Luteal-phase ovarian stimulation is feasible for producing competent oocytes in women undergoing in vitro fertilization/intracytoplasmic sperm injection treatment, with optimal pregnancy outcomes in frozen-thawed embryo transfer cycles

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Objective: To explore the feasibility of luteal-phase ovarian stimulation using hMG and letrozole in terms of ovarian response and pregnancy outcome using frozen-thawed embryo transfer.
Design: A prospective cohort study.
Setting: Academic tertiary-care medical center.
Patient(s): Two hundred forty-two female patients undergoing IVF/intracytoplasmic sperm injection (ICSI) treatment.
Intervention(s): Ovarian stimulation was initiated with hMG 225 IU and letrozole 2.5 mg daily after spontaneous ovulation. Letrozole administration was stopped when the dominant follicles reached diameters of 12 mm. Ovulation was induced with a GnRH agonist 100 μg when at least three follicles reached diameters of 18 mm or one dominant follicle reached 20 mm. The highest quality embryos were extracted and cryopreserved for later transfer.
Main Outcome Measure(s): The primary outcome measured was the number of oocytes retrieved. Secondary outcomes were the clinical pregnancy rate, ongoing pregnancy rate, and implantation rate after frozen embryo transfer (FET) cycles.
Result(s): Of the 242 women enrolled in the study, all participants succeeded in producing oocytes and 227 women had highest-quality embryos to cryopreserve. The average number of oocytes retrieved was 13.1, producing an average of 4.8 highest quality embryos. Moreover, no cases experienced a premature LH surge or moderate/severe ovarian hyperstimulation syndrome during the stimulation cycles. In FETs, the clinical pregnancy rate, ongoing pregnancy rate, and implantation rate were 55.46% (127/229), 48.91% (112/229), and 40.37% (174/431), respectively. Of all the pregnancies in the study, 68 resulted in live births and 44 were ongoing.
Conclusion(s): Luteal-phase ovarian stimulation is feasible for producing competent oocytes/embryos in women undergoing IVF/ICSI treatments, with optimal pregnancy outcomes in FET cycles. (Fertil Steril® 2014;101:105–11. ©2014 by American Society for Reproductive Medicine.)
Key Words: Ovulation induction, luteal phase, human menopausal gonadotropin, letrozole, frozen embryo transfer, LH surge, OHSS

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Ovarian follicles undergo dynamic morphological and endocrinological changes during the human menstrual cycle. The traditional process of folliculogenesis involves the recruitment of various antral follicles in each ovary during the late luteal phase of the preceding menstrual cycle. Then, during the initial or middle stage of the follicular phase, a single follicle is selected, while the others undergo atresia (1, 2). However, some studies have demonstrated that small antral follicles observed during the luteal phase may not necessarily be in atresia but may rather be in the early stages of follicular development (3, 4). This indicates waves of follicular development within a single interovulatory period, with the presence of healthy follicles in the luteal phase as determined by oocyte and granulosa cell viability (3, 4). Luteal-phase in vitro maturation provides sound evidence that the oocytes retrieved during the luteal phase can be competent to mature and be fertilized (5, 6). These observations suggest the possibility that follicles are continuously available for stimulation by gonadotropins during the menstrual cycle. However, standard regimens of ovarian stimulation are started during the early follicular phase of the menstrual cycle. One of the disadvantages of follicular-phase ovarian stimulation is that with the development of multiple follicles stimulated by exogenous gonadotropin, a premature LH surge is sometimes elicited. This is due to the positive feedback of high E_2 on the pituitary and results in premature luteinization and suboptimal oocyte quality. Therefore, GnRH analog cotreatment is believed to be necessary to prevent the premature LH surge that occurs in the current practices, but this regimen makes the stimulation complex (7). Another detriment of ovarian stimulation is the serious complications caused by ovarian hyperstimulation syndrome (OHSS), a rare but potentially life-threatening condition.

