ORIGINAL RESEARCH

Progestin-Primed Ovarian Stimulation with Clomiphene Citrate Supplementation May Be More Feasible for Young Women with Diminished Ovarian Reserve Compared with Standard Progestin-Primed Ovarian Stimulation: A Retrospective Study

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Purpose: The present study was designed to compare the efficiency of the progestin-primed ovarian stimulation (PPOS) protocol with clomiphene citrate (CC) supplementation (PPOS+CC) and the standard PPOS protocol for women of different ages with diminished ovarian reserve (DOR).

Patients and Methods: This retrospective cohort study included 364 DOR women who underwent controlled ovarian stimulation with PPOS+CC (n = 223) or standard PPOS (n = 141). They were divided into subgroups based on age: ≤35 years and >35 years. Differences in baseline characteristics, ovarian stimulation characteristics, endocrinological characteristics, and clinical outcome between the two groups were assessed. Statistical analyses were stratified by age.

Results: In all women with DOR, PPOS+CC was associated with a lower percentage of women with profound pituitary suppression than standard PPOS (0.0% vs 18.6%, $P < 0.001$ and 1.3% vs 11.0%, $P = 0.002$). In young women with DOR, more high-quality cleavage-stage embryos were harvested (1.96 vs 1.38, $P = 0.018$) and a lower dosage of gonadotropin per oocyte retrieved was required (558.37 vs 909.82, $P = 0.036$) in PPOS+CC. In older women with DOR, PPOS+CC led to an increase in the incidence of luteinizing hormone (LH) surge levels above 10 IU/L on trigger day (12.7% vs 4.9%, $P = 0.028$) and a decrease in the rate of oocyte maturation (84.7% vs 89.9%, $P = 0.034$) compared to standard PPOS.

Conclusion: Clomiphene citrate is an effective adjuvant to alleviate pituitary suppression in PPOS protocols; for young women with DOR, CC supplementation had a positive impact on the number of high-quality embryos. However, older women with DOR would be at risk of developing a premature LH surge and having poor oocyte maturation rate under the PPOS+CC protocol.

Keywords: diminished ovarian reserve, clomiphene citrate, medroxyprogesterone acetate, controlled ovarian stimulation, in vitro fertilization, luteinizing hormone surge

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Plain Language Summary

To evaluate clomiphene citrate (CC) supplementation in the progestin-primed ovarian stimulation (PPOS) protocol for women with diminished ovarian reserve (DOR), our study included 364 women with DOR who underwent controlled ovarian stimulation with the PPOS protocol and CC supplementation (PPOS+CC; n = 223) or standard PPOS (n = 141) from June 2018 to February 2020. Our results suggest that, for young women with DOR,

PPOS+CC led to an increase in the number of high-quality embryos and a reduction of gonadotropin dosage compared to standard PPOS. That is, PPOS+CC can be doubly beneficial for young women with DOR. However, older women (>35 years) with DOR would be at risk of developing a premature luteinizing hormone surge and poor oocyte maturation rate after PPOS+CC. CC supplementation in the PPOS protocol may not improve clinical outcomes for older women with DOR; rather it may lead to more risky outcomes.

Introduction

In assisted reproduction technologies (ART), the number of retrieved oocytes largely depends on a woman's ovarian reserve. Diminished ovarian reserve (DOR), which can be either age dependent or independent, is manifested mainly as increased follicle-stimulating hormone (FSH), decreased anti-Müllerian hormone (AMH) level, and low antral follicle count (AFC).[1] A reduced ovarian reserve often leads to some predictable clinical results, such as cycle cancellation, increased gonadotropin (Gn) consumption, or decreased number of oocytes retrieved, and consequent stress and disappointment to the women and their partners. As a decreased oocyte count is the major issue for DOR, it is critical to collect sufficient high-quality oocytes for subsequent embryo transfer in women with DOR. Although many stimulation protocols have been established to improve the number and quality of retrieved oocytes for women with DOR, identification of the most patient friendly protocol remains controversial.[2]

