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Single-dose administration of a short-acting gonadotropin-releasing hormone agonist does not affect cycle outcome in frozen–thawed embryo transfer cycles

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Abstract

Objective: This prospective study aimed to assess the effect of short-acting gonadotropin-releasing hormone agonist (GnRHa) administration on pregnancy outcomes in frozen–thawed embryo transfer (FET) cycles.

Methods: Patients who planned to have FET in Peking Union Medical College Hospital (China) were recruited for this study and randomly assigned into two groups. Patients in the experimental group (n = 460) received triptorelin acetate on the day of embryo transfer along with routine luteal support. Patients in the control group (n = 433) only received luteal support. One dose (0.1 mg) of a short-acting GnRHa was administered on the day of blastocyte transfer. The rates for clinical pregnancy, biochemical pregnancy, implantation, miscarriage, and ectopic pregnancy were compared between the groups.

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Results: There were no significant differences in the number and quality of blastocytes transferred between the two groups. In the experimental and control groups, the clinical pregnancy rate was 56.3% and 50.58%, the biochemical pregnancy rate was 15.78% and 18.94%, and the median implantation rate was 39.98% and 38.01%, respectively, with no significant difference between the groups. Biochemical pregnancy and abortion and the ectopic pregnancy rate were not significantly different between the two groups.
Conclusion: In FET cycles, a GnRHa does not affect the pregnancy outcome.

Keywords
Gonadotropin-releasing hormone agonist, in vitro fertilization–embryo transfer, frozen–thawed cycle, blastocyte, pregnancy, abortion

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Introduction

A gonadotropin-releasing hormone agonist (GnRHa) can suppress release of luteinizing hormone (LH) from the pituitary, thereby preventing unexpected ovulation during *in vitro* fertilization/embryo transfer (IVF-ET). In 2004, GnRHa therapy was proposed as a new method of luteal support.[1–7] Ata *et al*.[3] conducted a study on women who received embryo transfer using a long GnRHa protocol and reported that 0.1 mg of triptorelin acetate alone did not appear to increase the rate of pregnancy. However, some research has indicated that in patients who receive luteal support through administration of a single dose of a GnRHa, improved pregnancy, implantation, and live birth rates may be observed.[4,8] These applications are relevant to fresh embryo transfers. However, the mechanism of a GnRHa in the luteal phase and determination of whether a GnRHa supports luteinization by inducing LH flares, or through its direct effects on the ovaries, endometrium, or embryos, remain unknown.

During fresh embryo transfer, many factors can interfere with pregnancy outcomes. These include the supraphysiological endocrine milieu associated with IVF, embryo quality, endometrial receptivity, and luteal phase support. Some studies have investigated frozen–thawed embryo transfer (FET) (artificial/natural) cycles to evaluate the effects of GnRHa.[4,9–11] One study showed that single use of GnRHa after blastocyte transfer in natural cycles (79 vs. 84 cases) may increase the pregnancy rate.[9] However, regardless of whether a GnRHa was used, no effect was observed on the pregnancy outcome in these medicated cycles (228 vs. 266 cases).

Patients undergoing FET with medicated cycles were included in the present open-label, prospective, randomized, controlled trial. We wished to determine whether a single dose of a GnRHa could affect pregnancy outcomes. The present study aimed to determine whether a GnRHa affected pregnancy outcomes by affecting embryo implantation and clinical pregnancy rates.

Methods

Patients

In this prospective, single-center, quasi-randomized (odd/even allocation), controlled trial, which was conducted from October 2016 to June 2018, patients who underwent FET in the Reproductive Center of Peking

Union Medical College Hospital (China) were recruited. All patients were subjected to artificial FET cycles.

This trial was approved by the Ethics Committee of Peking Union Medical College Hospital (No. ZS-1905) and registered at the Chinese Clinical Trial Register website (www.chictr.org.cn, ChiCTR1900023232). Each patient provided written consent for inclusion in the study. Subjects were randomized using a randomization table method into two groups. Patients in the experimental group received triptorelin acetate (GnRHa, 0.1 mg) on the day of embryo transfer plus routine luteal support. Patients in the control group only received routine luteal support. The study was double-blinded to the subjects and investigators, and followed the relevant guidelines in the Enhancing the Quality and Transparency of Health Research network guidelines and the Consolidated Standards for Reporting of Trials statement.

