Clomiphene Citrate Priming Increases Sensitivity During Ovarian Stimulation in Poor Ovarian Responders Undergoing *In Vitro* Fertilization Treatment: A Retrospective Cohort Study

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Research

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Abstract

Background: Since ovarian stimulation was introduced as an assisted reproductive technology, poor ovarian response (POR) management has challenged clinicians. Guidance on optimally managing patients with poor response and/or low sensitivity to ovarian stimulation is still lacking. We aimed to investigate whether a clomiphene citrate (CC) priming protocol could increase ovarian sensitivity in poor ovarian responders.

Methods: This single-center retrospective cohort study included 294 patients (374 ovarian stimulation cycles). Of these, 193 cycles were treated by a CC priming antagonist protocol (study group) and 181 by the classical flexible gonadotropin-releasing hormone antagonist protocol (control group). Stimulation data and laboratory and clinical outcomes were compared between the groups.

Results: Total gonadotropin dosage and dosage per follicle were considerably lower, the follicle-to-oocyte index was significantly higher, and the gonadotropin duration was shorter in the study group. After adjusting for potential confounders, multivariate regression analysis showed that cumulative ongoing pregnancy remained comparable between the groups (adjusted odds ratio: 0.761, 95% confidence interval: 0.300-1.933, P = 0.566). Age, body mass index, gonadotropin dosage per follicle, and the follicle-to-oocyte index were directly associated with the reproductive outcomes. The result of the sensitivity analysis showed that patients stimulated by the CC priming antagonist protocol were administered less gonadotropin (1,739.09 ± 719.39 vs. 3,114.77 ± 1,171.23, P < 0.001) at a lower gonadotropin dosage per follicle (637.36 ± 373.05 vs. 1286.26 ± 976.66, P < 0.001) and for a shorter duration (6.58 ± 2.23 vs. 9.80 ± 1.90, P < 0.001).

Conclusions: The CC priming antagonist protocol offered a convenient and patient-friendly way to increase ovarian sensitivity during ovarian stimulation in poor ovarian responders.

Background

Since ovarian stimulation was introduced as an assisted reproductive technology, poor ovarian response (POR) management has challenged clinicians [1]. POR to ovarian stimulation usually indicates reduced follicular response and/or reduced oocyte number, resulting in a low live birth rate [2]. The most universally accepted definition of POR is the Bologna Criteria [3]. Multiple strategies have been suggested to enhance the outcomes of patients with inferior ovarian function. A simple approach is to vary the gonadotropin dosage or the stimulation initiation time, but the pregnancy rate remains very low [4]. Lekamge et al. found that higher gonadotrophin stimulation did not improve the *in vitro* fertilization (IVF) outcomes in patients with POR [5]. Another commonly used stimulation regimen is the administration of gonadotropin-releasing hormone (GnRH) agonist and gonadotropins during the follicular phase (the flare-up protocol). Non-stimulated (nature cycle) IVF treatment was also attempted. All these methods were used with limited success, and the results achieved remain controversial [6-8]. Guidance on optimally managing patients with poor response and/or low sensitivity to ovarian stimulation is still lacking.

Clomiphene citrate (CC) is a nonsteroidal triphenylethylene derivative that exhibits estrogen agonist and antagonist properties [9]. It increases the pulse frequency of hypothalamic GnRH and pituitary luteinizing hormone (LH) and follicle-stimulating hormone (FSH) by inhibiting the negative feedback effect of circulating estradiol [10]. Previous studies suggested that CC administration could reduce FSH consumption, and thus the costs incurred to the patient during ovarian stimulation [11-13]. However, few studies have compared ovarian sensitivity to CC-related protocols and gonadotropin plus GnRH protocols. Moreover, the index for assessing ovarian sensitivity to gonadotropin stimulation is controversial.

