The effectiveness and safety of the early follicular phase full-dose down-regulation protocol for controlled ovarian hyperstimulation: a randomized, paralleled controlled, multicenter trial

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Background

Since the first “tube baby”, Louise Brown, was born in the United Kingdom in 1978, many infertile couples have been benefitted from in vitro fertilization and embryo transfer (IVF-ET) and intracytoplasmic sperm injection (ICSI). It is reported that there are over 5 million babies born with the help of assisted reproductive technology (ART). According to the 2015 national data published by Human Fertility and Embryology Authority (HFEA, 48,147 women received 61,726 IVF/ICSI cycles and gave birth to 17,041 newborns [1]. In the United States, 169,602 IVF/ICSI cycles were performed in 2014 and 68,791 tubal babies were born [2]. China has a huge population base, and therefore has a substantial number of infertile couples. Although a late starter, China is developing rapidly in ART and playing a more and more important role in the area of reproductive medicine.

In spite of the continuous development in ART, so far, the overall success rate of IVF/ICSI is still hovering around 25-40%. The live birth rate per stimulated cycle is 25.6% in the UK in 2015, fluctuating from 1.9% in women aged 45 and elder to 32.2% in women younger than 35 years old [1]. The IVF/ICSI success rate in 2014 in the US is similar [2]. In China, according to the data submitted by 115 reproductive medicine centers on the ART data reporting system developed by Chinese Society of Reproductive Medicine, the delivery rate is about 40% [3]. Hence, there is much room for improvement regarding the live birth rate of IVF/ICSI, which is of great significance to infertile couples.

There are many factors influencing the success rate of IVF/ICSI, e.g. the infertile couples’ age, the controlled ovarian hyperstimulation (COH) protocol, the quality and number of embryos for transferring, the endometrium and luteal phase support protocol, etc. Among them, an appropriate COH protocol is directly associated with the number of oocyte retrieved, as well as the number and quality of embryos, which exert an important influence on the success rate of IVF/ICSI. The luteal phase pituitary down-regulation protocol is one of the most widely used COH protocols in clinical practice, particularly in China. In this protocol, gonadotropin releasing hormone agonist (GnRHa) administered in the previous luteal phase induces a state of down regulation of the pituitary gland via competitive occupying and further exhausting the GnRH receptors in the pituitary, which inhibits the endogenous luteinizing hormone (LH) peak and avoid spontaneous ovulation, decreasing the cycle cancellation rate. But the classic down-regulation protocol may lead to an increased incidence of ovarian hyperstimulation syndrome (OHSS), as well as a negative impact on endometrial receptivity due to the progesterone elevation after multiple oocytes development [4]. The coping strategy is to freeze all the embryos and transfer in the next cycle. Though avoiding the above-mentioned adverse effects, such strategy increases the time to pregnancy (TTP) and therefore results in certain psychological and economic burdens for infertile couples.

In recent years, some Chinese researches applied the early follicular full-dose down-regulation protocol that is always performed to women with endometriosis to a more general IVF/ICSI population and found a clinical pregnancy rate of 64% in the fresh embryo transfer cycle, much higher than that of the luteal phase down-regulation protocol [5-10]. The possible mechanism is that it may improve the down regulation of LH and the endometrial receptivity, therefore having a

better control of LH during COH, increasing the endometrial thickness on hCG day, as well as the embryo implantation rate and clinical pregnancy rate. Furthermore, since this protocol decrease the risk of progesterone elevation on hCG day, it increases the fresh embryo transfer rate and shortens TTP.

Given most studies regarding the effectiveness and safety of the early follicular phase full-dose down-regulation protocol are retrospective studies, the results may be biased by several confounding factors. Therefore, we would like to conduct a multicenter, randomized controlled trial to compare the pregnancy outcome and safety between the early follicular phase full-dose down-regulation COH protocol and the luteal phase down-regulation protocol.

Methods/Design

We will perform the multicenter, randomized controlled trial in 14 reproductive medicine centers all over mainland China. Included patients will be distributed in a 1:1 ratio to the intervention (early follicular phase full-dose down-regulation) group and the control (mid-luteal phase down-regulation) group. Their live birth rate of fresh embryo transfer and other secondary outcomes will be compared. This study has been approved by the institutional review boards at all study sites. Each participant will sign the consent form before inclusion. Reporting of the study results will follow the 2010 CONSORT statement [11].

Inclusion criteria

The following are the inclusion criteria for this study:

1. Women aged between 20 to 38 years old and with a history of infertility (fail to get pregnant after over one year's regular, unprotected sex), who receive IVF/ICSI for one of the following reasons:
   ① Tubal factor: e.g. peritubal adhesions, tubal obstruction, etc.. Patients with hydrosalpinx can be enrolled after salpingectomy or tubal ligation;
   ② Male factor: e.g. oligospermia, asthenozoospermia, teratozoospermia, etc.;
   ③ Unexplained infertility: patients with a history of infertility more than 1 year but with no specific cause for infertility (ovulation, tubal, endometrial and male factor), or still not get pregnant after the above-mentioned causes being removed.
2. Women with a normal ovarian reserve according to: ① basal steroid hormone on day 2-4 of menstrual cycle: basal FSH≤10mIU/ml, estradiol (E2) <50pg/ml; ② anti-Müllerian hormone (AMH)≥1.1ng/ml；③ antral follicle count (AFC) ≥7;
3. Women who start their first IVF/ICSI cycle;
4. Women who met the above-mentioned criteria and have given the informed consent.