Few studies consider the possibility of performing ovarian stimulation during the luteal phase of the menstrual cycle except for a small number of case reports on cancer patients (8–10). In the context of fertility preservation, luteal-phase administration of FSH and a GnRH antagonist was reported to produce mature oocytes/embryos for cryopreservation (8–10), but no data on pregnancy outcomes were available in the limited samples. This study is not generalized to all infertile women to evaluate the efficacy of ovarian stimulation in the luteal phase.

We drew inspiration from a case that initiated ovarian stimulation with hMG and letrozole during the luteal phase, with a satisfactory ovarian response and a pregnancy outcome. So we extended the concept to a routine IVF setting that can be used independently of menstruation. Given the asynchrony of the endometrium and embryo in such settings, all fertilized oocytes had to be cryopreserved for a later transfer.

This study aimed to explore the efficacy of initiation of ovarian stimulation in the luteal phase using hMG and letrozole in terms of ovarian response characteristics and pregnancy outcomes of frozen embryo transfers (FETs).

MATERIALS AND METHODS

Study Setting and Patients

A prospective cohort study was conducted at the Department of Assisted Reproduction of the Ninth People's Hospital of Shanghai Jiaotong University School of Medicine. Women undergoing IVF/intracytoplasmic sperm injection (ICSI) regimens for the treatment of infertility were recruited between July 2011 and September 2012. The study protocol was approved by the Ethics Committee (Institutional Review Board) of the Ninth People's Hospital of Shanghai. The trial was conducted according to the Declaration of Helsinki for medical research. All participants provided informed consent after counseling for infertility treatments and routine IVF procedures.

Patients planning to undergo IVF/ICSI treatments were eligible to participate. The inclusion criteria were women aged 20–38 years with a body mass index (BMI) of 18–30 kg/m^2, spontaneous ovulation, infertility caused by tubal factor infertility or male factor infertility, or unexplained infertility. The definition of spontaneous ovulation included at least one of the following criteria: an elevated LH level in the urine as measured by a urine test, the presence of collapsed follicles in an ultrasound examination, or an increase in the serum P level to 2.0 ng/mL or higher. Study exclusion criteria were [1] clinically significant systemic disease such as renal failure, [2] documented ovarian failure or basal FSH value >15 IU/L, [3] endometriosis grade 3 or higher, [4] subjects who had previous unsuccessful IVF/ICSI treatments, [5] any contraindications to ovarian stimulation treatment, and [6], for subjects prepared to undergo luteal-phase stimulation, largest antral follicle diameter of no more than 8 mm in 1–3 days after ovulation, as evidenced by ultrasound exam. Given that this research was exploratory, we planned to include 200 women.

Procedures

All participants were asked to prevent getting pregnant by using mechanical contraception or refraining from intercourse during their periovulatory phase. Participants were required to test their urine using an LH kit beginning on cycle day 10. When the LH surge indicator line appeared, they came to the clinic for an ultrasound and a serum hormone test. These ultrasound scans, along with serum P concentration testing, helped detect spontaneous ovulation. For the patients with follicles of <8 mm on 1–3 days remaining after ovulation, an hMG (Anhui Fengyuan Pharmaceutical Co.) 225 IU IM injection and letrozole (Jiangsu Hengrui Medicine Co.) 2.5 mg were administered every day; weekly follow-up visits were conducted. For monitoring, a transvaginal ultrasound examination was performed to record the number of developing follicles, and serum FSH, LH, E_2, and P_4 concentrations were measured. Letrozole administration was stopped when the dominant follicles each reached diameters of 12 mm. Daily administration of medroxyprogesterone acetate (MPA) 10 mg was added to the treatment regimen for cases in which on day 12 postovulation follicle size was smaller than 14 mm and stimulation needed to continue for several more days. This was done to postpone menstruation and avoid oocyte retrieval during menstruation, to prevent the risk of infection from the procedure. The final stage of oocyte maturation was induced with triptorelin (Decapeptyl, Ferring GmbH) 100 μg, injected when at least three follicles reached diameters of 18

mm or one dominant follicle reached 20 mm as measured by ultrasound. Transvaginal ultrasound–guided oocyte retrieval was conducted 32–36 hours later.