Progestin-primed ovarian stimulation (PPOS) has been approved for its effect in reducing cycle cancellation and preventing an endogenous luteinizing hormone (LH) surge during controlled ovarian stimulation.[3–7] Medroxyprogesterone acetate (MPA) is a simple, convenient, effective, and cheap treatment that can be delivered orally. Previous studies show that PPOS improves the quantity and quality of oocytes and is a more feasible controlled ovarian stimulation protocol for women with DOR or poor ovarian response compared with conventional protocols and mild/minimal stimulation.[8–10] However, the continuous supply of progestin during PPOS can lead to profound pituitary suppression.[4–6] Profound pituitary suppression is positively correlated with Gn consumption.[4–6] Clomiphene citrate (CC) is used to block negative feedback triggered by estrogen, thereby resulting in elevated FSH and LH.[11,12] PPOS supplemented with CC (PPOS+CC) significantly reduces the occurrence of profound pituitary suppression in women with normal ovarian reserve or polycystic ovarian syndrome, and the combination treatment is likely to reduce Gn consumption compared with PPOS treatment alone.[5,6]

Currently, the efficacy of PPOS+CC in women with DOR compared with standard PPOS is unclear. DOR reflects a quantitative rather than qualitative decline of the ovarian reserve, whereas age is considered a reliable marker for oocyte quality.[13] Relationship between maternal age and risk of chromosomal abnormalities has been clearly demonstrated by epidemiological studies.[14] Physiological decline in the ovarian follicle pool with ageing is often accompanied by decreased ovarian response to Gn stimulation with advancing age.[15] It is known that young women with DOR may have a different prognosis from older women.[16–18] Therefore, the present study was designed to comprehensively compare the efficiency of PPOS+CC and standard PPOS in young and older women with DOR.

Materials and Methods
Study Design and Participants

We conducted a retrospective cohort study at the Reproductive Medicine Center of the First Affiliated Hospital of Wenzhou Medical University. From June 2018 to February 2020, women who met two or more of the following conditions were diagnosed with DOR: (a) basal FSH level ≥10 IU; (b) FSH/LH ≥2; (c) AFC ≤8; and (d) AMH ≤1.1. Both in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) cycles were included. Women who were diagnosed with uterine cavity abnormalities, untreated hydrosalpinx, and immunologic disease were excluded. Every woman contributed to the study with one stimulation cycle. In women with multiple treatment cycles, the first chronological cycle was chosen. For analysis, couples were grouped as follows: women using PPOS+CC were grouped into the "PPOS+CC group," while women using standard PPOS were grouped into the "standard PPOS group". Subpopulations of women were classified based on different age cut-offs, namely ≤35 years and >35 years. All participants provided informed consent after counseling regarding infertility treatments and routine IVF procedures. Using the unique personal identification number, all data were retrospectively collected from computer databases and stored in an anonymous database. This study was approved by the ethics committee of the

First Affiliated Hospital of Wenzhou Medical University (no. 2020–02) and performed according to the principles embodied in the Declaration of Helsinki.

Stimulation Protocols and Pituitary Suppression

In PPOS+CC, HMG (Shanghai Livzon Pharmaceutical Company, Zhuhai, China) 150-225 IU; CC (Codal Synto Limited, Limassol, France) 100 mg; and MPA (Shanghai Xinyi Pharmaceutical Co., China) 10 mg daily were administered from menstrual cycle days 2–3 (MC2-3) or after an episode of withdrawal bleeding. In standard PPOS, HMG 150-225 IU and MPA 10 mg daily were initiated from menstrual cycle days 2–3 (MC2-3) or after an episode of withdrawal bleeding. The initial dose of Gn (150-225 IU) was based on patient age, basic FSH, AFC, and body mass index (BMI). Follicle monitoring and hormone assay (FSH, LH, estradiol [E_2], and progesterone [P]) were performed 5 days later. The dose of Gn was adjusted according to follicle development, and MPA dose was consistent up to trigger day. When the dominant follicle diameter reached >17 mm and the majority of growing follicles, if any, reached ≥14 mm, recombinant human chorionic gonadotropin (Ovitrelle, Serono, Bari, Italy) was administered to trigger ovulation. Oocytes were retrieved 36–38 h later. All follicles >10 mm in diameter were aspirated.

Fertilization was carried out in vitro after oocyte retrieval depending on the semen parameters and previous fertilization conditions. Embryos were examined for the number or regularity of blastomeres and the degree of fragmentation. One or two high-quality cleavage-stage embryos (7–9 cells and <20% fragmentation) were frozen via vitrification on day 3 after oocyte retrieval, and the remaining embryos were placed in extended culture.[19,20] Subsequently, blastocysts with good morphological grades were frozen on days 5 or 6 of culture.

