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Double stimulations during the follicular and luteal phases of poor responders in IVF/ICSI programmes (Shanghai protocol)




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Professor Yanping Kuang received his PhD from Fudan University School of Medicine in 1993. He has worked in the area of assisted reproductive technologies since 1995, and is presently director of the Department of Assisted Reproduction in the Ninth Hospital of Shanghai Jiaotong University School of Medicine. Professor Kuang has promoted a freeze-all policy since 2011, and has established a new approach of luteal-phase ovarian stimulation. He is also one of pioneers in performing natural cycle and mild IVF, and vitrification cryopreservation.

Abstract Previous studies have shown that existing antral follicles in the luteal phase enable ovarian stimulation. In a pilot study, the efficacy of double stimulations during the follicular and luteal phases in women with poor ovarian response was explored (defined according to the Bologna criteria). Thirty-eight women began with mild ovarian stimulation. After the first oocyte retrieval, human menopausal gonadotrophin and letrozole were administered to stimulate follicle development, and oocyte retrieval was carried out a second time when dominant follicles had matured. The primary outcome measured was the number of oocytes retrieved: stage one 1.7 ± 1.0 ; stage two 3.5 ± 3.2 . From the double stimulation, 167 oocytes were collected and 26 out of 38 (68.4%) succeeded in producing one to six viable embryos cryopreserved for later transfer. Twenty-one women underwent 23 cryopreserved embryo transfers, resulting in 13 clinical pregnancies. The study shows that double ovarian stimulations in the same menstrual cycle provide more opportunities for retrieving oocytes in poor responders. The stimulation can start in the luteal phase resulting in retrieval of more oocytes in a short period of time. This offers new hope for women with poor ovarian response and newly diagnosed cancer patients needing fertility preservation. 

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KEYWORDS: double stimulations, luteal-phase ovarian stimulation, mild stimulation, poor ovarian response

Introduction

Poor ovarian response (POR) during stimulation for IVF is a challenging and frustrating condition for patients and clinicians. The incidence of patients who respond poorly is estimated to be about 5–24% (Loutradis et al., 2003; Ubaldi et al., 2005; Ferraretti et al., 2011; Polyzos and Devroey, 2011). The cause of poor ovarian response may be associated with diminished ovarian reserve (Loutradis et al., 2007; Ferraretti et al., 2011). Various treatment protocols with high doses of gonadotrophins, along with various adjuvant drug therapies, were used to improve ovarian response and pregnancy rates (Loutradis et al., 2003; Ubaldi et al., 2005; Loutradis et al., 2007; Ferraretti et al., 2011; Polyzos and Devroey, 2011). Although standard definitions for POR are lacking, the European Society for Human Reproduction and Embryology has proposed that at least two of the following three features must be present in poor ovarian response: advanced maternal age or any other risk factor for poor ovarian response; previous poor ovarian response; or an abnormal ovarian reserve test (Bologna Criteria) (Ferraretti et al., 2011).

Recently, mild ovarian stimulation during a gonadotrophin-releasing hormone (GnRH) agonist cotreatment cycle using a low dose of exogenous gonadotrophins, ovarian stimulation for IVF using oral compounds (e.g. anti-oestrogens or aromatase inhibitors), or both, have been proposed as cost-effective, patient-friendly regimens that optimize the balance between outcomes and risks of treatment (Fauser et al., 2010; Mohsen and El Din, 2013; Pelinck et al., 2006; Yoo et al., 2011). For patients with POR, mild ovarian stimulation resulted in IVF outcomes similar to conventional ovarian stimulation, and even yielded slightly better pregnancy rates than conventional ovarian stimulation in poor responders older than 37 years (Yoo et al., 2011).

The classic IVF procedure starts with ovarian stimulation in the early follicular phase and retrieves oocytes when follicles are mature. Luteal-phase stimulation was originally used to produce mature oocytes and embryos for cryopreservation in case reports of emergency fertility preservation (Bedoschi et al., 2010; Sönmezer et al., 2011). In the present study, antral follicles in the luteal phase had similar development potential, with potential pregnancy outcomes in subsequent cryopreserved embryo transfers (Kuang et al., 2013). The efficacy of luteal-phase ovarian stimulation in patients with poor ovarian response after follicular phase stimulation, however, has not yet been examined. We therefore hypothesized that more oocytes and embryos could be obtained by continuing ovarian stimulation after the first oocyte retrieval from the mild stimulation cycle. This is a pilot study that was designed to investigate the efficacy of double stimulations during both the follicular and luteal phases in patients with poor ovarian response undergoing IVF and intracytoplasmic sperm injection (ICSI) treatments.