Many methods have been proposed to evaluate the ovarian response and/or ovarian sensitivity to gonadotropin stimulation. Follicle output rate (FORT) was first introduced by Genro et al. in 2011 [14], suggesting that FORT < 0.30 indicates low ovarian sensitivity [14, 15]. However, this index does not assess the actual number of oocytes retrieved, which is strongly associated with live birth rates [16]. Alternatively, the follicle-to-oocyte index (FOI) was proposed to address the ovarian response to gonadotropin stimulation, indicating that FOI $\leq$ 0.50 indicates low ovarian sensitivity and FOI > 0.50 indicates normal ovarian sensitivity [17]. Another parameter used to predict ovarian response to gonadotropin stimulation is the ovarian sensitivity index [18]. Although both indexes seem to be useful in evaluating ovarian sensitivity, some drawbacks should be considered. For example, technical aspects such as triggering the final oocyte maturation and ovum pick-up are related to oocyte retrieval and can influence FOI and ovarian sensitivity index results. Therefore, neither of these indexes alone could be strong enough to assess the dynamical aspect of the ovarian response to gonadotropin stimulation. We proposed a new biomarker, namely, gonadotropin (Gn) dosage per follicle. This index is calculated as the ratio between the total FSH dosage administered and the number of preovulatory follicles developed in response to the ovarian stimulation. Our unpublished data demonstrate that it could reflect on ovarian sensitivity and is positively related to the IVF outcomes.

In the present study, we evaluated a CC priming antagonist protocol in POR patients. The objective was to investigate whether this protocol could increase ovarian sensitivity among POR patients over what could be achieved with the classical flexible antagonist protocol. FORT, FOI, and Gn dosage per follicle were used to evaluate the dynamic nature of follicular growth in response to the exogenous gonadotropin treatment. Reproductive outcomes were compared between the treatment protocols.

Materials And Methods

Study design and participants

This is a retrospective, observational single-center cohort study. The patients were recruited from the Medical Centre for Human Reproduction, Beijing Chao-Yang Hospital, Capital Medical University from January 1, 2017, to October 31, 2019. The study was approved by the Ethics Committee of Beijing Chao-Yang Hospital, Capital Medical University. Written informed consent was waived due to the retrospective

nature of the study. Analyses of data was performed in accordance with the rules and regulation with approvals from the ethics committee of our hospital.

Patients were included in the study if they fulfilled at least two of the following conditions according to the Bologna Criteria: 1) advanced maternal age (≥40 years) or any other risk factor for POR; 2) a previous POR (≤3 oocytes with a conventional stimulation protocol); 3) an abnormal ovarian reserve test [i.e., antral follicle count of 5-7 or anti-müllerian hormone of 0.5-1.1 ng/mL]. Patients were excluded from the study when at least one of the following features was present: 1) body mass index higher than 36 kg/m^2; 2) age over 45 years; 3) severe endometriosis, or autoimmune or metabolic disorders, 4) severe azoospermia for the partner.

A total of 374 cycles performed in 294 patients were included in this study. They were divided into two groups based on the stimulation protocols: CC priming antagonist protocol (study group, *n* = 193) and classical flexible antagonist protocol (control group, *n* = 181).

Ovarian stimulation, oocyte retrieval, and embryo transfer

The study group protocol was used to stimulate 193 cycles in 145 women. Briefly, ovarian stimulation was initiated with 100 mg/day CC for five days, from day 3 to 7 of the menstrual cycle. A recombinant FSH (rFSH; Gonal F, Merck Serono, Germany) dose of 225-300 IU was administered daily, starting from day 6 of the cycle. The initial Gn dosage was individualized based on the patient's age, antral follicle count (AFC), body mass index (BMI), and, if available, the ovarian response in previous cycles. During ovarian stimulation, Gn dosage may have been adjusted according to hormone levels and follicular development. A daily dose of 0.25 mg of GnRH antagonist (Cetrotide, Merck Serono, Germany) was administered when the dominant follicle reached 14 mm in diameter. The co-treatment continued until the trigger day (included).

In the control group, 181 cycles in 149 women were stimulated by the classical flexible antagonist protocol. A daily dose of 225-300 IU rFSH was administered from day 3 of the menstrual cycle. The rFSH dosage adjustments and GnRH antagonist administration were the same as in the study group. In both groups, 250 μg recombinant human chorionic gonadotropin (rhCG; Ovidrel, Merck Serono, Germany) was administered when the leading follicle reached a diameter of 18-20 mm or when the diameter of at least two follicles reached 17-18 mm (Figure 1).