The present study was a superiority trial. We assumed that the pregnancy rate in the experimental group (patients who received a GnRHa) would be higher compared with that in the control group. Inclusion criteria for participants were as follows: patients aged 20 to 45 years and patients who had previously undergone IVF with either conventional insemination or intracytoplasmic sperm injection (ICSI) and who had at least one or two blastocyte-stage embryo(s) cryopreserved. The history of implantation failure was equal to or less than before times for each patient. Patients who had undergone FET cycles were included. Exclusion criteria were as follows: patients who experienced infertility for longer than 10 years; chromosomal abnormalities were detected from either the male or female partner; a known presence of hydrosalpinx, uterine malformations, or submucosal leiomyoma autoimmune disorders; a history of tuberculosis or any uncontrolled endocrine disorder that may affect pregnancy; and a history of endometrial hyperplasia.

IVF-ET and FET protocols

Controlled ovarian stimulation, oocyte retrieval, fertilization, and embryo transfer were carried out according to routine methods of the clinical center. Controlled ovarian stimulation was formulated on the basis of the estradiol (E2) level on the second day of menstruation and the condition of the ovarian antral follicles. The dose for follicle-stimulating hormone preparation was adjusted on the basis of follicular growth and the E2 response during monitoring. When two or more dominant follicles with a diameter $\geq$18 mm were observed by ultrasound, 250 μg of human chorionic gonadotropin (hCG, 250 μg/dose; Merck Serono, S.p.A., Modugno [BA], Italy) was subcutaneously administered to trigger the final maturation of oocytes. Ultrasound-guided oocyte retrieval was then performed after 36 to 37 hours. Semen washing was performed after oocyte retrieval by conventional gradient centrifugation, and the conventional IVF or ICSI approach was decided on the basis of the patient's sperm analysis findings. Fresh embryo transfer was conducted on the third day of embryo development. The remaining embryos continued to be cultured until day 5 or 6 for cryopreservation of embryos at the blastocyte stage. The blastocytes' quality score was based on the criteria in the Assisted Reproductive Technology guidelines.[11] FET with cryopreserved blastocyte transfer was planned for patients who did not become pregnant following fresh embryo transfer. Natural or artificial cycles were chosen for the FET cycles according to the patient's preference. Only patients who opted for the FET cycle were recruited for the present study.

Evaluation of pregnancy outcomes

Biochemical pregnancy was defined as a positive pregnancy test in the absence of

any ultrasonographic evidence of pregnancy and no evidence or treatment for ectopic pregnancy with declining hCG levels. The implantation rate was defined as the number of gestational sacs observed by transvaginal ultrasound divided by the number of embryos transferred.[12] The abortion rate was defined as patients with a positive hCG test and at least one intrauterine sac as a pregnancy loss at <20 weeks of clinical pregnancy. An ectopic pregnancy was evaluated as a verified ectopic pregnancy when diagnosed by sonography or laparoscopy (presence of an extrauterine gestational sac).

Statistical analysis

Sample size calculation. The clinical pregnancy rate was adopted as the primary outcome measure. Data from our center suggested that a clinical pregnancy with FET should be 50% in the control group. An absolute 10% increase from 50% in this measurement was required in the current study to detect a significant difference. With an alpha error level of 0.05 and a beta error level of 0.2, 408 patients needed to be included in each group. The Stata 12.0 software program (StataCorp., College Station, TX, USA) was used for this calculation.

Data analysis. IBM SPSS Statistics 21.0 software (IBM Corp., Armonk, NY, USA) was used to conduct data analysis. Categorical data are shown by case number (n), and statistical differences between the groups were tested using the χ^2 test. Measurement data are shown as mean ± standard deviation and comparison between the two groups was made by using the independent *t*-test. Numerical data that were not normally distributed are expressed as P50 (P25, P75) and were compared using the Mann–Whitney test. $P < 0.05$ was considered statistically significant.