Fertilization was carried out in vitro, by either conventional insemination or ICSI, depending on semen parameters. Embryos were examined for the number and regularity of blastomeres and the degree of embryonic fragmentation and graded according to Cummins's criteria (11). All highest quality embryos (including grade 1 and grade 2 8-cell blastomere embryos) were frozen by vitrification on the third day after oocyte retrieval. The embryos that were not of top quality were placed in extended culture until the blastocyst stage. At this stage, on day 5 or day 6, only good-morphology blastocysts were frozen. The procedure for freezing and thawing cleavage-stage embryos and blastocysts has been described elsewhere (12).

Endometrial preparation and FET were performed in either a natural cycle or a stimulation cycle as described elsewhere (13). In a natural or stimulated cycle, the transfer time that depends on hCG priming is more accurate than a spontaneous LH surge that varies by individual in duration and configuration.

Our method of embryo and endometrium synchronization in FET was as follows: for natural FET cycles, we monitored follicular growth by serum hormones and ultrasound from cycle day 10. When the diameter of the dominant follicle was >16 mm and endometrial thickness was >8 mm, with E_2 >150 pg/mL and P <1.0 ng/mL, one of two procedures was performed depending upon the LH value. If LH was <20 IU/L, hCG 10,000 IU was administrated at night (21:00) to trigger ovulation and the transfer of the 3-day-old embryos was arranged for 5 days later. If the LH value was >20 IU/L, hCG 10,000 IU was injected the same afternoon and the transfer time was conducted 4 days later. The transfer of blastocysts was arranged for the sixth or seventh day depending on serum hormones and ultrasound results. Duphaston (Abbott Biologicals B.V., America) 40 mg/day was used for luteal support beginning on the third day after hCG injection.

For some cases with irregular menstrual cycles, we used letrozole and, if necessary, hMG to stimulate monofollicular growth. The common method used was as follows: letrozole 2.5–5 mg was administered from cycle day 3 to 7, and then follicle growth was monitored beginning on day 10. At times treatment included a low dose of hMG (75 IU/day) to stimulate follicular and endometrial lining growth. Administration of 10,000 IU of hCG and the timing of FET were performed according to the above criteria.

For patients with thin endometria during either natural cycles or stimulation cycles, hormone therapy was recommended for endometrial preparation, specifically, oral E_2 (ethinylestradiol; Shanghai Xinyi Pharma) 75 μg/day from cycle day 3 onwards. Once the endometrial lining was >8 mm thick, Femoston (Solvay Pharmaceuticals B.V.) 8 mg/day was started. The time of thaw/transfer was determined on the third day after Femoston administration. The maximum number of transferred embryos was two per patient. When pregnancy was achieved, the P supplement was continued until 8 weeks of gestation.

Statistical Analysis

The primary outcome measure was the number of oocytes retrieved. The secondary measures included the clinical pregnancy rate, ongoing pregnancy rate, and FET implantation rate. Clinical pregnancy was defined as the presence of a gestational sac with fetal heart activity during ultrasound examination 7 weeks after ET. The implantation rate was defined as the number of gestational sacs divided by the number of embryos transferred. The miscarriage rate was defined as the proportion of patients with spontaneous termination of pregnancy.

In the table presented in this study, data are presented as the mean ± SD, and in Figure 1, the hormone profile is presented as the mean ± SEM. Data were analyzed by the one-way analysis of variance method, using Bonferroni and Dunnett's test where appropriate. $P<.05$ was considered statistically significant. All data were analyzed using the Statistical Package for the Social Sciences for Windows (ver. 16.0, SPSS Inc.).