Materials and methods

Study setting and patients

A pilot 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–ICSI regimens for the treatment of infertility were recruited between July 2012 and June 2013. The study protocol was approved by the Ethics Committee (Institutional Review Board) of the Ninth People's Hospital of Shanghai on 9th May 2012 (IRB reference number: 2012-80; Trial registration number: ChiCTROCH-13003992; (<http://www.chictr.org/cn/proj/show.aspx?proj=6192>)). The trial was conducted according to the Declaration of Helsinki for medical research. All participants provided informed consent after counselling for infertility treatments and routine IVF procedures.

Patients planning to undergo IVF–ICSI treatments were eligible for participation in the study. In order to participate, patients had to meet at least two of the following criteria: age over 40 years; a history of ovarian surgery; previous treatment using conventional protocols that yielded less than three oocytes; antral follicle count of less than 5 on menstrual cycle day 2–3; and basal serum FSH concentration between 10 and 19 IU/L.

Study exclusion criteria were documented ovarian failure including basal FSH above 20 IU/L or no antral follicle by ultrasound examination; endometriosis grade 3 or higher; or any contraindications to ovarian stimulation treatment.

Procedures

Stage one of treatment protocol

Patients were screened by transvaginal ultrasound and serum FSH testing on day 3 of their menstrual cycle. Clomiphene citrate (Fertilan; Codal-Synto Ltd., France) 25 mg/day cotreatment and letrozole (Jiangsu Hengrui Medicine Co., China) 2.5 mg/day were given from cycle day 3 onwards. Letrozole was only given for 4 days and clomiphene citrate was continuously used before the trigger day. Patients started to inject human menopausal gonadotrophin (HMG) 150 IU (Anhui Fengyuan Pharmaceutical Co., China) every other day beginning on cycle day 6. Follicular monitoring started on cycle day 10 and was carried out every 2–4 days using a transvaginal ultrasound examination to record the number of developing follicles and serum FSH, LH, oestradiol and progesterone concentrations.

When one or two dominant follicles reached 18 mm in diameter, the final stage of oocyte maturation was induced with triptorelin (Decapeptyl; Ferring GmbH, Germany) 100 µg, followed by ibuprofen 0.6 g (Ibuprofen Sustained Release Capsules; Tianjin Glaxo Smith Kline Pharmaceuticals, China), which was used on the triggering day and the next day, for

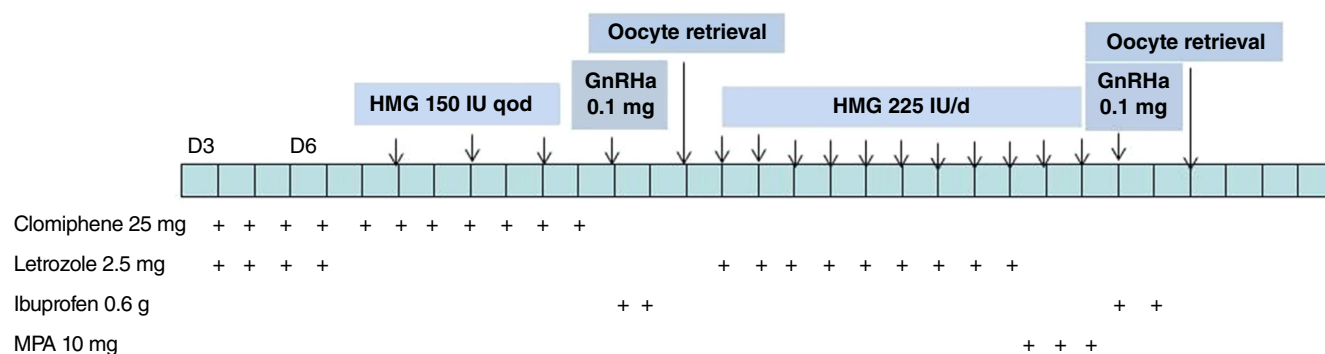


Figure 1 The protocol of double stimulation during the follicular and luteal phases in patients with poor ovarian response. GnRHa, gonadotrophin-releasing hormone agonist; HMG, human menopausal gonadotrophin; MPA, medroxyprogesterone acetate; qod, every other day.

preventing possible follicle rupture before oocyte retrieval (Kadoch et al., 2008). Transvaginal ultrasound-guided oocyte retrieval was conducted 32–36 h after GnRH agonist administration. All follicles of less than 10 mm were not retrieved and left for the second-stage stimulation in the luteal phase.