Transvaginal ultrasonography-guided ovum pick-up was performed 36 h after rhCG administration. Retrieved oocytes were fertilized by either IVF or intracytoplasmic sperm injection, depending on sperm quality. No more than three embryos were transferred on day 3 after ovum pick-up. Extra good-quality embryos were vitrified. Fresh embryo transfer was canceled if the patient had an unfavorable endometrium (endometrial thickness of ≤6 mm or ≥16 mm, endometrial polyp, or fluid in the cavity), progesterone level ≥1.5 ng/mL on the triggering day, or when no good quality embryos have developed. At the treating doctor's decision, the endometrium was prepared through a natural or artificial cycle regimen for frozen embryo transfer.

Blood samples and hormone assays

Serum hormone concentrations were measured by an automated immunometric assay. Serum FSH, LH, and estradiol were collected on day 3 of a previous basal cycle as the baseline (within three months) and at the start of the stimulation protocol. During ovarian stimulation, hormone analyses were performed regularly, 4-5 times in most cases, until the day of rhCG administration. All measurements were performed according to the manufacturer's instructions.

Outcomes and measures

The indexes used to evaluate ovarian sensitivity were FORT, FOI, and Gn dosage per follicle. FORT was defined as the ratio between the number of preovulatory follicles obtained in response to ovarian stimulation and the pre-existing small antral follicle pool. FOI was defined as the ratio between the total number of oocytes collected following ovarian stimulation and the number of antral follicles available at stimulation initiation. Gn dosage per follicle was calculated as the ratio between the total FSH dosage administered and the number of preovulatory follicles obtained in response to ovarian stimulation.

A β-hCG level above 10 IU/L was defined as a positive biochemical pregnancy. Clinical pregnancy was diagnosed when the ultrasonographic examination revealed a gestation sac and fetal heartbeat 2-3 weeks from the positive β-hCG test. Ongoing pregnancy was defined as a pregnancy progressing for over 12 weeks from ovum pick-up. Implantation rate was defined as the number of fetal sacs divided by the number of embryos transferred. The cancellation rate was defined as the number of cycles with no oocytes retrieved, or no embryos available for transfer divided by the number of ovum pick-up cycles.

Statistical analysis

Continuous data are expressed as means ± standard deviation unless otherwise stated. An independent samples *t*-test was used to compare continuous variables that were normally distributed, while the Kruskal-Wallis test was applied for variables with skewed distribution. Categorical data are represented as number and percentage; differences in these variables were assessed by the chi-squared test or Fisher's exact test. Cumulative ongoing pregnancy per cycle was assessed both crudely and using multivariate logistic regression analysis. The decision to add the measured potential confounders to the model was based on previous scientific evidence.

Fifty-five patients were treated in different cycles by both protocols. We performed a sensitivity analysis in these patients (110 cycles) to assess if the results we obtained with the full sample set were biased by including multiple ovarian stimulation cycles in the same patient.

Statistical analysis was performed using the IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp, Armonk, NY, USA). All statistical tests were two-sided. Differences with a *P*-value < 0.05 were considered statistically significant.

Results

This study included 374 cycles performed in 294 patients that were divided into the study and control groups based on the stimulation protocol used. Baseline patient characteristics and demographic data are summarized in Table 1. The AFC was lower, and the basal FSH was higher in the study group patients. This suggests that the ovarian reserve may have been lower in these patients.

Table 1
Baseline patient characteristics and demographic data

	CC priming antagonist group (*n* = 193)	GnRH antagonist group (*n* = 181)	*P*-value
Age (years)	37.74 ± 5.14	37.72 ± 4.94	0.982
Duration of infertility (years)	3.81 ± 3.26	3.69 ± 3.28	0.472
BMI (kg/m^2)	22.68 ± 3.37	22.07 ± 3.46	0.084
AFC	4.76 ± 2.47	5.36 ± 2.14	0.012
AMH	0.62 ± 0.67	0.62 ± 0.59	0.979
Basal FSH (IU/L)	11.42 ± 4.35	10.13 ± 4.26	0.004
Basal LH (IU/L)	5.00 ± 1.85	4.85 ± 2.07	0.716
Basal E_2 (pg/mL)	44.30 ± 23.20	45.63 ± 18.37	0.768

Note: Values are presented as mean ± standard deviation, or *n*. CC = clomiphene citrate; BMI = body mass index; AFC = antral follicle count; AMH = Anti-Müllerian hormone; FSH = follicle stimulating hormone; LH = luteinizing hormone; E_2= estradiol.

