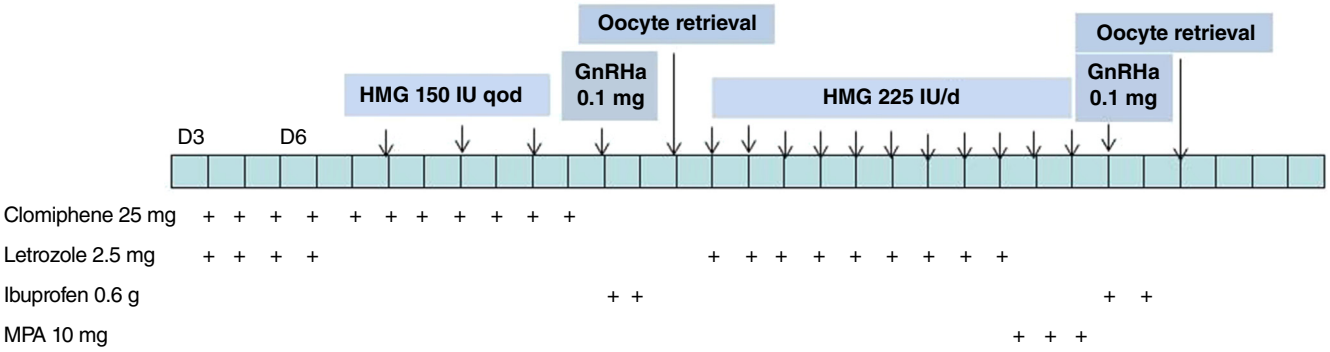

Figure 1 The protocol of double stimulation during the follicular and luteal phases in patients with poor ovarian response. GnRHa, gonadotrophin-releasing hormone agonist; HMG, human menopausal gonadotrophin; MPA, medroxyprogesterone acetate; qod, every other day.

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, oestradil 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 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 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 ws 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