



# Luteal-phase protocol in poor ovarian response: a comparative study with an antagonist protocol

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## Abstract

**Objective:** This retrospective study compared the effect of the luteal phase ovarian stimulation protocol (LP group) with the gonadotrophin-releasing hormone (GnRH) antagonist protocol (AN group) in women with poor ovarian responses.

**Methods:** Ovarian stimulation was initiated with 225 IU of human gonadotrophin (hMG) daily. When the dominant follicle diameter exceeded 13 mm, 0.25 mg of a GnRH antagonist was used daily until human chorionic gonadotrophin (HCG) administration in the AN group. A GnRH antagonist was not used in the LP group. Ovulation was induced with HCG for all patients when at least one follicle reached a diameter of 16 mm or one dominant follicle reached 18 mm. The highest quality embryos were transferred or cryopreserved for later transfer.

**Results:** From January 2013 to December 2015, 274 women with poor ovarian response were included. A total of 108 patients underwent the luteal phase ovarian stimulation protocol while 166 patients underwent the GnRH antagonist protocol. hMG was used for more total days in the LP group than in the AN group. Oestradiol levels on the day of HCG administration in the LP group were significantly lower than those in the AN group. The mean number of oocytes retrieved in the LP and AN groups was  $3.5 \pm 2.5$  and  $3.5 \pm 2.9$ , respectively. The mean number of embryos of the highest quality was  $1.7 \pm 1.2$  and  $1.7 \pm 1.5$ , respectively. The clinical pregnancy and implantation rates in the LP and AN groups were 26.2% (22/84) and 25% (29/116), and 15.5% (24/155) and 16.3% (35/215), respectively.

**Conclusions:** The luteal phase ovarian stimulation protocol can be applied in women with poor ovarian response and attain comparable clinical pregnancy and implantation rates to those of the GnRH antagonist protocol.

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## Keywords

Ovarian stimulation, poor responder, luteal phase, GnRH antagonist, frozen embryo transfer

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## Introduction

One of the most important prognostic factors is ovarian reserve for achievement of pregnancy in *in vitro* fertilization (IVF) cycles.<sup>1</sup> Patients with a poor ovarian response (POR) are often faced with high cycle cancellation and low pregnancy rates.<sup>2,3</sup> Some strategies have been suggested for management of poor responders. The gonadotrophin-releasing hormone (GnRH) antagonist protocol has received much attention recently. This protocol has some advantages, including less time and cost with late follicular phase administration of a GnRH antagonist. Recent evidence has indicated that follicles developing in the luteal phase can ovulate in the presence of a luteinizing hormone (LH) surge. Therefore, this situation has created more opportunities to use ovarian stimulation.<sup>4</sup>

Many investigators have confirmed that oocytes that are retrieved in the luteal phase can be fertilized and developed into embryos *in vitro*.<sup>5-9</sup> Kuang *et al.*<sup>10</sup> observed that luteal phase ovarian stimulation can produce competent embryos in women aged 20–38 years with infertility caused by tubal, male, or unexplained factors. Some researchers have shown that luteal phase ovarian stimulation is feasible for obtaining mature oocytes and good embryos in women with PORs.<sup>9</sup> This study aimed to examine the efficacy of luteal phase ovarian stimulation in women with PORs, by comparing IVF outcomes with GnRH antagonist protocols.

## Patients and methods

### Study setting and patients

The data for this study were retrieved from patients' medical records from January 2013

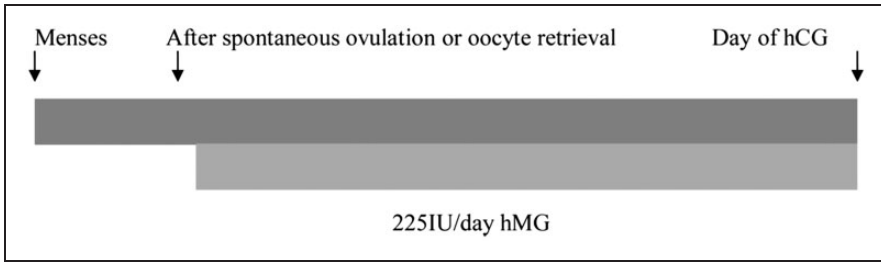
to December 2015. According to the Bologna criteria,<sup>11</sup> the patients had at least two of the following characteristics: (1) advanced maternal age ( $\geq 40$  years old); (2) a previous POR ( $\leq 3$  oocytes with a conventional stimulation protocol); and (3) an abnormal ovarian reserve test (antral follicle count of  $< 5-7$  follicles). Two episodes of POR after maximal stimulation were sufficient to define a patient as a poor responder in the absence of advanced maternal age or abnormal ovarian reserve test.