Hormone Measurement

Serum FSH, LH, E_2, and P were measured on menstrual cycle days 2–3, days 7–10, and trigger day. Hormone levels were determined by immunofluorescence assays (Roche Diagnostics, Mannheim, Germany). The lower limits of sensitivity were as follow: FSH, 0.06 IU/L; LH, 0.09 IU/L; E_2, 10 pg/mL; and P, 0.1 ng/mL. A serum LH concentration <1.0 IU/L on trigger day was set as the cut-off for profound pituitary suppression.[21]

Endometrium Preparation and Frozen Embryo Transfer

All women in the present study received frozen-thawed embryo transfer (FET). Hormone replacement treatment was used for endometrial preparation. Briefly, the timing of embryo transfer was scheduled on day 3 or day 5 after progestin administration depending on the embryo stage. All embryo transfers were performed under abdominal ultrasound guidance in our center. Each patient received no more than two embryos at one time. Women received luteal support after embryo transfer in the form of intra-vaginal progestin 200 mg, twice daily. Once pregnancy was achieved, the luteal support was continued until 10 weeks of gestation.

Outcome Measures

We analyzed pregnancy outcomes of the chronological first cycle after oocyte retrieval that transferred at least one high-quality cleavage-stage embryo or good morphology blastocyst (blastocysts better than grade 322).[19] Clinical pregnancy was defined as the presence of fetal cardiac activity confirmed by transvaginal ultrasound. Ongoing pregnancy rate was defined as the proportion of women with ongoing pregnancy after a gestational age of 12 weeks. The implantation rate was calculated as the number of gestational sacs visualized on transvaginal ultrasound divided by the number of transferred embryos.

Statistical Analysis

Data were evaluated by Student's *t*-test for continuous variables of normal distribution, Mann–Whitney *U*-test for continuous variables of non-normal distribution, and chi-square test or Fisher's exact for categorical variables, as appropriate. All tests were two-sided, and $P < 0.05$ was considered statistically significant. SPSS software (version 25; IBM Corporation, Armonk, NY, USA) was used for data analysis. The dynamic changes in hormones during controlled ovarian stimulation were presented by a broken line graph created using Microsoft Excel.

Results
Patient Characteristics

In total, 364 women were enrolled. Table 1 shows the comparison of basic patient characteristics between ovarian stimulation groups stratified by age. No statistically significant differences were found between the groups with respect to age, duration of infertility, proportion of

Table 1 Basic Patient Characteristics Between Ovarian Stimulation Groups Stratified by Age

	≤35 Years			>35 Years		
	PPOS+CC Group (n = 73)	**Standard PPOS Group (n = 59)**	*P*-value	**PPOS+CC Group (n = 150)**	**Standard PPOS Group (n = 82)**	*P*-value
Age (years)	30.82±2.66	30.71±3.19	0.825	40.25±3.02	39.90±3.31	0.423
Duration of infertility (years)	3.29±2.46	3.96±2.80	0.148	3.62±3.28	4.36±4.07	0.138
Secondary infertility, % (n)	64.4 (47/73)	69.5 (41/59)	0.536	65.3 (98/150)	62.2 (51/82)	0.634
Cause of infertility, % (n)			NE[a]			NE[a]
Tubal factor	67.1 (49/73)	50.8 (30/59)		61.33 (92/150)	62.2 (51/82)	
Male factor	56.16 (41/73)	37.3 (22/59)		52 (78/150)	47.6 (39/82)	
Other factors	17.8 (13/73)	8.5 (5/59)		16.7 (25/150)	15.9 (13/82)	
Unknown factor	13.7 (10/73)	11.9 (7/59)		6.7 (10/150)	11.0 (9/82)	
Failed cycles	1.81±1.45	2.11±1.37	0.226	1.77±1.52	2.07±1.65	0.164
BMI (kg/m^2)	22.47±3.58	21.39±3.14	0.067	22.56±2.69	22.17±2.75	0.295
AMH (ng/mL)	0.88±0.61	0.95±0.64	0.530	0.88±0.58	0.86±0.49	0.785
Antral follicle counts	6.22±2.08	6.15±2.03	0.854	5.69±2.25	6.01±2.31	0.298
Baseline hormones						
FSH (IU/L)	11.13±5.33	11.23±4.18	0.904	11.10±5.02	12.19±4.96	0.116
LH (IU/L)	4.49±2.45	4.13±2.05	0.370	4.22±2.22	4.68±2.29	0.131
E$_2$ (pg/mL)	53.44±34.97	53.29±29.55	0.980	58.57±44.35	59.78±45.34	0.843
P (ng/mL)	0.55±0.31	0.60±0.41	0.380	0.52±0.46	0.51±0.40	0.848