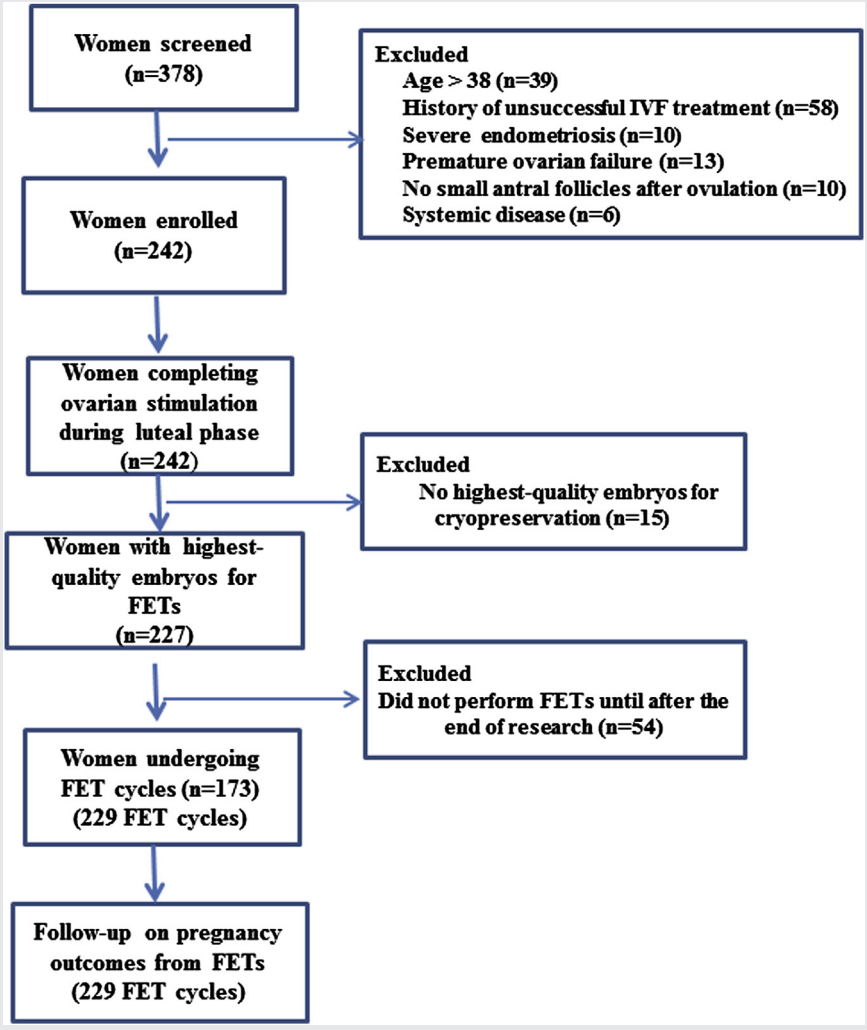
RESULTS

Three hundred seventy-eight women were screened for the study. A total of 242 women were selected, enrolled, and treated according to study protocol. The remaining 136 women were rejected in accordance with predefined study exclusion criteria. All participants succeeded in producing oocytes, with a range of 1–44. Two hundred twenty-seven women (93.8%) had highest-quality embryos to cryopreserve, while 15 patients were excluded from the study because they did not produce highest-quality embryos. Of the 227 women, 173 (71.5%) completed a total of 229 FETs by the end of the research period (March 2013), including 119 women who underwent one FET, 52 women who completed two FETs, and two women who finished three FET cycles. The remaining 54 women did not complete their FET cycles before the end of the study (Fig. 1). Sixty-eight women completed postnatal outcome follow-up after FET.

The mean patient age in the study was 30.8 ± 3.6 years, with minimum and maximum ages of 21 and 38 years, respectively. The mean BMI was 21.2 ± 2.9. The mean duration of patient infertility was 4.0 ± 2.9 years. In terms of the cause of infertility, primary infertility accounted for 48.4% of the cases (117/242) and secondary infertility accounted for 51.6% of the cases (125/242).