The study was approved by the Ethical Committee of Reproductive Medicine of Linyi People's Hospital (Shandong Province, China). Written informed consents were obtained from all participants.

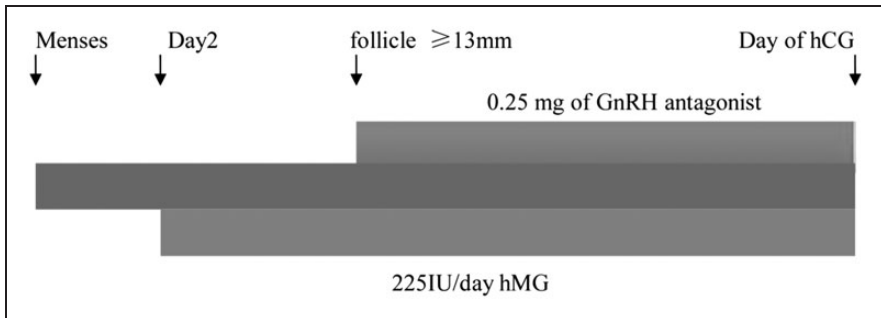
### Procedures

A total of 274 patients (337 cycles) were enrolled from our computerized IVF database. A total of 108 patients (113 cycles) underwent the luteal phase ovarian stimulation protocol (LP). Between 0 and 24 hours after spontaneous ovulation or oocyte retrieval, patients with at least one follicle of  $< 8$  mm remaining were administered Highly Purified Menotrophin (Ferring GmbH, Wittland 11, Germany) in a 225-IU IM injection until the day of human chorionic gonadotrophin (HCG) administration (Figure 1).

During the same time interval, 166 patients (224 cycles) underwent the GnRH antagonist protocol (AN). On day 2 of the menstrual cycle, 225 IU of Highly Purified Menotrophin (Ferring GmbH Wittland 11) daily was commenced. When the leading follicle exceeded 13 mm in diameter, 0.25 mg of GnRH antagonist (Cetrotide; Merck-Serono, Switzerland) was started daily



**Figure 1.** The luteal phase ovarian stimulation protocol (LP) group.



**Figure 2.** The GnRH antagonist protocol (AN) group.

until the day of HCG administration (Figure 2).

Transvaginal ultrasound examination for all patients was performed to record the diameter of developing follicles, and serum oestradiol (E2) concentrations were measured. The criterion for recombinant HCG (Profasi; Merck-Serono, Switzerland) administration was that at least one follicle diameter reached 16 mm or one dominant follicle reached 18 mm. A total of 36 hours after recombinant HCG injection, oocyte aspiration was performed, which was guided by transvaginal ultrasound.

Fertilization was performed *in vitro*, by IVF or intracytoplasmic sperm injection, relying on semen parameters. The embryos were examined and graded depending on the number and regularity of the blastomeres and embryonic fragmentation according to Cummins criteria.<sup>12</sup> All of the highest quality embryos (including the 2–4 rated

embryos) were transferred or frozen by vitrification on the second or third day after oocyte retrieval.

Endometrial preparation in frozen embryo transfer was performed in an artificial cycle or a natural cycle as previously described.<sup>10,13</sup>

A  $\beta$ -HCG blood test was used to detect pregnancy on day 14 after transplantation. If pregnancy was achieved, progesterone application was continued until 12 weeks of gestation.

### Statistical analysis

The clinical definition of pregnancy was defined as a gestational sac being detected by ultrasonography. The implantation rate was the total number of gestational sacs demonstrated by ultrasonography divided by the total number of transferred embryos. The miscarriage rate was defined as the

**Table 1.** Baseline characteristics of the LP and AN protocols.

Parameters (mean $\pm$ SD)	LP	AN	P
Number of patients	108	166	
Previous IVF attempts	2.4 $\pm$ 1.9	2.5 $\pm$ 1.4	0.56
Number of cycles	113	224	
Number of cancelled cycles (%)	25 (22.1)	48 (21.4)	0.88
Age, years	37.3 $\pm$ 5.1	37.7 $\pm$ 4.9	0.41
BMI, kg/m <sup>2</sup>	23.7 $\pm$ 2.9	24.4 $\pm$ 3.2	0.06
Basal E2, pg/mL	27.4 $\pm$ 13.6	28.5 $\pm$ 18.6	0.76
Basal FSH, mIU/ml	10.9 $\pm$ 4.2	11.0 $\pm$ 3.6	0.81
Basal LH, mIU/ml	4.5 $\pm$ 2.3	4.8 $\pm$ 3.7	0.73
Duration of infertility years	5.1 $\pm$ 4.7	4.1 $\pm$ 2.5	0.80

All values are expressed as mean  $\pm$  SD.  $P < 0.05$ , significant difference.