Notes: Data are presented as mean ± standard deviation or percentage (number); [a]Because someone may have more than 1 cause of infertility.
Abbreviations: PPOS, progestin-primed ovarian stimulation; CC, clomiphene citrate; BMI, body mass index; AMH, anti-Müllerian hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E$_2$, estradiol; P, progesterone; NE, not estimable.

secondary infertility, BMI, AMH, AFC, and baseline hormones ($P > 0.05$).

Controlled Ovarian Stimulation and Embryological Outcomes

The controlled ovarian stimulation and embryological outcomes of both groups are presented in Table 2. No statistical differences were found in Gn duration, two pro-nucleate (2PN) rate, number of 2PN cleavage, and cancellation rate prior to oocyte retrieval between the groups ($P > 0.05$). PPOS+CC had lower doses of Gn per oocyte retrieved than standard PPOS, especially for women ≤35 years (558.37 ± 499.71 vs 909.82 ± 1183.15, $P = 0.036$). In addition,

women aged ≤35 years in the PPOS+CC group showed a significantly higher number of high-quality cleavage-stage embryos compared with those in standard PPOS group (1.96 vs 1.38, $P = 0.018$). For women >35 years, the mean number of oocytes retrieved in PPOS+CC was significantly higher than those in standard PPOS group (4.23 vs 3.50, $P = 0.034$). However, the oocyte maturation rate in PPOS+CC was significantly lower than those in standard PPOS (84.7% vs 89.9%, $P = 0.034$).

Hormone Profile During Treatment

Serum concentrations of FSH, LH, E$_2$, and P in the two groups stratified by age are presented in Figure 1. In both

Table 2 Controlled Ovarian Stimulation and Embryological Outcomes of Both Groups

	≤35 Years			>35 Years		
	PPOS+CC Group (n = 73)	**Standard PPOS Group (n = 59)**	**P-value**	**PPOS+CC Group (n = 150)**	**Standard PPOS Group (n = 82)**	**P-value**
Gn duration (days)	9.32±2.45	9.90±3.75	0.284	9.71±2.28	10.05±3.06	0.386
Total dosage of Gn (IU)	1975.34±555.86	2052.54±1448.86	0.700	2050.53±575.89	1993.29±971.05	0.626
No. of >14mm follicles on trigger day	4.34±2.29	3.20±1.57	0.001	3.51±1.89	3.28±1.93	0.374
No. of oocytes retrieved	5.10±2.72	4.44±3.97	0.264	4.23±2.64	3.50±2.16	0.034
Dosage of Gn per oocyte retrieved (IU)	558.37±499.71	909.82±1183.15	0.036	739.29±606.90	895.92±941.00	0.176
ICSI MII oocytes rate, % (n)	87.3 (165/189)	80.0 (56/70)	0.140	82.5 (274/332)	88.3 (121/137)	0.118
Oocyte maturation rate, % (n)	85.2 (317/372)	82.8 (217/262)	0.416	84.7 (537/634)	89.9 (258/287)	0.034
2PN rate of IVF oocytes, % (n)	78.9 (120/152)	72.0 (116/161)	0.157	70.7 (186/263)	79.6 (109/137)	0.057
2PN rate of ICSI oocytes, % (n)	79.4 (131/165)	82.1 (46/56)	0.656	80.7 (221/274)	79.3 (96/121)	0.762
No. of 2PN cleavage	3.54±2.17	2.89±2.00	0.089	2.75±1.82	2.55±1.57	0.407
No. of high-quality cleavage-stage embryos	1.96±1.44	1.38±1.26	0.018	1.47±1.28	1.46±1.28	0.983
High-quality cleavage-stage embryos cycles rate, % (n)	11.0 (8/73)	33.9 (20/59)	0.001	(38/150)	(19/82)	0.715
Cancellation rate prior to oocyte retrieval, % (n)	2.7 (2/73)	0.0 (0/59)	0.502	0.0 (0/150)	2.4 (2/82)	0.124