The following data detail the medication administration, cycle characteristics of ovulation induction during the luteal phase, and clinical outcomes. The mean duration of hMG stimulation was 10.2 ± 1.6 days, with a mean dose of 2,211.3 ± 422.7 IU. The mean duration of letrozole administration was 8.3 ± 2.1 days, with a mean dose of 21.8 ± 8.1 mg. A total of 189 women were administrated MPA, with a mean duration of 3.7 ± 3.1 days and a mean dose of 36.8 ± 31.4 mg. The mean number of antral follicles before luteal-phase ovarian stimulation was 11.4 ± 5.5. The mean number of follicles with diameters larger than 10 mm on the day of ovulation triggering was 13.9 ± 7.8. The mean number of follicles with diameters larger than 14 mm on the day of ovulation triggering was 11.1 ± 5.5. The mean number of oocytes

FIGURE 1



The flowchart of the study.

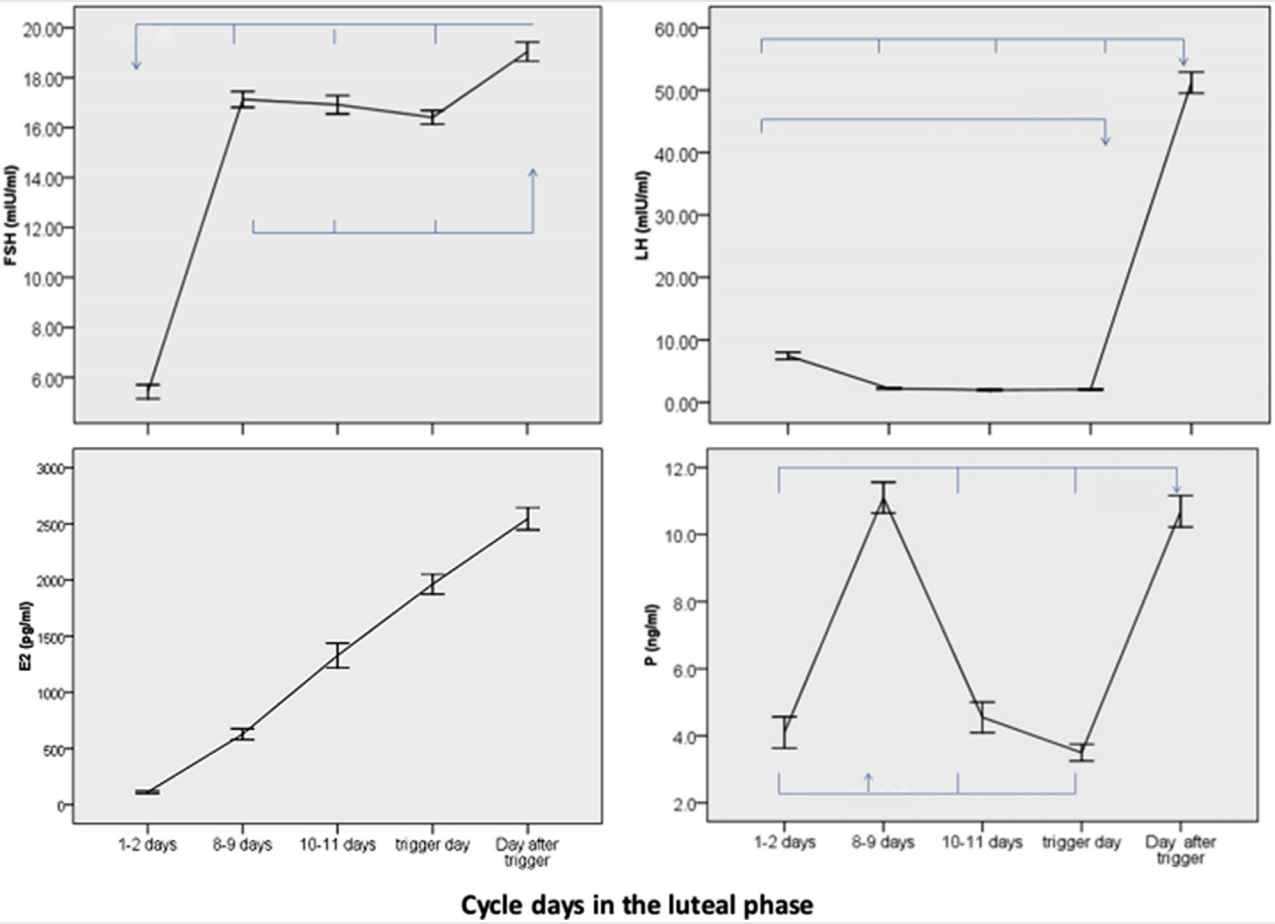
Kuang. Luteal-phase ovarian stimulation for IVF. Fertil Steril 2014.