IVF *in vitro* fertilization, BMI body mass index, E2 oestradiol, FSH follicle-stimulating hormone, LH luteinizing hormone, LP luteal-phase ovarian stimulation protocol, AN gonadotropin-releasing hormone antagonist protocol.

proportion of patients with spontaneous abortion of the total pregnancy. The data are shown as the mean  $\pm$  SD and were analysed by the one-way analysis of variance method. When the analyses showed significance, the groups were compared using the LSD test. The chi-square or Fisher's exact test was used to analyse the clinical pregnancy rate, implantation rate, and miscarriage rate.  $P < 0.05$  was considered statistically significant. Windows SPSS 18.0 software was used for statistical analysis (SPSS, USA).

## Results

Among the 337 cycles, no patient experienced a premature LH surge or moderate/severe ovarian hyperstimulation. The baseline characteristics of patients in the two groups are shown in Table 1. The mean age of female patients in the study was 37.3  $\pm$  5.1 years and 37.7  $\pm$  4.9 years. The mean body mass index was 23.7  $\pm$  2.9 and 24.4  $\pm$  3.2 kg/m<sup>2</sup>. The mean basal follicle-stimulating hormone (FSH) level was 10.9  $\pm$  4.2 mIU/mL and 11.0  $\pm$  3.6 mIU/mL. Seventy-three cycles were cancelled because of no oocyte or embryo. Among the cancelled cycles, two and 11 cycles were cancelled because of no

oocytes in the LP and AN groups, respectively. Among the cancelled cycles, 23 and 37 cycles had no embryo in the LP and AN groups, respectively. The two groups were similar in terms of age, body mass index, mean basal FSH levels, and cancelled cycles (Table 1).

The mean duration of hMG administration was 9.9  $\pm$  2.3 days in the LP group and 8.7  $\pm$  2.2 days in the AN group ( $p < 0.001$ ). The total dose of gonadotrophin stimulation was higher in the LP group than in the AN group (Table 2) ( $p < 0.001$ ). E2 levels on the day of hCG administration were lower in the LP group compared with the AN group. However, the mean number of oocytes was 3.5  $\pm$  2.5 and 3.5  $\pm$  2.9, and the mean number of high quality embryos was 1.7  $\pm$  1.2 and 1.7  $\pm$  1.5 in the LP and AN groups, respectively (Table 2). There were no significant differences in these variables between the two groups.

A total of 159 embryos were first thawed in the LP protocol, and the survival rate was 97.5%. A total of 155 embryos were transferred. A total of 215 embryos were transferred in the AN protocol (Table 3). The clinical pregnancy, implantation and miscarriage rates were compared between

**Table 2.** The controlled ovarian hyperstimulation response of the LP and AN protocols.

Parameters(mean $\pm$ SD)	LP	AN	P
Total days of hMG used	9.9 $\pm$ 2.3	8.7 $\pm$ 2.2	<0.001
Total dose of hMG used	2088.2 $\pm$ 561.3	1690.5 $\pm$ 668.1	<0.001
Peak E2 levels, pg/ml	832.5 $\pm$ 675.5	1057.3 $\pm$ 889.9	0.02
Number of oocytes aspirated	3.5 $\pm$ 2.5	3.5 $\pm$ 2.9	0.90
Number of embryos	1.7 $\pm$ 1.2	1.7 $\pm$ 1.5	0.85

hMG human gonadotrophin, E2 oestradiol.

**Table 3.** Pregnancy outcomes of the LP and AN protocols.

Parameters	LP (N = 119)	AN (N = 224)	P
First transferred cycles	84	116	
Thawed embryos	159		
Transferred embryos	155	215	
Clinical pregnancy	22	29	
Total implantation sacs	24	35	
Pregnancy rate (per transfer)	26.2% (22/84)	25% (29/116)	0.849*
Implantation rate	15.5% (24/155)	16.3% (35/215)	0.837*
Single embryo	18	22	
Twin embryos	2	6	
Miscarriage rate	22.7% (5/22)	13.8% (4/29)	0.647**
Ectopic pregnancies	2	1	0.571**
Delivery	12	15	
Delivery rate	60% (12/20)	53.6% (15/28)	
Single births	11	13	
Twin birth	1	2	
Ongoing	3	9	

\*Analysed by the chi-square test; \*\*analysed by Fisher's exact test.

the two groups (Table 3). A trend of a higher miscarriage rate (22.7% vs 13.8%;  $P=0.647$ ) was observed in the LP group, but this was no significant because of the small number of patients.