Fertilization of the aspirated oocytes 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 (Cummins et al., 1986). All highest-quality embryos (including grade 1 and grade 2, eight-cell blastomere embryos) were cryopreserved on the third day after oocyte retrieval. The non-top-quality embryos were placed in extended culture until the blastocyst stage. During this stage, on day 5 or day 6, only good morphology blastocysts were cryopreserved. Both cleavage-stage embryos and blastocysts were cryopreserved by vitrification. In brief, the cryotop carrier system (Kitazato Biopharma Co Ltd, Japan) was used for vitrification and 15% (v/v) ethylene glycol, 15% (v/v) Dimethylsulphoxide and 0.5 M sucrose as the cryoprotectant. For warming, 1 M, 0.5 M and 0 M sucrose solutions were used for cryoprotectants dilution step by step. All vitrification and warming steps were carried out at room temperature except the first warming step at 37°C.

Stage two of treatment protocol: ovarian stimulation and oocyte retrieval

Transvaginal ultrasound examination was carried out after oocyte retrieval to determine whether to continue the second ovarian stimulation. The criterion for continued stimulation was the presence of at least two antral follicles 2–8 mm in diameter. A total of 225 IU HMG and letrozole 2.5 mg were administered daily from the day of, or the day after, oocyte retrieval. The initial second stage follicular monitoring was conducted 5–7 days later, and then every 2–4 days, using a transvaginal ultrasound examination to record the number of developing follicles, and serum FSH, LH, oestradiol and progesterone concentrations. Letrozole administration was stopped when the dominant follicles reached diameters of 12 mm, given that large follicles have redundant LH and FSH receptors, and good response to exogenous hormone stimulations. Daily administration of medroxyprogesterone acetate 10 mg was added beginning on stimulation day 12 for cases in which post-ovulation follicle size was smaller than 14 mm

in diameter 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. When three dominant follicles reached diameters of 18 mm or one mature dominant follicle exceeded 20 mm, the final stage of oocyte maturation was induced again with triptorelin 100 µg by injection. Again, ibuprofen 0.6 g was used on the day of oocyte-maturation triggering and the day after. Transvaginal ultrasound-guided oocyte retrieval was conducted 36–38 h after GnRH agonist administration. All oocytes collected were treated as in study stage one.

The protocol of double stimulation during the follicular and luteal phases is presented in Figure 1.

Endometrial preparation and cryopreserved embryo transfer

Embryo and endometrium synchronization in cryopreserved embryo transfer cycles in this study was according to the method described earlier (Kuang et al., 2013). In brief, for natural cryopreserved embryo transfer cycles, follicular growth was monitored by measuring serum hormone levels and by ultrasound beginning on cycle day 10. When the diameter of the dominant follicle exceeded 16 mm and endometrial thickness was more than 8 mm, with oestradiol greater than 150 pg/ml, one of two procedures was carried out, depending on the LH and progesterone value. If LH was less than 20 IU/l and progesterone was less than 1.0 ng/ml, HCG 10,000 IU was administered 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 more than 20 IU/l or the progesterone value was more than 1.0 ng/ml, HCG 10,000 IU was injected the same afternoon and the transfer of the 3-day-old embryos 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 cases with irregular menstrual cycles, letrozole was used and, if necessary, HMG, to stimulate mono-follicular growth. The common method used was letrozole 2.5–5 mg 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 HCG 10,000 IU and the timing of cryopreserved embryo transfer were determined according to the above criteria.

For patients with thin endometria during either natural cycles or stimulation cycles, hormone replacement treatment was recommended for endometrial preparation, specifically, oral ethinyl oestradiol (Shanghai Xinyi Pharmaceutical Co., China) 25 µg three times a day from cycle day 3 onwards. Once the endometrial lining was greater than 8 mm thick, the following medications were started: two yellow femoston tablets twice a day (Solvay Pharmaceuticals B.V., France) (each tablet contains 2 mg oestradiol and 10 mg dydrogesterone) and vaginal progesterone soft capsules 200 mg twice a day (Laboratoires Besins International). The timing of warming and transfer was determined on the third day after femoston administration. The maximum number of transferred embryos was two per patient. When pregnancy was diagnosed by a positive beta-HCG test, the progesterone supplementation was continued until 10 weeks of gestation.