retrieved was 13.1 ± 8.5, including 11.2 ± 7.2 mature oocytes. There is a positive correlation between the number of antral follicles and both oocytes retrieved and mature oocytes ($r = 0.681$ and $r = 0.663$, respectively, $P<.01$). The oocyte retrieval rate, fertilization rate, and cleavage rates were 65.5%, 69.59%, and 87.83%, respectively. The mean number of fertilized oocytes and cleaved embryos were 8.3 ± 6.0 and 7.4 ± 5.7, respectively. The mean number of highest quality embryos was 4.8 ± 4.1. The mean number of blastocysts on day 5/6 was 1.4 ± 2.1, and the mean number of cryopreserved embryos and blastocysts was 6.2 ± 5.7. In addition, no patients experienced moderate or severe OHSS during the study.

The associations among circulating concentrations of FSH, LH, E_2, and P in women undergoing ovarian stimulation during the luteal phase are presented in Figure 2. The FSH values increased after hMG administration ($P<.05$). The serum E_2 values showed a gradual increase accompanying the growth of follicles ($P<.05$). The P level reached its peak on the ninth day (11.1 ng/mL) and then decreased to 4.55 ng/mL on the trigger day ($P<.05$). The P level increased again the day after ovulation triggering. There was a decreasing trend in serum LH levels during early and middle luteal phases; the significant difference was found on days 1–2 compared with the LH values on trigger days ($P<.05$). The mean LH value was 2.07 mIU/mL on the trigger day, with a range of 0.1 to 11.0 mIU/mL; the LH value increased dramatically on the day after triggering ($P<.05$).

FET pregnancy outcomes are presented in Table 1. The mean number of frozen-thawed embryos was 1.9 ± 0.3, and the thawed embryo survival rate was 96.0%. Two hundred twenty-nine FET cycles were completed, and a total of 431 embryos were transferred, including 412 highest quality embryos. The clinical pregnancy rate was 55.5% (127/229). The implantation rate was 40.4% (174/431). Ten women were reported to have miscarried in the first trimester (7.9%), and one woman's pregnancy ended in miscarriage at a gestational age

FIGURE 2



Serum hormone profiles are present during ovarian stimulation in the luteal phase. The mean ± SEM values show the temporal associations among circulating concentrations of FSH, LH, E_2, and P in women undergoing ovarian stimulation during the luteal phase (n = 242). The *arrows* on the graphs indicate significant differences in the comparisons of different cycle days, post hoc ($P<.05$). The comparisons between every two groups are significant with respect to E_2 values during the ovulation induction in the luteal phase ($P<.05$).

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of 18 weeks (0.8%). Four women experienced ectopic pregnancies (3.2%). The ongoing pregnancy rate was 48.9% (112/229). The cumulative pregnancy rate per cycle initiated was 64.7% (112/173). The cumulative pregnancy rate was the total number of pregnancies divided by the total number of patients who underwent FET. A total of 173 women underwent a total of 229 FET cycles, including 119 patients who completed one FET, 52 who finished two FETs, and two women who underwent three FET cycles. The FET cycle details are shown in Figure 1. Of the unsuccessful cases, 33 women each had one to six cryopreserved embryos available (an average of 2.8), for a maximum possible 45 FETs.