Some of the ovarian stimulation characteristics were comparable between the treatment groups (Table 2). However, the total Gn dosage was considerably lower, and the Gn administration duration was shorter in the study group (Table 2). The endometrium in the study group was thinner on the ovulation triggering day, as expected from the anti-estrogen effect of CC on the endometrium. Although the difference was statistically insignificant, FORT was higher in the study group. Significantly less Gn was administered to obtain a pre-ovulatory follicle, and FOI was much higher in the study group (Table 2). Although the number of oocytes retrieved was similar between the groups, more available embryos were obtained in the study group (Table 2).

Table 2
Ovarian stimulation and laboratory outcomes

	CC priming antagonist group (*n* = 193)	GnRH antagonist group (*n* = 181)	*P*-value
Total Gn dosage (IU)	1850.58 ± 717.34	3138.09 ± 1052.72	<0.001
Gn duration (days)	6.87 ± 1.91	9.68 ± 1.85	<0.001
LH on day of trigger (IU/L)	5.48 ± 3.74	4.88 ± 2.89	0.489
E_2 on day of trigger (pg/mL)	1422.62 ± 962.99	1624.42 ± 1099.36	0.353
P on day of trigger (ng/mL)	0.56 ± 0.41	0.62 ± 0.42	0.492
Em thickness on day of trigger (mm)	7.17 ± 2.75	9.51 ± 2.38	<0.001
FORT (%)	0.94 ± 0.68	0.81 ± 0.47	0.249
Gn dosage per follicle (IU)	696.90 ± 604.10	1087.10 ± 824.76	<0.001
FOI (%)	1.13 ± 0.76	0.84 ± 0.42	0.006
No. of oocytes retrieved	4.38 ± 3.06	4.06 ± 2.58	0.623
No. of available embryos	1.79 ± 1.12	1.34 ± 1.04	<0.001

Note: Values are presented as mean ± standard deviation. *P*-values were calculated using the chi-squared or Fisher's exact test for categorical data and the *t*-test for continuous data. FORT: follicular output rate; FOI: follicle-to-oocyte index

Seventeen (8.8%) cycles in the study group and 47 (26.0%) in the control group were canceled as no oocyte was retrieved or no embryos were available for transfer ($P < 0.001$, Table 3). As of the time of statistical analysis, embryos remained cryopreserved without transfer for 25 cycles in the study group and 22 in the control group. The numbers of transferred cycles were 151 and 112 in the study and control groups, respectively. The crude reproductive outcomes were similar between the groups (Table 3).

Table 3
Reproductive outcomes

	CC priming antagonist group	GnRH antagonist group	Rate ratio in CC priming antagonist group (95% CI)	*P*-value
Cancellation	17/193 (8.8%)	47/181 (26.0%)	0.34 (0.20 - 0.57)	<0.001
No. of transferred cycle [a]	151	112		
Positive pregnancy	54 (35.8%)	39 (34.8%)	1.03 (0.74 - 1.43)	0.875
Implantation	51(17.3%)	44 (22.3%)	0.77 (0.54 - 1.11)	0.590
Cumulative clinical pregnancy	45 (29.8%)	39 (34.8%)	0.86 (0.60 - 1.22)	0.388
Cumulative ongoing pregnancy	34 (22.5%)	33 (29.5%)	0.70 (0.40 - 1.21)	0.201
Cumulative live birth	33 (21.9%)	33 (29.5%)	0.74 (0.49 - 1.12)	0.159

P-values were calculated using the chi-squared or Fisher's exact test. [a] For 25 cycles in CC priming antagonist group and 22 cycles in GnRH antagonist group, embryos remained cryopreserved without transfer until statistical analysis. CI, confidence interval

Table 4 summarizes the results of a multivariate regression analysis. After adjusting for potential confounders, the multivariate regression analysis showed that cumulative ongoing pregnancy was still comparable between the treatment groups (adjusted odds ratio: 0.761, 95% confidence interval: 0.300-1.933, $P = 0.566$, Table 4). The results showed that age, BMI, Gn dose per follicle, and FOI were directly associated with reproductive outcomes.