Exclusion criteria

1. Women with a negative reproductive history, including a history of:
   ① recurrent miscarriage: women with twice and more than twice spontaneous miscarriage, missed abortion, biochemical pregnancies, etc.;
   ② fetal malformation or chromosomal abnormalities;

③ intrauterine death.

2. Women with a history of one side adnexectomy;
3. Women with a poor ovarian response or diminished ovarian reserve (based on Bologna' criteria);
4. Women with ovulation dysfunction;
5. Women with PCOS (based on Rotterdam's criteria);
6. Women with endometriosis;
7. Women with the following uterine abnormalities: uterine malformation (unicornuate uterus, uterus bicornis, uterus duplex, mediastinum uterus), adenomyosis, submucosa myoma, intrauterine adhesion;
8. Chromosomal abnormality for either or both of the couple;
9. Women with contraindications for ART or pregnancy: uncontrolled diabetes mellitus, cardiac disease, undiagnosed liver and/or renal function, vaginal bleeding, suspected or a past history of cervical cancer, endometrial cancer, breast cancer, and a history of deep venous thrombosis, pulmonary embolism, stroke, etc.;
10. Women who are enrolled in other clinical trials.

Randomization

After the evaluation, patients met the eligible criteria will be informed, sign the consent form and be included in this study. We will randomly assign women (1:1) to the two groups, using a central randomization system with block sizes of 4 to 6 (changing constantly) and setting hospital as a stratification factor. The researchers (physicians, nurses and embryologists) and patients are not blinded due to the nature of both interventions while the data analysts are blinded.

Interventions

Pituitary down-regulation and controlled ovarian hyperstimulation(COH)

1. *Pituitary down-regulation and starting dose determination*

   After inclusion and randomization, qualified participants will be in either intervention or control group and receive one of the following treatments：

1) *Intervention group* (early follicular phase full-dose down-regulation group)

   Patients have an injection of 3.75mg long-acting triptorelin acetate (Diphereline®, IPSEN, France) on the 1^{st}-4^{th} day of menstrual cycle and receive the endocrinology and ultrasound examinations 28-42 days thereafter. If complete pituitary down-regulation is achieved (endometrial thickness≤5mm, basal FSH≦5mIU/mL, LH≦5mIU/mL, E2≦50pg/mL) and the diameter of follicles between 4-6mm under ultrasound, a supplementation of recombinant FSH (Gonal-F®, Merck, Switzerland) will be given according to the participants' BMI (18≤BMI≤22, starting dose 112.5IU/d; 22＜BMI≤25, starting dose 150IU/d.

2) *Control group* (luteal phase long down-regulation group)

   Patients have an injection of short-acting triptorelin acetate (Decapeptyl®, Ferring, Germany) 0.1mg every day, 10-12 days before the menstruation and receive the endocrinology and ultrasound examinations 14-21 days thereafter. If complete pituitary down-regulation is achieved (endometrial thickness≤5mm, basal FSH≦5mIU/mL, LH≦5mIU/mL, E2≦50pg/mL) and the diameter of follicles between 4-6mm under ultrasound,

a supplementation of recombinant FSH (Gonal-F®, Merck, Switzerland) will be given according to the participants' BMI (18≤BMI≤22, starting dose 112.5IU/d; 22＜BMI≤25, starting dose 150IU/d).

2. *Ovarian response monitoring and gonadotropin dose adjustment:* 4 days after the daily injection of rFSH, the physician will perform ultrasound to monitor the follicular growth, measure the serum hormone level and adjust the dose of exogenous gonadotropins accordingly.

Trigger and oocyte retrieval

1. *hCG trigger for final oocyte maturation:* When the desired follicle size is reached (at least one follicle ≥ 19mm or two follicles ≥ 18mm or three follicles ≧ 17mm), 250 μg recombinant human chorionic gonadotropin (HCG) (Luveris®, Merck, Switzerland) will be administered subcutaneously.
2. *Oocyte retrieval*: Oocyte retrieval will be performed transvaginally 36-38 hours after pre-ovulatory hCG injection under ultrasound monitoring.
3. *In vitro fertilization and embryo culture:* Oocyte retrieved will be cultured in vitro for 3-6h before being fertilized via IVF or ICSI. Two top-quality Day 3 cleavage embryos will be transferred 72h after retrieval. If more than 15 oocytes are retrieved, the participant should receive ultrasound examination on the day of embryo transfer. For those complaining abdominal or stomach distension, with an ovary≥7cm in diameter, estradiol ≧4500 pg/ml, there is a high risk of developing moderate or severe OHSS, and all the embryos should be frozen.