Statistical analysis

The primary outcome measurement for this study was the number of oocytes retrieved. The secondary measures included the number of mature oocytes, fertilization rate, cleavage rate, the number of valid embryos and the pregnancy outcomes from cryopreserved embryo transfers. The criteria of cancel cycle were no viable embryos for cryopreservation. Clinical pregnancy was defined as the presence of a gestational sac during ultrasound examination 7 weeks after embryo transfer. The implantation rate was defined as the number of gestational sacs divided by the number of embryos transferred. The spontaneous abortion rate was defined as the proportion of patients with spontaneous loss of a clinical pregnancy before 12 weeks gestation. In the study, data are presented as the mean \pm standard deviation (SD) in text and tables. Data were analysed by Student's *t*-test, Mann-Whitney U test and chi-squared test where appropriate. The one-way analysis of variance method was used for the comparison of hormone concentrations at different time-points. The Mann-Whitney U test was used for the variables of non-normal distribution. The significance was accepted for $P < 0.05$. All data were analysed using the Statistical Package for the Social Sciences for Windows (SPSS, Version 16.0, SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics

A total of 178 women were screened, of which 38 women were enrolled according to the Bologna criteria and completed the mild stimulation cycles. Six women did not continue stimulation after first oocyte retrieval for the cause of low antral follicle count (<2) or poor response after luteal-phase ovarian stimulation. Another two women discontinued treatment out of personal choice after the first oocyte retrieval. Thirty women completed the double stimulation regimen.

The basic characteristics of the patients in the study are shown in Table 1. Of all patients, 15 out of 38 patients were

Table 1 Basic characteristics of patients with poor ovarian response ($n = 38$).

Parameter	Values
Age (years) mean \pm SD (range)	36.4 \pm 5.0 (27–48)
Body mass index (kg/m ²) mean \pm SD (range)	22.6 \pm 3.7 (16.4–30.1)
Infertility duration (year) mean \pm SD (range)	4.4 \pm 3.8 (1–15)
Basal FSH (IU/l) mean \pm SD (range)	6.9 \pm 2.3 (3.4–12.9)
Antral follicle counts in follicular phase mean \pm SD (range)	3.8 \pm 1.8 (1–8)
Primary infertility <i>n</i> (% , range in years)	24/38 (63.2, 0–8)
Secondary infertility <i>n</i> (% , range in years)	14/38 (36.8, 0–2)
Previous IVF failure <i>n</i> (%)	
0	12/38 (31.6)
1–2	15/38 (39.5)
≥ 3	11/38 (28.9)

over 40 years. Five women had high FSH level on cycle day 3 (10–15 IU/ml). Thirty-one out of 38 patients had less than five antral follicles in bilateral ovaries at the beginning of stage 1; 13 cases had a history of ovarian surgery. Twenty-six out of 38 (68.4%) had previous failed IVF-ICSI treatments, including 17 cases of previous poor response (no more than three oocytes) using classical long protocol or short protocol.

Ovarian stimulation, follicle development and oocyte performance

The antral follicle counts after first oocyte retrieval were similar with those in the early follicular phase (4.2 \pm 2.1 versus 3.8 \pm 1.8, respectively). Six out of 38 (15.8%) patients had HMG withdrawn owing to high FSH levels during stage 1. Thirty-seven out of 38 (97.4%) participants succeeded in obtaining oocytes in stage one and 29 out of 30 (96.8%) in stage two. The cancellation rate (no cryopreserved embryos) was 20 out of 38 (52.6%) in stage one and 13 out of 30 (43.3%) in stage two owing to no oocyte retrieval, immature oocytes, unfertilized oocytes or fragmented embryos. A total of 19 patients received medroxyprogesterone acetate to postpone menstruation during stage 2 to avoid possible infection risk of oocyte retrieval during menstruation.

A total of 26 women had one to six viable embryos cryopreserved after double stimulation. In total, out of 38 egg collection attempts in stage one and 30 attempts in stage two, a total of 167 eggs were collected, and 26 women had a total of 72 cryopreserved embryos. A profile summary of the pilot study is shown in Figure 2.