Table 4
OR for cumulative ongoing pregnancy by multivariate regression analysis

Exposure	OR	95%CI	*P* value	OR adj.	95%CI	*P* value
		Univariate			Multivariate	
protocol						
GnRH antagonist protocol	Ref.	Ref.		Ref.	Ref.	
CC priming antagonist protocol	0.669	0.382 - 1.172	0.160	0.761	0.300 - 1.933	0.566
Age (years)	0.888	0.836 - 0.942	<0.001	0.899	0.834 - 0.969	0.005
Duration of infertility (years)	0.924	0.836 - 1.021	0.120	0.954	0.847 - 1.074	0.434
BMI (kg/m^2)	0.871	0.791 - 0.958	0.004	0.879	0.794 - 0.973	0.013
Basal FSH (IU/L)	1.030	0.964 - 1.101	0.382	1.021	0.942 - 1.107	0.615
Total Gn dosage (per 100 IU)	1.006	0.981 - 1.031	0.650	1.028	0.962 - 1.100	0.412
Gn duration (days)	1.068	0.946 - 1.205	0.288	1.011	0.768 - 1.330	0.940
FORT (%)	1.228	0.796 - 1.896	0.353	0.906	0.503 - 1.634	0.743
Gn dosage per follicle (per 100 IU)	0.881	0.812 - 0.956	0.002	0.864	0.770 - 0.971	0.014
FOI (%)	0.879	0.762 - 0.913	0.021	0.856	0.758 - 0.904	0.027

Ref., reference; OR, odds ratio; OR adj, adjusted odds ratio

Details of the sensitivity analyses are presented in Tables 5 and 6. Fifty-five patients were treated in different cycles by both protocols. Stimulation characteristics and laboratory outcomes were compared. With comparable numbers of retrieved oocytes and available embryos, patients were administered less Gn (1,739.09 ± 719.39 vs. 3,114.77 ± 1,171.23, $P < 0.001$), and the duration of Gn administration was shorter (6.58 ± 2.23 vs. 9.80 ± 1.90, $P < 0.001$) when stimulated by the CC priming antagonist protocol, suggesting a lower cost per treatment cycle. Gn dosage per follicle, indicating how much Gn was administered to obtain one preovulatory follicle, was considerably lower after the CC priming antagonist protocol, hinting at better ovarian sensitivity. The reproductive outcomes following the two protocols were similar.

Table 5
Sensitivity analysis of ovarian stimulation and laboratory outcomes

	CC priming antagonist group	GnRH antagonist group	*P*-value
n	55	55	
Total Gn dosage (IU)	1739.09 ± 719.39	3114.77 ± 1171.23	<0.001
Gn duration (days)	6.58 ± 2.23	9.80 ± 1.90	<0.001
LH on day of trigger (IU/L)	5.42 ± 2.57	4.89 ± 3.41	0.547
E_2 on day of trigger (pg/mL)	951.87 ± 554.80	1729.46 ± 994.06	0.016
P on day of trigger (ng/mL)	0.63 ± 0.45	0.63 ± 0.44	0.891
Em thickness on day of trigger (mm)	6.57 ± 2.79	8.73 ± 2.69	<0.001
FORT (%)	0.92 ± 0.79	0.71 ± 0.40	0.443
Gn dosage per follicle (IU)	637.36 ± 373.05	1286.26 ± 976.66	<0.001
FOI (%)	1.05 ± 0.66	0.83 ± 0.64	0.248
No. of oocytes retrieved	4.20 ± 3.64	4.04 ± 2.89	0.745
No. of available embryos	1.53 ± 1.14	1.15 ± 0.93	0.086

Note: Values are presented as mean ± standard deviation, or *n*. *P*-values were calculated using the chi-squared or Fisher's exact test for categorical data and the *t*-test for continuous data. Kruskal-Wallis test was applied for the variables with a skewed distribution.