Forty of the pregnant women had single embryos and eight women had twin embryos. Nine women had miscarriage in the first trimester, and there were three ectopic pregnancies. Among the pregnancies, 27 women had given birth, and 12 had ongoing pregnancies at the end of the study. There were 24 single births and three twin births, and no malformations were found in the newborns (Table 3).

## Discussion

Women whose ovarian reserves are too poor to retrieve sufficient mature oocytes have less hope of having their own children. There have been many reports on management of a POR to improve ovarian response and increase IVF outcomes, including the following: increasing the gonadotrophin dose; decreasing the GnRH agonist dose, aromatase inhibitors, and natural cycle; and using a GnRH antagonist protocol.<sup>14</sup> Several studies have shown that luteal phase ovarian stimulation might be feasible. Immature oocytes retrieved in the luteal phase can be successfully matured *in vitro*.

One possible explanation for the existence of viable follicles during the luteal phase was proposed by Baerwald *et al.*<sup>4</sup> Studies have suggested that oocytes that are obtained during the luteal phase are able to mature *in vitro* in women requiring imminent chemotherapy.<sup>5,6</sup> One patient in the luteal phase had two mature oocytes on the day of retrieval.<sup>5,6</sup> A bovine model showed that the maturation and fertilization rates in the normal menstrual cycle were equal to those in luteal oocyte retrieval.<sup>15</sup> Additionally, there were no significant differences in the rates of cleavage and blastocyst formation in the two groups. After maturing, mature oocytes that have been recovered in the luteal phase are able to be fertilized and form good quality embryos *in vitro*.<sup>7,8</sup> Xu and Li<sup>9</sup> reported a patient who was diagnosed with a POR. A mature oocyte obtained through luteal phase ovarian stimulation resulted in a cleavage embryo (8-cell, grade 2). Findings in their case showed that luteal phase ovarian stimulation might be equally feasible in poor responders. In our study, oocytes that were retrieved during the luteal phase were capable of maturing *in vitro* and being fertilized.

Two groups were compared in our study. The baseline characteristics of the groups were similar, including cancelled cycles, female age, basal FSH levels, and body mass index. However, the total number of days of stimulation, total dose of gonadotrophin administered, and E2 levels on the day of HCG administration were different between the groups. The total number of days of HMG used was longer in the LP protocol compared with the AN protocol. E2 levels on the day of HCG administration were significantly lower in the LP protocol compared with the AN protocol. These differences may be due to luteal phase stimulation with the LP protocol. There were no significant differences in the pregnancy and implantation rates between the two groups.

The trend of a higher miscarriage rate in the LP group than that in the AN group might have been related to the small sample size.

Fertility preservation for cancer patients involves freezing embryos and oocytes, and the rate of pregnancy from vitrified oocytes is acceptable.<sup>16,17</sup> Whether oocytes and embryos that are obtained and frozen in the luteal phase would result in a comparable pregnancy rate is unknown. A previous study showed that the clinical pregnancy, ongoing pregnancy, and implantation rates of frozen embryo transfer were 55.46%, 48.91%, and 40.37%, respectively.<sup>10</sup> However, the criteria were women aged 20–38 years with a high ovarian reserve, and exclusion of PORs.<sup>10</sup> The current study demonstrated that the clinical pregnancy, ongoing pregnancy, and implantation rates of frozen embryo transfer with the LP protocol were 26.2% (22/84), 15% (3/20) and 15.5% (24/155), respectively, in women with PORs. These results were better than the microdose GnRH agonist flare-up protocol, which was reported by Yarali *et al.* and Cenksoy *et al.*<sup>18,19</sup> Yarali *et al.*<sup>18</sup> showed that the clinical pregnancy and implantation rates were 17.4% and 9.8%, respectively, in the microdose GnRH agonist flare-up protocol. The miscarriage rate with the LP protocol in the current study was 22.7%, which is consistent with previous reports (23%).<sup>18</sup> The cancellation rate (24.3%) and total dosage of gonadotrophins (4221 IU) were higher in the current study (22.1%, 2088 IU) than in Cenksoy *et al.*'s study.<sup>19</sup> Therefore, continuous stimulation during the luteal phase could be used in women with PORs.

An ideal protocol for poor responders has not been determined. The long protocols of GnRH agonist administration show benefits in follicular synchronization. However, they have the disadvantage of inhibiting the ovarian response to gonadotrophins, particularly in patients PORs. The current study showed that luteal phase

ovarian stimulation was feasible for women with a POR, and ovarian stimulation may be used in special circumstances. However, this result might have been caused by the small sample size. Further prospective, randomized trials are required to evaluate the effect of such protocols.

### Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

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