Results

Consort flowchart

A consort flowchart for the process of selecting patients is shown in Figure 1. A total of 1070 patients who were scheduled for FET were invited to participate in the present study. One hundred fifty patients

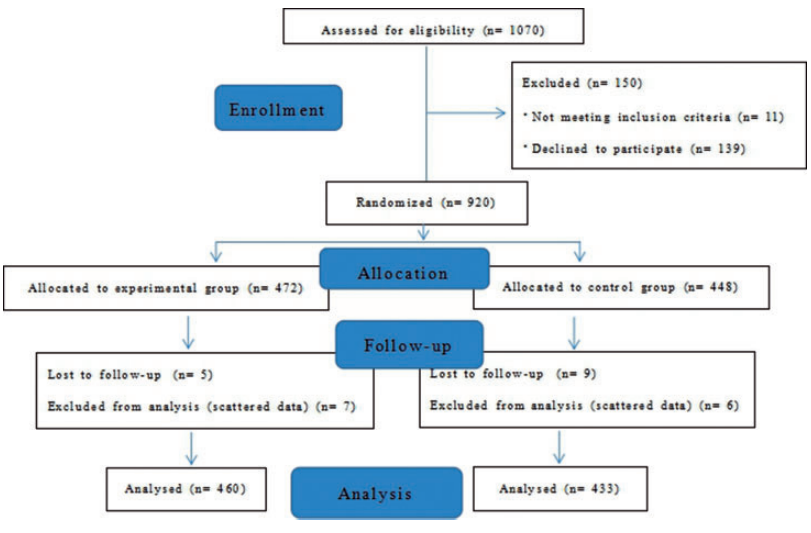

Figure 1. Consort flowchart for recruitment of candidates.

were excluded for either not meeting the inclusion criteria (n = 11) or declining to further participate in the study (n = 139). A total of 920 women were randomized into the two following groups: 472 women were assigned to the experimental group and 448 were assigned to the control group. Among these patients, six from the experimental group and nine from the control group dropped from the study and were considered lost to follow-up. Furthermore, seven patients from the experimental group and five patients from the control group left the study because of incomplete data. The data for the remaining 460 and 433 patients in the experimental and control groups, respectively, were used for statistical analysis.

Comparison of baseline data between the two groups

There were no significant differences in baseline criteria, such as age, years of infertility, the numbers of oocytes retrieved, optimal embryos, blastocytes, FETs, transferred embryos (1/2), and the type of transferred blastocyte, between the two groups (Table 1).

Comparison of pregnancy outcomes between the two groups

Comparison of pregnancy outcomes between the experimental and control groups is shown in Table 2. There was no significant difference in the clinical pregnancy rate, biochemical pregnancy rate, implantation rate, abortion rate, or ectopic pregnancy rate between the experimental and control groups.

Discussion

A GnRHa may act directly on endometrial GnRH receptors (GnRHRs) to improve

Table 1. Comparison of baseline data between the two groups.

Items	Experimental group (with triptorelin acetate, n = 460 patients)	Control group (without triptorelin acetate, n = 433 patients)	*P*
Age (years)	34.03 ± 4.12	33.88 ± 4.34	0.623*
Years of infertility	3.86 ± 2.43	4.24 ± 2.68	0.057*
BMI (kg/m^2)	21.78 ± 2.69	22.16 ± 3.16	0.154*
Basic FSH (IU/L)	6.74 ± 2.43	6.97 ± 2.42	0.096*
Basic E2 (ng/mL)	48.54 ± 31.39	45.51 ± 21.97	0.649*
E2 levels on the triggered date (ng/mL)	4114.22 ± 3114.65	4193.55 ± 1906.54	0.075*
Number of oocytes retrieved (n)	11.93 ± 4.88	12.53 ± 5.23	0.090*
Number of MII oocytes	10.72 ± 4.74	11.28 ± 5.05	0.900*
Number of optimal embryos	1.45 ± 1.77	1.46 ± 1.54	0.718*
Number of blastocytes	3.87 ± 3.24	3.95 ± 2.97	0.669*
Endometrial thickness on the day before FET (mm)	10.86 ± 2.12	11.02 ± 2.26	0.284*
Number of transferred embryos (1/2)	119/341	104/329	0.523*
Type of transferred blastocyte (optimal/not optimal)	312/148	301/132	0.587#

Values are number or mean ± standard deviation.

*t-test; #chi-square test.

The type of optimal blastocyst was scored on the basis of the shape of the blastocyst. Optimal included AA, AB, and BB, and was designated as 2, while others were classified as mediocre grade blastocysts and designated as 1.