Table 6
Sensitivity analysis of reproductive outcomes

	CC priming antagonist group	GnRH antagonist group	Rate ratio in CC priming antagonist group (95% CI)	*P*-value
Cancellation	13/55 (23.6%)	17/55 (30.9%)	0.76 (0.41 - 1.42)	0.392
No. of transferred cycle [a]	34	31		
Positive pregnancy	11 (32.4%)	4 (12.9%)	2.51 (0.89 - 7.06)	0.063
Implantation	12 (17.1%)	4 (7.3%)	2.36 (0.80 - 6.91)	0.101
Cumulative clinical pregnancy	10 (29.4%)	4 (12.9%)	2.28 (0.80 - 6.53)	0.106
Cumulative ongoing pregnancy	8 (23.5%)	2 (6.5%)	3.65 (0.84 - 15.88)	0.057
Cumulative live birth	8 (23.5%)	2 (6.5%)	3.65 (0.84 - 15.88)	0.057

P-values were calculated using the chi-squared or Fisher's exact test.

[a] For 8 cycles in CC priming antagonist group and 7 cycles in GnRH antagonist group, embryos remained cryopreserved without transfer until statistical analysis.

Discussion

POR patients undergoing IVF/intracytoplasmic sperm injection (ICSI) usually suffer from a limited number of oocytes, poor embryo quality, and a low pregnancy rate per cycle. Considerable attention has been paid to developing strategies to improve oocyte quality and quantity. Clinicians were the first to perform modifications to the ovarian stimulation protocol. Multiple stimulation protocols were reported, including GnRH antagonist protocol, luteal phase ovarian stimulation, mild/minimal stimulation, progestin-primed ovarian stimulation, and modified natural cycles [19-22]. Various Gn and/or starting doses have also been administered [23, 24]. Although there is insufficient evidence to support their administration, supplements such as growth hormone, dehydroepiandrosterone, coenzyme Q10, and multi-nutrients have all been used in an attempt to improve oocyte quality [25-29]. To date, the most effective protocol for POR patients remains controversial, and the management of these patients is still a challenge for clinicians.

An increasing number of researchers believe that an efficient, patient-friendly regimen that can improve ovarian response and decrease the costs involved is needed for POR patients. Oral ovulation induction medications such as CC fulfill the concept of patient-friendly IVF. CC is a selective estrogen receptor modulator that binds competitively to estrogen receptors. By the negative feedback of estrogen, secretion

of gonadotropin hormones increases, and follicular growth is induced. The present study aimed to compare ovarian response and clinical outcomes in POR patients treated by a CC priming protocol vs. a flexible GnRH antagonist protocol.

Previous studies suggested that FORT could act as an efficient quantitative and qualitative marker and be used to evaluate the ovarian sensitivity to gonadotropins and predict IVF/ICSI outcomes [30]. In the present study, patients in the study group presented a higher FORT, although the difference was statistically insignificant. It might be partially due to the limited sample size of this study. Furthermore, patients in the study group had fewer antral follicles and a higher basal FSH level, suggesting a lower ovarian reserve. FOI also reflects the ovarian sensitivity to Gn [17, 31]. The present study demonstrated a much higher FOI in the study group, even though they had a lower AFC than the control group. We also evaluated ovarian sensitivity to stimulation by Gn dosage per follicle, which showed promising results in our unpublished data. The results indicated that the Gn dosage required to obtain a pre-ovulatory follicle was considerably lower in the study group. Growing evidence suggests that increased Gn stimulation cannot improve clinical outcomes, but it increases the treatment costs for poor ovarian responders [32]. Therefore, the lower Gn dosage per pre-ovulatory follicle in the study group suggested a better ovarian sensitivity to Gn than in the control group. We conclude that patients stimulated by the CC priming antagonist protocol achieved a better ovarian response or sensitivity.

