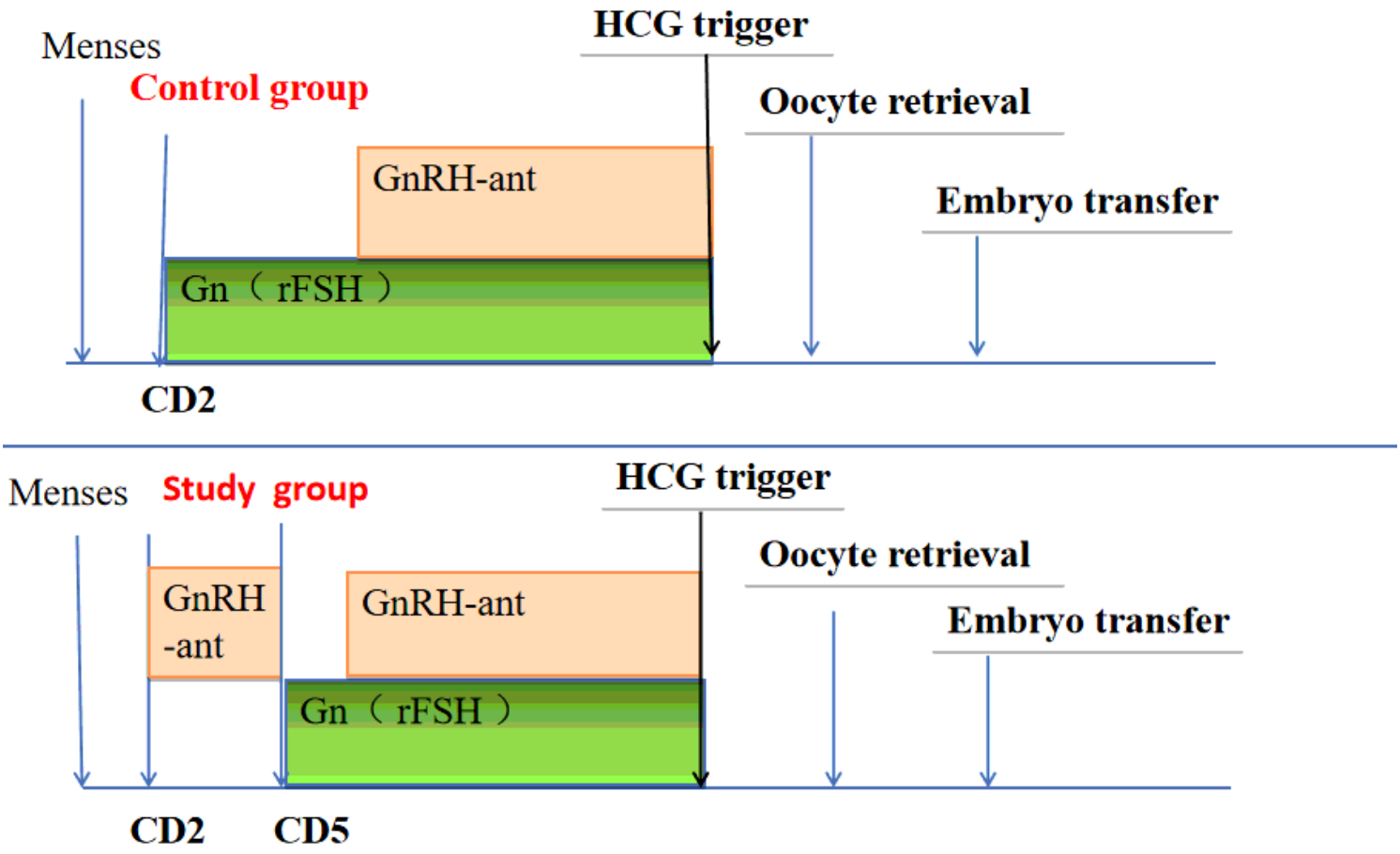

Figure 2

Protocols of the two groups Note:CD-menstrual cycle day;GnRH-ant-GnRH antagonist. In control group, ovarian stimulation with Gn was started on day 2 of the menstrual cycle. In study group, ovarian stimulation was started after three days of GnRH antagonist pre-treatment.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.xlsx
- Table2.xlsx
- Table3.xlsx
- Additionalfile1.xlsx
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