The clinical and cycle characteristics of ovulation induction in a single menstrual cycle is shown in Table 2. The mean stimulation durations in two stages were about 10 days, respectively. The number of follicles with diameters larger than 14 mm and the oocytes retrieved in stage one were less than those in stage two (1.5 \pm 0.6 versus 3.5 \pm 2.0; 1.7 \pm 1.0 versus 3.5 \pm 3.2; $P < 0.001$ and $P = 0.001$ respectively). Between the two stages, no significant differences were found in the rate

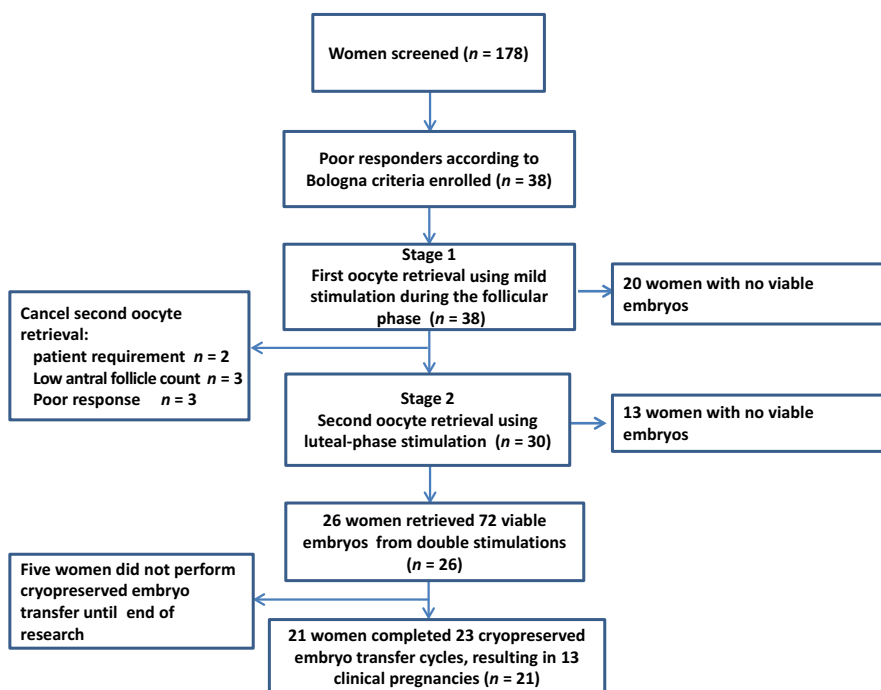


Figure 2 Profile summary of the pilot study.

Table 2 The cycle characteristics in double stimulations in patients with poor ovarian response.

Characteristic	First oocyte retrieval (n = 38)	Second oocyte retrieval (n = 30)	P-Value
Stimulation duration (days)	10.2 ± 2.4	10.8 ± 3.1	NS
Human menopausal gonadotrophin dose (IU)	326.4 ± 248.9	1802.5 ± 712.7	<0.001
Number of follicles >10 mm on trigger day	1.9 ± 0.9	4.3 ± 2.8	<0.001
Number of follicles >14 mm on trigger day	1.5 ± 0.6	3.5 ± 2.0	<0.001
Number of oocytes retrieved	1.7 ± 1.0	3.5 ± 3.2	0.001
Number of metaphase II (metaphase II) oocytes	1.4 ± 1.0	2.7 ± 2.7	0.008
Number of immature oocytes	0.2 ± 0.5	0.8 ± 1.0	0.011
Number of fertilized oocytes	1.0 ± 1.0	2.1 ± 2.5	0.019
Number of cleaved embryos	1.0 ± 1.0	2.0 ± 2.4	0.045
Number of top-quality embryos	0.7 ± 1.0	1.2 ± 1.5	NS
Number of cryopreserved embryos	0.9 ± 1.0	1.3 ± 1.4	NS
Oocyte retrieval rate per follicle n (%)	62/78 (79.5)	105/183 (57.4)	<0.001
Mature oocyte rate n (%)	53/62 (85.5)	82/105 (78.1)	NS
Fertilization rate n (%)	37/53 (69.8)	62/82 (75.6)	NS
Cleavage rate n (%)	37/37 (100)	59/62 (95.2)	NS
Cancellation rate n (%)	20/38 (52.6)	13/30 (43.3)	NS

NS, not statistically significant.

of mature oocytes (85.5% versus 78.1%), fertilization rate (69.8% versus 75.6%) and cleavage rate (100.0% versus 95.2%). The oocyte retrieval rate in stage one was higher than those in stage two (79.5% versus 57.4%, $P < 0.001$). The number of top-quality embryos (0.7 ± 1.0 versus 1.2 ± 1.5) and cryopreserved embryos (0.9 ± 1.0 versus 1.3 ± 1.4) was no significant difference between the first oocyte retrieval and the second oocyte retrieval.