Such an improved ovarian response could have several causes. First, through a negative feedback mechanism. CC may have occupied the hypothalamic estrogen receptors for a longer time than estrogen [33], increasing GnRH release, and thus the endogenous gonadotropin levels in the patients, including FSH and LH. FSH could stimulate follicular development and synthesis of estrogen. As a result, the administration of CC improved follicular growth and reduced the exogenous FSH dose needed. Second, CC increased the release of endogenous LH through the feedback increase of GnRH. POR patients, in whom LH activity is usually insufficient, may have benefited from the additional LH. Many researchers commented that adding LH to hypo-responders could increase the number of mature oocytes and improve implantation rate while significantly reducing the total FSH dosage administered [4, 34-37]. Moreover, CC has a relatively long half-life of 5 to 7 days, going through liver metabolization and stool excretion. This suggests that residual CC continues to work for some time after terminating its administration. In this case, CC increased the endogenous FSH and LH content for a long time, improving the ovarian response to the stimulation process.

CC is a selective estrogen modulator that can negatively impact endometrial development, resulting in a thinner endometrium. Previous studies reported that a negative effect of CC on the endometrium was behind the inferior fertility outcomes [38, 39]. In the present study, endometrial thickness in the study group on the ovulation triggering day was significantly lower than in the control group. Recent studies suggested that under CC treatment, an endometrial thickness cut-off value of $\geq$8 mm at midcycle was associated with a better outcome [40]. It is well known that time-to-pregnancy should be considered when making decisions related to infertility treatment, especially for POR patients whose ovarian reserve suffers from a considerable decline. Therefore, we propose that when sufficient endometrial thickness is

observed on ovulation triggering day in the study group, the negative effect of CC during ovarian stimulation will be avoided, and a good pregnancy outcome could be achieved by fresh embryo transfer, resulting in a shorter time-to-pregnancy.

The study group had a significantly lower total Gn dosage and shorted Gn administration time than the control group. Such results imply lower costs and more convenient and patient-friendly treatment when using the CC priming protocol. These aspects are important, considering that POR patients often suffer from economic and time stresses due to the need for repeated ovarian stimulation.

There were no differences in the rates of cumulative clinical pregnancy, cumulative ongoing pregnancy, or live birth per cycle between the two groups. The cumulative ongoing pregnancy rate was comparable between treatment groups even after adjusting for possible confounders. Age, BMI, Gn dosage per follicle, and FOI were all directly associated with reproductive outcomes (Table 4). Considering the limited size of the present study and limited number of available embryos for transfer per cycle in POR patients, the comparison of reproductive outcomes between the two protocols needs further research.

POR patients often suffer from repeated, unsuccessful ovarian stimulation cycles. We performed a sensitivity analysis of 55 patients stimulated by both protocols in separate cycles as a comparative self-control study. Most stimulation parameters and laboratory outcomes were in line with the data on the entire POR patient group. FORT, FOI, and the number of available embryos were higher in the study group, although the differences were statistically insignificant. This insignificant outcome could, at least partially, be because of the limited sample size available for the comparison. Although there were no statistically significant differences, reproductive outcomes in the self-control study appeared to have improved following CC priming stimulation.

Guidance on how to most optimally manage POR patients is still lacking till now. Many clinicians may be keener on developing new ovarian stimulation protocols, but the old things, including drugs, may still have new uses, such as CC priming protocol in the present study for POR patients. CC has been used for ovarian stimulation since decades. However, the application of CC in this study still achieved very inspiring results. For clinicians, the results could inspire them raising concerns regarding the use of this convenient and patient-friendly protocol during ovarian stimulation in POR patients to increase ovarian sensitivity. For POR patients, it is helpful to enhance the treatment confidence of them, better cooperate with doctors and shorten time-to-pregnancy.

In conclusion, the current study suggests that the CC priming protocol offers a convenient and patient-friendly way to reduce the costs and increase ovarian sensitivity to stimulation in POR patients. The study has some limitations arising from its retrospective nature and small sample size. Secondly, the patients are from a single center with similar ethnicities. Thirdly, the Bologna criteria is used for definition of POR in the present study. Further large scale multicenter randomized clinical trials are warranted to verify the utility of this CC priming antagonist protocol and should be based on the POSEIDON criteria.