BMI, body mass index; FSH, follicle-stimulating hormone; E2, estradiol; FET, frozen–thawed embryo transfer.

Table 2. Comparison of pregnancy outcomes between the two groups.

Outcome	Experimental (GnRHa) group (n = 460)	Control group (n = 433)	*P*
Clinical pregnancy rate	56.30 (259/460)	50.58 (219/433)	0.086
Biochemical pregnancy rate	15.87 (73/460)	18.94 (82/433)	0.230
Abortion rate	10.43 (48/460)	12.01 (52/433)	0.460
Ectopic pregnancy rate	0.43 (2/460)	0.69 (3/433)	0.947
Implantation rate	39.98 (321/803)	38.01 (290/763)	0.425

Values are % (n).
GnRHa, gonadotropin-releasing hormone agonist.

endometrial receptivity.[9,12] GnRHRs may play a role in early placental development[5] and directly affect the embryo.[3] GnRHa administration can potentially result in higher hCG levels,[9] reflecting its possible direct effect on embryos. Furthermore, a GnRHa may have a direct effect on the endometrium through GnRHRs.[5] The present prospective, randomized, controlled trial was conducted to determine whether single-dose administration of a GnRHa shows any of these effects, potentially through the endometrium or embryo itself, in terms of increasing the pregnancy rate in women undergoing FET. During FET cycles, endometrial development is the direct result of exogenous E2. Accordingly, the potential effect of luteal support of a GnRHa does not apply to the current study population. The present comparative study suggested that a GnRHa did not improve pregnancy outcome. Furthermore, there was no evidence to indicate that a GnRHa improved endometrial receptivity or affected embryos through GnRHRs.

The above-mentioned results appear to differ from those reported in previous studies. Li *et al.*[9] performed a retrospective comparative study of 657 FET cycles and found that a GnRHa significantly increased the pregnancy rate after blastocyte transfer in natural cycles, but not in medicated cycles. This finding is consistent with the present study. A meta-analysis of six studies was conducted to compare progesterone versus progesterone + GnRHa for luteal support after IVF.[5] This meta-analysis showed that the pregnancy outcome was better in the progesterone + GnRHa group compared with the progesterone only group. Furthermore, there were significant differences in the birth rate (0.40, 95% confidence interval: 0.26–0.61), clinical pregnancy rate (0.74, 0.60–0.90), sustained pregnancy rate (0.76, 0.60–0.97), abortion rate, and multiple pregnancy rate in favor addition of a GnRHa. However, this analysis was only relevant for fresh embryo transfers, and the effect of luteal support from a GnRHa may have been the primary mechanism of action with increased LH secretion. These published studies suggest that a GnRHa can improve pregnancy outcomes by promoting the corpus luteum. However, the present study appears to rule out the putative direct endometrial and embryonic effects of a GnRHa because no differences were detected in the studied pregnancy outcomes following administration of single-dose GnRHa on the day of FET in medicated cycles. Therefore, a GnRHa in this setting may not improve endometrial receptivity or quality of the embryo.

The present study has some limitations. Because the research was conducted with the aim of achieving a 10% difference, the observed 6% difference in favor of experimental intervention must be verified by additional research. LH levels on the day before and after single-dose GnRHa administration

were not assessed. Therefore, LH dynamics were not determined in the present medicated FET protocol. Additionally, the present study was an open-label trial because a placebo injection was not used in the control group, which may have affected the research findings. The clinical pregnancy rate was chosen as the primary outcome measure (rather than live birth), despite being able to report abortion rates. Therefore, the present study can still be relevant to ongoing assessment of pregnancy. Additionally, this was a single-center study with a set protocol. Consequently, our findings may not be relevant to other centers or different FET protocols. Additionally, we examined artificial or natural cycles for patients before FET, but did not address the distribution principle of the two methods in the experimental and control groups. The different protocols may have had an effect on the experimental results. We also did not perform a sample size calculation. The present study's main strength is that a large number of patients were included.

Conclusion

The present study suggests that GnRHa administration plays a role in luteal support, primarily by supporting functioning of the corpus luteum, rather than by improving endometrial receptivity or imposing direct effects on embryos. Therefore, use of a single dose of a GnRHa on the day of embryo transfer in medicated FET cycles is not recommended. However, based on the present data, administration of a GnRHa does not introduce any additional risks.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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