No patients experienced moderate or severe ovarian hyperstimulation syndrome during the study.

Hormonal profile during treatment

The values of circulating concentrations of FSH, LH, oestradiol and progesterone in women undergoing ovarian double stimulation are presented in Figure 3. The FSH and LH values on the day after the first GnRH agonist administration were significantly higher than those after the second trigger ($P < 0.001$). Serum oestradiol values showed a gradual increase accompanying the growth of follicles during stage one, and no significant difference in values was found between the

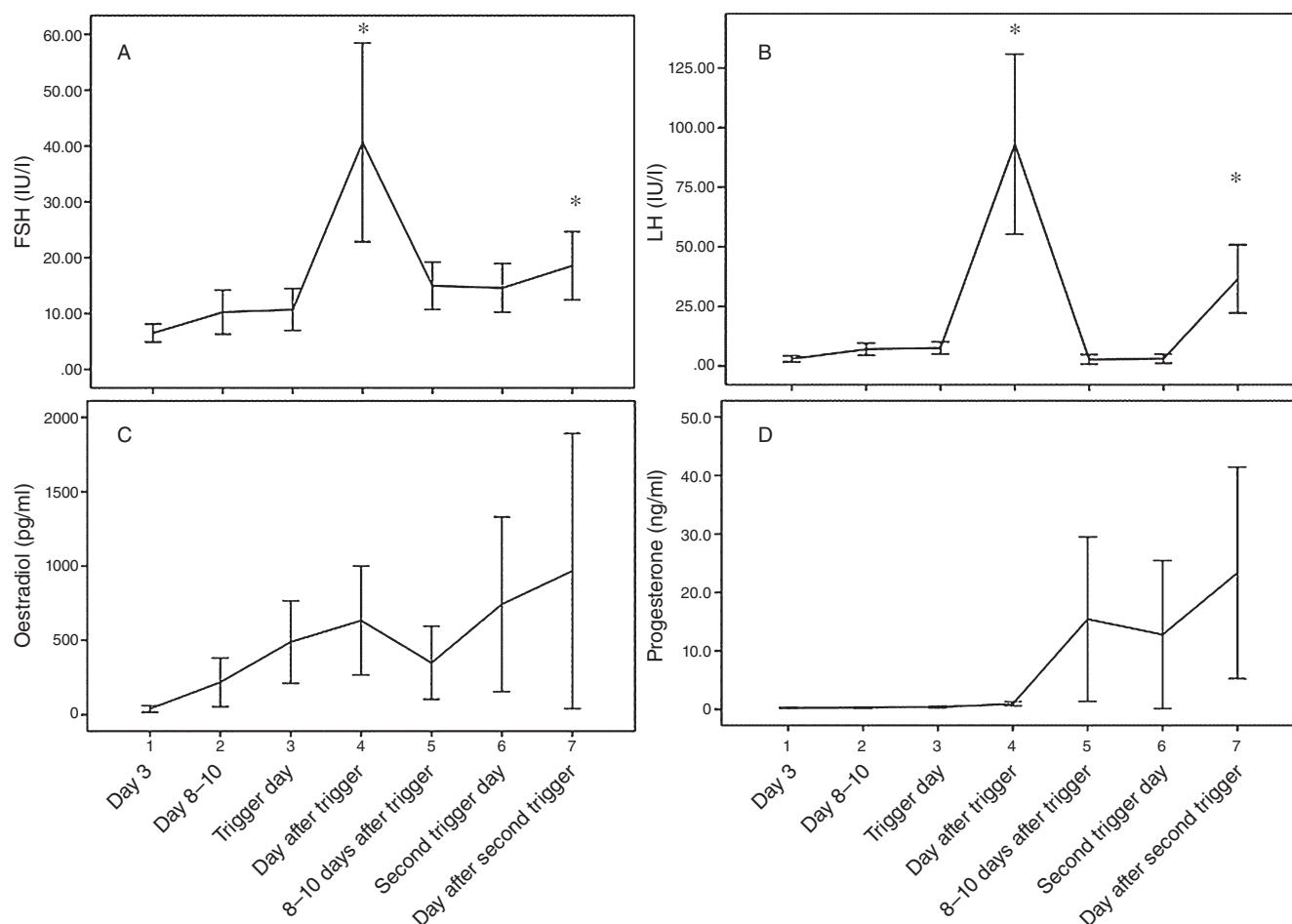


Figure 3 The hormone profiles during the whole process of double stimulation are shown in panel A (FSH), panel B (LH), panel C (oestradiol) and panel D (progesterone). The FSH and LH levels on the day after the first trigger were significantly higher than the levels on the day after the second trigger ($P < 0.001$).

day of GnRHa trigger and the next day. The progesterone value showed wide variation among individuals during the luteal phase (0.2–58.4 IU/l). Progesterone levels on the day of the second GnRH agonist trigger and the day after were negatively associated with the duration of HMG stimulation (Pearson correlation coefficient = -0.75 , $P < 0.01$). During the second stage, if the duration of HMG stimulation was longer than 9 days, the progesterone level was significantly lower compared with shorter-duration stimulation (<9 days) on the day of the second trigger and the day after trigger ($P < 0.01$).

Pregnancy

Twenty-one women completed 23 cryopreserved embryo transfer cycles until the end of study period (Table 3). A total of 43 embryos were warmed and the implantation rate was 36.6% in cryopreserved embryo transfer cycles (15/41). Clinical pregnancy was observed in 13 cases, representing a clinical pregnancy rate per transfer of 56.5% (13/23), 11 had single pregnancies and two had twin pregnancies. Two spontaneous abortions occurred before the gestational age of 8 weeks (15.4%). The similar implantation rate of embryos derived from