Note: Data are presented as mean ± standard deviation or percentage (number).
Abbreviations: PPOS, progestin-primed ovarian stimulation; CC, clomiphene citrate; Gn, gonadotropin; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; 2PN, two pro-nucleate; MII, metaphase of second meiosis; No, number.

groups, FSH levels increased after Gn administration, and no difference was found between the groups ($P > 0.05$). Serum E_2 levels and P levels increased gradually in both groups after Gn administration and on trigger day in PPOS+CC they were significantly higher than those in all sub-groups of standard PPOS (1974.68 ± 1239.05 pg/mL vs 1143.19 ± 742.72 pg/mL, $P < 0.001$; 1777.37 ± 1148.10 pg/mL vs 1220.22 ± 796.84 pg/mL, $P < 0.001$; 1.02 ± 0.50 ng/mL vs 0.83 ± 0.53 ng/mL, $P = 0.043$; and 0.99 ± 0.87 ng/mL vs 0.69 ± 0.40 ng/mL, $P < 0.001$, respectively).

LH levels of the two groups showed different trends. LH levels in PPOS+CC initially increased and then remained steady at a range of 5.52–6.61 IU/L; by contrast, the LH levels in standard PPOS remained at a certain level initially and then decreased slightly. There was no significant difference in the value of basal LH between the groups ($P > 0.05$),

whereas LH levels on days 7–10 and on the day of trigger in PPOS+CC were significantly higher than those in all sub-groups of standard PPOS ($P < 0.05$).

The percentage of women with profound pituitary suppression was significantly lower in PPOS+CC than in standard PPOS (0.0% [0/73] vs 18.6% [11/59], $P < 0.001$ and 1.3% [2/150] vs 11.0% [9/82], $P = 0.002$). In addition, compared with standard PPOS, the percentage of women with LH levels >10 IU/L on the trigger day was higher in PPOS+CC, especially for women aged >35 years (12.7% [22/150] vs 4.9% [4/82], $P = 0.028$). For all women with LH levels >10 IU/L on trigger day, the presence of sonolucent follicles was confirmed by transvaginal ultrasound just before the planned oocyte retrieval. Of all these women who underwent oocyte retrieval, two had no oocytes retrieved and one woman had no mature oocytes for retrieval.
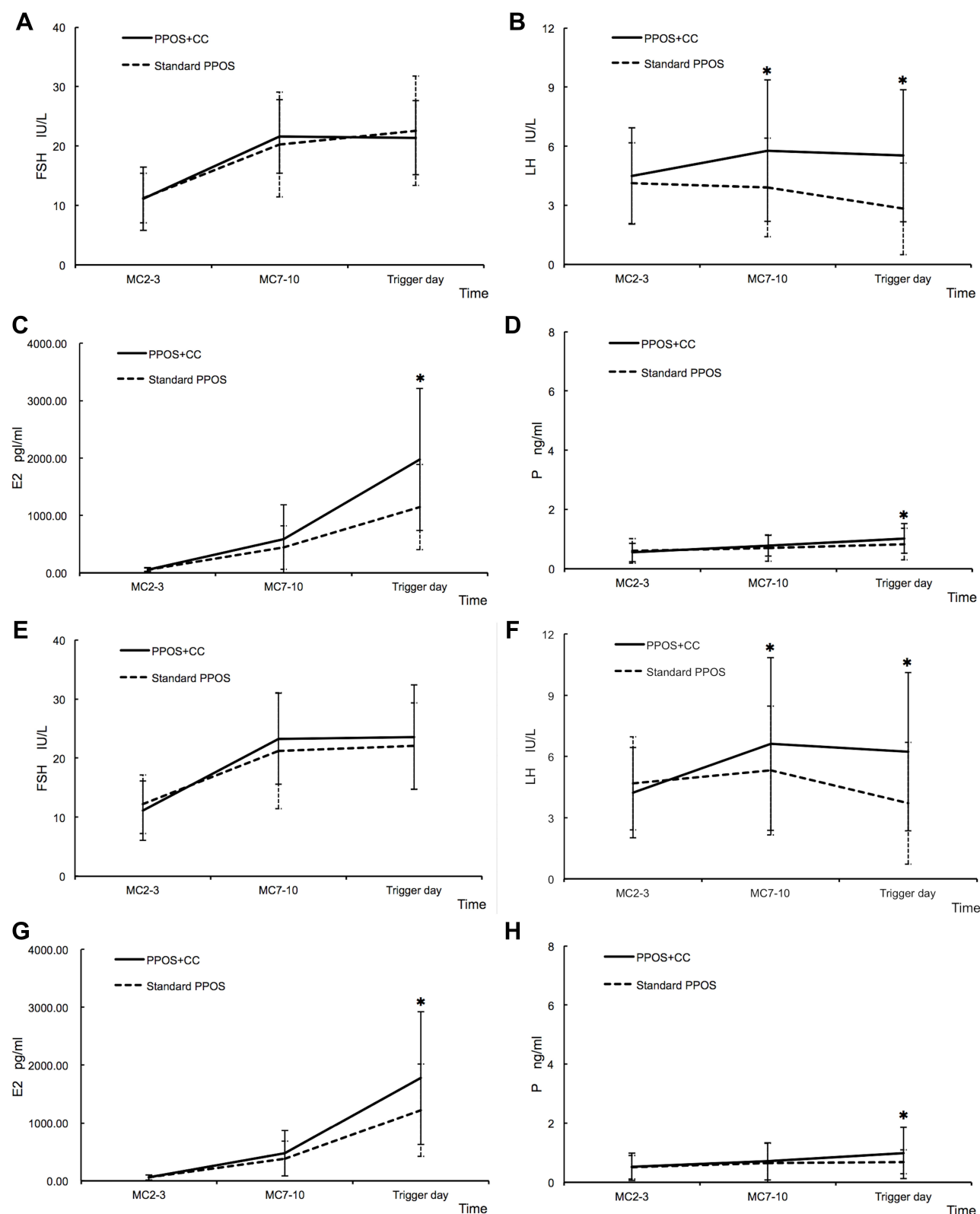
Figure 1 The dynamic changes in hormones during ovarian stimulation in the two groups stratified by age. (**A–D**) Hormone measurement for women aged ≤35 years, (**E–H**) Hormone measurement for women aged >35 years. The asterisk (*) represents P <0.05 at the time point.
Abbreviations: PPOS, progestin-primed ovarian stimulation; CC, clomiphene citrate; MC, menstrual cycle day; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2, estradiol; P, progesterone.