Of all pregnancies, 68 women had live births and 44 had ongoing pregnancies by the end of the research period. Delivery follow-up showed 30 twin births and 38 single births; no malformations were reported in the newborns. The mean newborn birth weight was 2,819 ± 541 g (range, 1,500–4,150 g), and the mean birth height was 48.6 ± 2.8 cm (range, 40–56 cm). All newborns were healthy during follow-up except one twin baby, who, at 2 weeks of age, was hospitalized to treat a neonatal gastric perforation.

Of the 68 pregnancies, 63 women had live births from their first FETs, including 29 twin births and 34 single births; and five women delivered after their second FETs, including one twin birth and four single births. The mean birth weight of first FET cycle newborns was 2,804 ± 542 g (range, 1,500–4,150 g), and the mean birth height was 48.6 ± 2.8 cm (range, 40–56 cm).

DISCUSSION

This is the first clinical study using ovarian stimulation with hMG and letrozole during the luteal phase for women undergoing IVF/ICSI treatment. The efficacy of this novel protocol is promising. The mean number of oocytes retrieved was 13.1, producing a mean of 4.8 highest quality embryos. The clinical pregnancy rate, ongoing pregnancy rate, and implantation rate of FETs were 55.46% (127/229), 48.91% (112/229), and

TABLE 1

Pregnancy outcomes from frozen-thawed embryos originating from ovarian stimulation during the luteal phase.

	First FETs	Second and third FETs	Total FETs
No. of thawed cycles	173	56	229
No. of thawed embryos	2.0 ± 0.3	1.9 ± 0.3	1.9 ± 0.3
Thawed survival rate, %	97.4 (333/342)	91.6 (98/107)	96.0 (431/449)
No. of thawed highest-quality embryos	1.8 ± 0.4	1.7 ± 0.4	1.8 ± 0.4
Endometrial thickness, mm	12.4 ± 2.6	12.1 ± 2.9	12.3 ± 2.7
Pregnancy outcome of FET			
Positive hCG rate per transfer, %	63.0 (109/173)	58.9 (33/56)	62.0 (142/229)
Clinical pregnancy rate per transfer, %	59.0 (102/173)	55.6 (25/56)	55.5 (127/229)
Implantation rate, %	43.9 (145/330)	28.7 (29/101)	40.4 (174/431)
First trimester miscarriage rate, %	4.9 (5/102)	25.0 (5/25)	7.9 (10/127)
Second trimester miscarriage, %	1.0 (1/102)	0.0 (0/25)	0.8 (1/127)
Multiple pregnancy rate, %	42.2 (43/102)	16.0 (4/25)	37.0 (47/127)
Ectopic pregnancy rate, %	3.9 (4/102)	0.0 (0/25)	3.2 (4/127)
Ongoing pregnancy rate per transfer, %	53.2 (92/173)	35.7 (20/56)	48.9 (112/229)
Cumulative pregnancy rate per cycle initiated, %			64.7 (112/173)

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40.37% (174/431), respectively. No cases incurred a premature LH surge or moderate/severe OHSS. The results provided evidence of a breakthrough in overcoming the limitations in the current ovulation induction protocols that are used during the menstrual cycle and provide a new approach to follicular recruitment. The method can also serve as an emergency procedure for newly diagnosed cancer patients who wish to preserve fertility, with stimulation to be performed immediately upon diagnosis.

Ovarian follicles undergo dynamic morphological and endocrinological changes during human menstrual cycles. In this study, ovarian stimulation during the luteal phase resulted in lower serum LH concentrations on the day of ovulation triggering; no cases incurred a premature LH surge. In contrast, a high occurrence of premature LH surges (20%–25%) was reported during the cycles with hMG administration during the follicular phase; the consequent premature luteinization resulted in suboptimal oocyte quality and a high cancellation rate (14). Our study provided sound evidence of luteal-phase LH suppression and the need to artificially induce an LH surge by a GnRH agonist. The phenomenon, which is related to the absence of a spontaneous LH surge in women undergoing luteal-phase ovarian stimulation, will have far-reaching significance in removing the scruples of blocking an endogenous LH surge for IVF treatment. It primarily simplifies ovarian stimulation protocols and makes it easy to handle procedure monitoring. Determining the potential mechanism of LH suppression awaits further research. Corpus luteum secretions, including steroidal and nonsteroidal substances (such as inhibins, activins, and follistatin), may play a specific role in regulating LH release in luteal-phase ovarian stimulation (15).