stage one and stage two indicated embryos derived from double stimulations having similar development potential.

Discussion

The poor responders, according to the Bologna criteria, represent a subgroup of patients with relatively poor pregnancy prognosis, and their live-birth rate in natural-cycle IVF is consistently low, with a range from 6.8–7.9% (Ferraretti *et al*, 2011; Polyzos *et al*, 2012). The efficacy of mild stimulation during the follicular phase and continuous ovarian stimulation during the luteal phase was explored, and it was found that 68.4% of patients with poor ovarian response had one to six viable embryos cryopreserved after double stimulations. The outcomes of cryopreserved embryo transfers indicated that the embryos originating from double stimulations had good development potential. The double stimulations during the same menstrual cycle and cryopreserved embryo transfers provided a promising approach for patients with poor ovarian response.

Several factors may be associated with poor ovarian response; diminished ovarian reserve in both older patients and

Table 3 Cryopreserved embryo transfer cycle outcomes using embryos derived from double stimulation in patients with poor ovarian response.

	<i>Total</i>	<i>Embryos from first oocyte retrieval</i>	<i>Embryos from second oocyte retrieval</i>	<i>Two embryos from two oocyte retrievals</i>
Number of patients	21	12	6	3
Cryopreserved embryo transfer cycles	23	13	7	3
Embryos warmed	43	22	15	6
Embryo transferred	41	21	14	6
Embryo survival rate (%)	41/43 (95.3)	21/22 (95.5)	14/15 (93.3)	6/6 (100)
Clinical pregnancy rate (%)	13/23 (56.5)	8/13 (61.5)	5/7 (71.4)	0/3
Implantation rate (%)	15/41 (36.6)	10/21 (47.6)	5/14 (35.7)	0/6
Spontaneous abortion rate (%)	2/13 (15.4)	1/8 (12.5)	1/5 (20.0)	0
Ongoing pregnancy rate (%)	11/23 (47.8)	7/13 (53.8)	4/7 (57.1)	0/3