Table 3 Pregnancy Outcomes of Both Groups

	≤35 Years			>35 Years		
	PPOS+CC Group (n = 56)	**Standard PPOS Group (n = 35)**	**P-value**	**PPOS+CC Group (n = 92)**	**Standard PPOS Group (n = 57)**	**P-value**
No. of transferred embryos	1.86±0.35	1.74±0.44	0.202	1.79±0.41	1.79±0.41	0.954
Endometrial thickness (mm)	9.86±1.87	9.37±1.89	0.233	9.69±1.86	9.21±1.69	0.119
Clinical pregnancy rate, % (n)	44.6 (25/56)	54.3 (19/35)	0.371	35.9 (33/92)	28.1 (16/57)	0.325
Ongoing pregnancy rate, % (n)	39.3 (22/56)	51.4 (18/35)	0.256	22.8 (21/92)	17.5 (10/57)	0.440
Implantation rate, % (n)	29.8 (31/104)	41.0 (25/61)	0.143	21.8 (36/165)	17.6 (18/102)	0.410
Miscarriage rate, % (n)	12.0 (3/25)	5.3 (1/19)	0.622	36.4 (12/33)	25.0 (4/16)	0.526
Ectopic pregnancy rate, % (n)	0	0	NE	3.0 (1/33)	6.3 (1/16)	1.000
Live birth, % (n)	39.3 (22/56)	51.4 (18/35)	0.256	21.7 (20/92)	17.5 (10/57)	0.535

Note: Data are presented as mean ± standard deviation or percentage (number).
Abbreviations: PPOS, progestin-primed ovarian stimulation; CC, clomiphene citrate; NE, not estimable.

Pregnancy Outcomes

A total of 279 women achieved at least one high-quality cleavage-stage embryo or good morphology blastocyst after oocyte retrieval, among which 35 women declined FET for personal reasons before the end of the study, and four women were transferred with embryos collected from two oocyte retrievals in subsequent FET. Thus, only 240 FET cycles were included in the analysis of pregnancy outcomes (Table 3). The rate of blastocyst transfers was similar between the groups and in all subgroups (5.4% [3/56] vs 14.3% [5/35]; 6.5% [6/92] vs 0% [0/57]; $P > 0.05$). The clinical pregnancy rate, ongoing pregnancy rate, implantation rate, miscarriage rate, ectopic pregnancy rate, and live birth rate were comparable between the two groups across all subgroups ($P > 0.05$).