The fluctuating P_4 levels directly reflect the functions of the corpus luteum during the stimulation process. The first peak of P occurred during the middle of the luteal phase. Then the corpus luteum receded, as shown by the decreased P level. MPA was used during the late luteal phase to support endometrial development and postpone menstruation, in order to avoid ovum pick-up during menstruation (and prevent the risk of infection from the procedure). These results showed that the existing high P level had no negative impact on oocyte/embryo quality. One of the preconditions in our study was targeting antral follicles no larger than 8 mm in diameter during the early luteal phase, so we inferred that the small antral follicles had strong potential to produce competent oocytes in the context of gonadotropin stimulation during the luteal phase. The larger follicles with diameters greater than 10 mm after spontaneous ovulation with abundant FSH/LH receptors (16) may be luteinized during the stimulation process, and it was difficult to obtain viable oocytes/embryos from them.

Because of the asynchrony between the endometrium and embryo in this study, all fertilized embryos had to be cryopreserved for a later transfer. Using vitrification, the quality of the frozen-thawed embryos was confirmed and the thawed embryo survival rate had the potential to reach over 96% in our study. The safety of FET was proved to be similar to that of fresh embryo transfer (17). The high implantation rate in FET cycles was associated with superior endometrial receptivity in the relatively normal hormone environment (18). No patients in this study reported moderate or severe OHSS. Even of the women who produced more than 20 oocytes ($n = 38$), no one complained of abdominal distension, nausea, or vomiting during ovarian stimulation. There were two reasons why OHSS did not occur, namely, the GnRH agonist used to trigger ovulation lowered the risk of OHSS compared with hCG (7) and FET reduced late-onset OHSS. We speculate that other factors related to the role of the corpus luteum may also be associated with the low incidence of OHSS; however, any benefit of this protocol in such a context needs to be further investigated.

Letrozole is a potentially important factor in this study. Letrozole helps increase follicular growth by inhibiting the

aromatization of androgens into estrogens, which releases the hypothalamic-pituitary axis from negative estrogenic feedback, whereas the increase of intraovarian androgens enhances early follicular growth and results in improved IVF outcomes (19). In a preliminary trial of our study, we had attempted to perform ovarian stimulation in the luteal phase with only hMG and found that the stimulation duration was very long (20.6 days) with low efficiency. The adjuvant administration of letrozole appeared to increase the sensitivity of follicles to gonadotropins and reduce the stimulation duration, but the specific mechanism should be further investigated.

In our study, hMG was consistently administered at a dose of 225 IU. Owing to the scarce evidence about the responsiveness of follicles to exogenous FSH during the luteal phase, we chose a relatively generous dose of hMG to increase the probability of reaching the FSH threshold window of dominant follicle selection, resulting in satisfactory effects. However, more clinical trials are necessary to determine the optimal dose for luteal-phase ovarian stimulation.

Another obvious weakness in this study is that there was no control group. The comparison of ovulation induction characteristics between the luteal phase and the follicular phase should be further investigated by well-designed prospective randomized controlled clinical trials. At the same time, the study points to many interesting opportunities in follicular development during ovulation induction of the luteal phase that await exploration, especially the enigma of no spontaneous LH surge. In addition, further research is required to determine whether this method can be successfully applied to patients of advanced maternal age or low-responder patients.

In conclusion, this prospective study demonstrated the feasibility of initiating ovarian stimulation during the luteal phase with hMG and letrozole to produce competent oocytes/embryos, with optimal pregnancy outcomes from subsequent FET. The study extended the concept of ovarian induction to a routine IVF setting that can be used regardless of menstruation. The new approach will be promising for patients undergoing emergency fertility preservation.

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