younger patients represents the most frequent cause. When the ovarian reserve is reduced, the induction of multi-follicular growth remains a challenge (Saldeen et al., 2007). In this study, both clomiphene citrate and letrozole were used at early follicular phase to increase the ovarian response. Clomiphene citrate acts to increase pituitary FSH secretion by reducing the negative oestrogen feedback. Letrozole is a potent non-steroidal aromatase inhibitor. It blocks aromatization of androgens into oestrogens and releases the hypothalamic-pituitary axis from negative oestrogen feedback, whereas the increase of intraovarian androgens enhance early follicular growth and results in improved IVF outcomes (Garcia-Velasco et al., 2005). We presumed that they may have co-ordinated actions in concomitant use. Mostly patients retrieved oocytes through the first stage of mild stimulation with letrozole and clomiphene, but cancel rate of no viable embryos after mild stimulation was high in this study. The cancel rate decreased into 31.6% (12/38) through double stimulation. Double stimulations during the follicular and luteal phases provide a promising alternative or a rescue approach for the patients with poor ovarian response. The number of antral follicles after first oocyte retrieval was similar with the counts in the early follicular phase in our study, which offers an exciting potential target for extending ovarian stimulation and an additional oocyte retrieval. The main benefit of double stimulations is to obtain more oocytes and produce more viable embryos through continuous ovarian stimulation in a single menstrual cycle. To a large extent, this avoids cycle cancellation and increases the possibility of pregnancy.

For patients with poor ovarian response undergoing IVF-ICSI treatment, successful pregnancy achieved from one oocyte retrieval procedure and fresh embryo transfer is usually attributed to luck; it is not the expected outcome. The strategy of separating the oocyte retrieval and embryo transfer processes can provide ample time for doctors to accomplish the sequential tasks of oocyte retrieval and implantation. Sometimes, it is necessary to accumulate viable embryos from several oocyte retrieval events (Cobo et al., 2012). Although this approach seems to be tedious, it is feasible and efficacious for women with poor ovarian response. The study protocol is based on optimal cryopreservation techniques and cryopreserved embryo transfer, which has been proven to increase the implantation and pregnancy rates, with even better delivery outcomes (Cohen and Alikani, 2013; Shapiro et al., 2011). Our findings indicate that the implantation rate was

still satisfactory in poor responders if they have embryo cryopreserved. Therefore, this protocol could be useful for patients who have undergone recurrent failed oocyte retrieval procedures or for patients using conventional IVF protocols with no viable embryos.

We presume it is also beneficial for cancer patients needing emergency fertility preservation. As the number of women who survive cancer increases, the demand for effective and individualized fertility preservation options grows. Among the strategies for the preservation of fertility, embryo cryopreservation or oocyte vitrification seem to be the most effective approach (Chung et al., 2013; Garcia-Velasco et al., 2013). This protocol would minimize delays and allow more oocytes and embryos to preserve in a limited period of time for the cancer patients before starting chemotherapy, radiotherapy, or both.

Another important finding in reproductive endocrinology was the relative ovarian insensitivity to HMG stimulation during the luteal phase compared with the follicular phase. We used the relative larger dose of HMG to perform ovarian stimulation after first oocyte retrieval in the pilot study. A previous study reported that the mean dose of HMG per oocyte retrieved in the luteal phase stimulation was nearly two times more than those of classical protocol (Buendgen et al., 2013). In the pituitary gland, the FSH surge and LH surge induced by the same dose of GnRH agonist were much higher in the first trigger than the second trigger. This suggested that ovarian sensitivity to the HMG stimulation was significantly reduced during the luteal-phase ovarian stimulation. One of the impacting factors was pituitary suppression of co-existing high progesterone during the luteal phase. The inherent mechanism of the corpus luteum on follicle growth waits for further study.

We also noticed a wide range of oestradiol and progesterone levels on the day of and the day after the second trigger. Further analysis showed the fluctuation of progesterone was negatively associated with HMG duration during the second stimulation. For the cases with longer luteal-phase HMG stimulation (>9 days), the progesterone level had a decreasing trend which approximately coincided with the formation and recession of the corpus luteum. In addition, this pilot study opened the door for the possibility of performing double stimulation in patients with POR. A large-scale, prospective, randomized controlled trial should be further investigated to draw solid conclusions.

As mentioned above, double stimulation during the follicular and luteal phases in the same menstrual cycle provided more opportunities to retrieve oocytes in poor responders, with the resulting embryos having similar development potential. Double stimulation and subsequent cryopreserved embryo transfer is a promising approach both for patients with POR, especially for the cases that repeatedly did not have oocytes retrieved or viable embryos using conventional IVF regimens, and for cancer patients needing emergency fertility preservation.

Acknowledgements

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