All newborns were examined for congenital malformation: one infant from the PPOS+CC group had esophageal atresia, and another infant from standard PPOS had cleft lip and palate.

Furthermore, we assessed the clinical outcomes of women with LH levels above 1 IU/L, but below 10 IU/L on the trigger day (Tables 4 and 5). We found a similar trend of outcomes in these women as in the whole population.

Discussion

This retrospective cohort trial showed that the percentage of women with profound pituitary suppression was significantly lower in PPOS+CC than in standard PPOS, in all subgroups. For young women with DOR, PPOS+CC, compared to standard PPOS, led to an increase in the number of high-quality cleavage-stage embryos and a decrease in the dosage of Gn per number of oocytes retrieved. For older women with DOR, the efficiency of the two protocols in embryological outcomes was similar, but a significantly higher incidence of LH levels >10 IU/L on trigger day and significantly lower rate of oocyte maturation were found in PPOS+CC than in standard PPOS.

According to the action of progesterone in the blockade of estradiol-induced gonadotropin discharges, concurrent administration of progestin with estrogen inhibits an estrogen-positive feedback, resulting in suppression of a premature LH surge.[22,23] It was shown that the number of retrieved oocytes and high-quality embryos in standard PPOS was significantly higher than that in the group of conventional protocols and mild/minimal stimulation.[8–10] However, several previous studies have indicated that the application of progestin from the beginning of ovarian stimulation may lead to profound pituitary suppression, and the extent of pituitary suppression is directly associated with the total dosage of Gn.[4–6] This was consistent with our results showing that women in standard PPOS exhibited higher rates of profound pituitary suppression and required a higher dosage of Gn per number of oocytes retrieved than those in PPOS+CC, especially for women ≤35 years. CC interacts with hypothalamic estrogen receptors and increases endogenous FSH and LH secretion by

Table 4 Controlled Ovarian Stimulation and Embryological Outcomes of Women with LH Levels >1 IU/L, but <10 IU/L on the Trigger Day

	≤35 Years			>35 Years		
	PPOS+CC Group (n = 66)	**Standard PPOS Group (n = 46)**	***P*-value**	**PPOS+CC Group (n =126)**	**Standard PPOS Group (n = 69)**	***P*-value**
Gn duration (days)	9.32±2.21	10.00±3.99	0.297	9.69±2.10	9.70±2.71	0.989
Total dosage of Gn (IU)	1995.83±548.47	2086.96±1585.99	0.710	2047.38±533.98	1850.36±752.16	0.057
No. of >14mm follicles on trigger day	4.50±2.82	3.15±1.61	<0.001	3.66±1.85	3.51±1.99	0.595
No. of oocytes retrieved	5.38±2.59	4.65±4.32	0.269	4.43±2.67	3.72±2.23	0.064
Dosage of Gn per oocyte retrieved (IU)	481.16±335.40	970.02±1321.86	0.018	695.40±558.44	764.60±725.16	0.459
ICSI MII oocytes rate, % (n)	86.9 (159/183)	78.3 (47/60)	0.110	83.0 (230/277)	89.5 (102/114)	0.106
Oocyte maturation rate, % (n)	84.5 (300/355)	81.3 (174/214)	0.322	85.7 (478/558)	90.7 (233/257)	0.047
2PN rate of IVF oocytes, % (n)	78.0 (110/141)	70.1 (89/127)	0.138	70.2 (174/248)	79.4 (104/131)	0.053
2PN rate of ICSI oocytes, % (n)	79.2 (126/159)	80.9 (38/47)	0.810	83.0 (191/230)	79.4 (81/102)	0.428
No. of 2PN cleavage	3.58±2.18	2.76±2.10	0.051	2.90±1.85	2.67±1.59	0.383
No. of high-quality cleavage-stage embryos	2.02±1.47	1.22±1.25	0.003	1.55±1.31	1.54±1.31	0.954
High-quality cleavage-stage embryos cycles rate, % (n)	9.1 (6/66)	37.0 (17/46)	<0.001	21.4 (27/126)	18.8 (13/69)	0.669
Cancellation rate prior to oocyte retrieval, % (n)	3.0 (2/66)	0.0 (0/46)	0.512	0.0 (0/126)	2.9 (2/69)	0.124