Embryo transfer and luteal phase support

Patients will receive luteal phase support with intramuscular progesterone (60mg/day) since the day of oocyte retrieval. On day 3 after oocyte retrieval, two top quality cleavage-stage embryos will be transferred via a catheter under transabdominal ultrasound guidance. The patients will lie in bed for 15 minutes after the procedure. After embryo transfer, 8% progesterone sustained-releasing vaginal gel (Crinone®, Merck, Switzerland) 90mg and dydrogesterone (Duphaston®, AbbottBiologicals, Netherlands) 20mg every day will be used as luteal phase support, continuing for at least 2 weeks. For those who get pregnancy, luteal phase support will be continued to 10 weeks of gestation. The dosage adjustment will be determined by physicians of each study site according to their personal experience.

Pregnancy evaluation and follow-up

All the information will be recorded in our follow-up forms designed specifically for each follow-up visit.

1. *Biochemical determination:* 14±4 days after embryo transfer, serum β-hCG will be tested to determine pregnancy.
2. *Clinical pregnancy determination*: 28±4 days after embryo transfer, a transvaginal ultrasound scan will be performed to confirm the presence of fetal sac, yolk sac and fetal heart.
3. *Ongoing pregnancy determination*: A transvaginal ultrasound scan will be performed at 10-12 weeks of gestation to confirm ongoing pregnancy. The presence of first-trimester pregnancy complications (e.g. OHSS, ectopic pregnancy, miscarriage etc.) will be registered according to the participant's medical record and a telephone follow-up.
4. *28 weeks of gestation*: A telephone follow-up will be conducted to collect the information regarding the second-trimester complications, e.g. pre-eclampsia, gestational diabetes,

prenatal diagnosis, miscarriage, etc.

5. 37 weeks of gestation: A telephone follow-up will be conducted to collect the information regarding the third-trimester complications, e.g. pre-eclampsia, gestational diabetes, preterm birth, etc.
6. *Delivery information:* A telephone follow-up will be made to collect delivery information including gestational age, delivery mode, delivery complications and infant information such as birth weight, any birth defect, etc.
7. *Postpartum information:* Postpartum information includes complications of both the mother and the infant, which will be collected 6 weeks after delivery.

For those not receiving a fresh embryo transfer (e.g. due to OHSS), and those not achieving live birth and with surplus cryopreserved embryos, the outcome of frozen-thawed embryo transfer cycles will be followed up and recorded as well.

Outcome measurements

Primary outcome

The primary outcome of this study is live birth rate per transfered cycle, defined as delivery of any viable infant ≥28 gestational weeks divided by all transferred cycles.

Secondary outcomes

Secondary effectiveness endpoints will include implantation rate, biochemical pregnancy, clinical pregnancy and ongoing pregnancy. *Biochemical pregnancy* will be achieved if serum β-hCG ≥ 10U/L. *Clinical pregnancy* is defined as the presence of an intrauterine gestational sac 28±4 days after embryo transfer. Ongoing pregnancy is defined as a viable pregnancy at 10-12 gestational week.

The safety endpoints will include moderate and severe OHSS, pregnancy loss, pregnancy complications, neonatal birth weight and adverse fetal outcomes (e.g. malformation, intrauterine fetal death, etc.)

Statistical analysis

Sample size and power calculation

Sample size will be calculated based on superiority test. The significance level will be set at α =0.05 and the statistical power will be set as 1- β =0.80. The reported live birth rate is about 30%, while the live birth rate of the prolonged protocol is predicted to be around 40% based on previous retrospective studies. We set the delta value as 6%, and the ratio between groups will be 1:1; therefore, the sample size of each group is 851. Taking into consideration a drop rate of 10%, we expect to ultimately have a total of 1892 enrollees, with 946 participants in each group.

Statistical analysis method

1. Statistical description: We will adopt descriptive statistical analysis to summarize the participants' demographic characteristics, physical examination, laboratory tests, other health information and the outcome indicators. The continuous variables will be described with means and standard deviation (or median and interquartile range), while the categorical variable will be described with frequency and percentage. The proportion of drop-off cases will be calculated and analyzed.
2. Comparable analysis It will be used to compare the demographic and other baseline characteristics to make sure the comparability of the two groups. If the continuous variables follow the normal distribution, t test will be performed; if not, the rank sum

test or a normal transformation will be performed. For the categorical variables, chi-square test or Fisher's exact test will be performed.

3. Statistical analysis for the outcome measures Between-group differences for categorical and continuous outcome variables will be assessed by logistic regression and linear regression, respectively.
4. Safety analysis: Adverse events and reaction will be listed and analyzed using the chi-square test.
5. The analysis for the primary outcome and important outcome measurements will be based on both the intention-to-treat analysis and per-protocol analysis. The results of these two analyses will be compared in the final analysis.

Reference

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Figure 1. CONSORT diagram