Abbreviations

POR
poor ovarian response
CC
clomiphene citrate
IVF
in vitro fertilization
GnRH
gonadotropin-releasing hormone
(LH)luteinizing hormone
FSH
follicle-stimulating hormone
FORT
follicle output rate
FOI
follicle-to-oocyte index
Gn
gonadotropin
rFSH
recombinant FSH
AFC
antral follicle count
BMI
body mass index
rhCG
recombinant human chorionic gonadotropin
ICSI
intracytoplasmic sperm injection

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Beijing Chao-Yang Hospital, Capital Medical University. Written informed consent was waived due to the retrospective nature of the study. Analyses of data was performed in accordance with the rules and regulation with approvals from the ethics committee of our hospital.

Consent for publication

Not applicable.

Availability of data and materials

Data are available from the authors upon reasonable request and with permission of Professor Yuan Li.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

SL: data collection and statistical analysis; drafting of the manuscript. XL, HL, ML, and YLv: data collection and revision of the manuscript; YLi: supervision of the study, conception, and review of the manuscript. All authors performed revision of intellectual content and approved the final version of the article.

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Figures

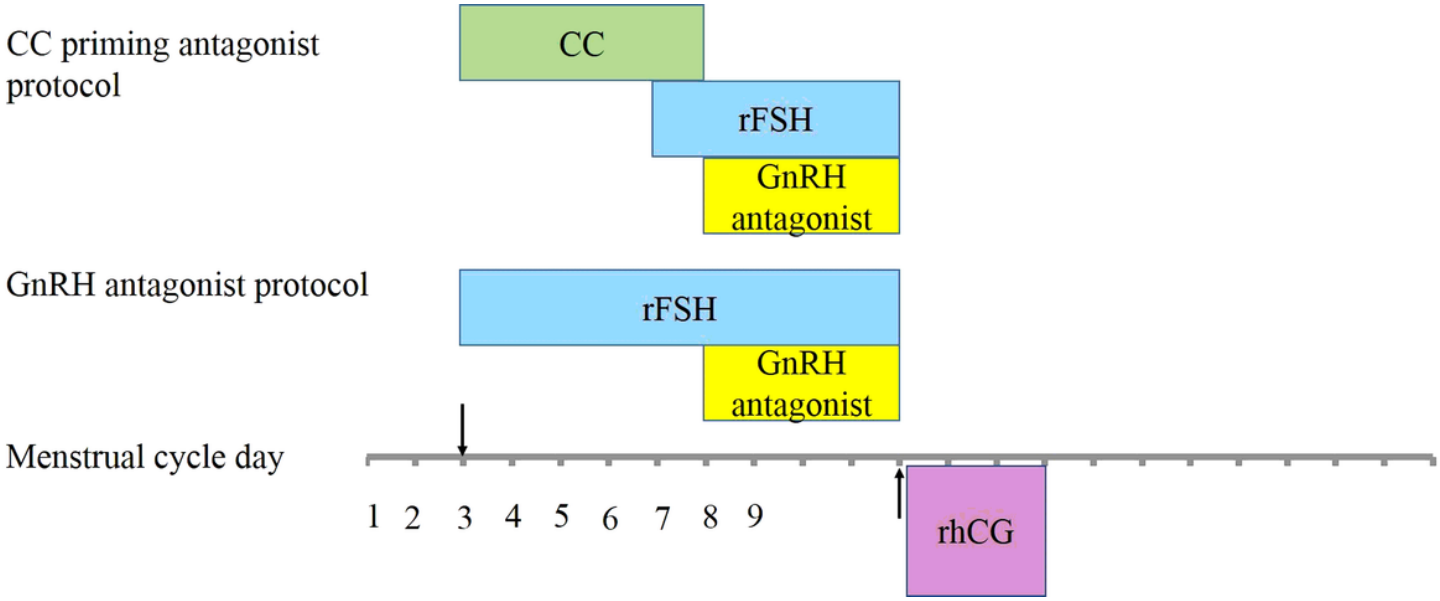

Figure 1

Ovarian CC priming and flexible GnRH antagonist stimulation protocols. CC, clomiphene citrate; GnRH, gonadotropin-releasing hormone; rFSH, recombinant follicle-stimulating hormone; rhCG, recombinant human chorionic gonadotropin.

Supplementary Files

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