Note: Data are presented as mean ± standard deviation or percentage (number).
Abbreviations: PPOS, progestin-primed ovarian stimulation; CC, clomiphene citrate; Gn, gonadotropin; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; 2PN, two pro-nucleate; MII, metaphase of second meiosis.

blocking the estrogen-negative feedback mechanism.[11,24,25] Our data are consistent with previous findings[5,6] and showed that LH levels on days 7–10 and the day of trigger in PPOS+CC were significantly higher than in all subgroups of standard PPOS ($P < 0.05$). Furthermore, the E_2 levels and P levels on trigger day were significantly higher in PPOS+CC than in standard PPOS ($P < 0.01$). The key role of LH in synthesizing and secreting androgens, which are required for further production of E_2 and P is widely acknowledged.[26] Our results support previous findings that CC supplementation alleviates profound LH suppression.

According to the concept of a therapeutic window for LH, proposed by Hillier, there is a threshold requirement for LH for an optimal cycle outcome.[27] In the present study, the number of high-quality cleavage-stage embryos was higher among younger women treated with PPOS+CC

than those treated with standard PPOS. These findings further support the notion that LH plays a critical role in normal follicular development.[28] However, for older women with DOR, although the number of >14 mm follicles on trigger day was comparable between the groups ($P > 0.05$), PPOS+CC led to a decrease in the rate of oocyte maturation compared to standard PPOS. In addition, we found a similar trend of outcomes in women with LH levels >1 IU/L, but <10 IU/L, on trigger day in the entire population. Thus, our study indicated that elevated serum LH during controlled ovarian stimulation might be associated with decreases in the oocyte maturation rate for elderly women with DOR. A possible explanation is that women of different ages with DOR may require a different range of therapeutic window for LH;[28] however, the optimal LH level required for folliculogenesis and oocyte maturation for women of different ages remains unknown.

Table 5 Pregnancy Outcomes of Women with LH Levels >1 IU/L, but <10 IU/L on the Trigger Day

	≤35 Years			>35 Years		
	PPOS+CC Group (n = 52)	Standard PPOS Group (n = 25)	*P*-value	PPOS+CC Group (n =79)	Standard PPOS Group (n = 51)	*P*-value
No. of transferred embryos	1.87±0.35	1.72±0.46	0.168	1.81±0.40	1.80±0.40	0.931
Endometrial thickness (mm)	9.90±1.91	9.32±1.94	0.221	9.89±1.87	9.34±1.73	0.092
Clinical pregnancy rate, % (n)	44.2 (23/52)	56.0 (14/25)	0.333	36.7 (29/79)	29.4 (15/51)	0.391
Ongoing pregnancy rate, % (n)	38.5 (20/52)	52.0 (13/25)	0.261	21.5 (17/79)	19.6 (10/51)	0.793
Implantation rate, % (n)	29.9 (29/97)	44.2 (19/43)	0.100	22.4 (32/143)	18.5 (17/92)	0.473
Miscarriage rate, % (n)	13.0 (3/23)	7.1 (1/14)	1.000	37.9 (11/29)	20.0 (3/15)	0.314
Ectopic pregnancy rate, % (n)	0	0	NE	3.4 (1/29)	6.7 (1/15)	1.000
Live birth, % (n)	38.5 (20/52)	52.0 (13/25)	0.261	21.5 (17/79)	19.6 (10/51)	0.793

Note: Data are presented as mean ± standard deviation or percentage (number).
Abbreviations: PPOS, progestin-primed ovarian stimulation; CC, clomiphene citrate; NE, not estimable.

Nevertheless, pregnancy outcomes were comparable between the groups in all subgroups, demonstrating a similar developmental potential of embryos despite different LH levels. On the basis of these results, we suggest that early stage embryo development may differ slightly between oocytes retrieved after PPOS+CC compared with standard PPOS. Additional fundamental research should be performed to determine alterations in the follicular microenvironment, which may help determine the optimal range of LH levels, elucidate the mechanisms by which LH affects oocyte quality, and provide evidence for the use of PPOS.

In the present study, the incidence of LH levels >10 IU/L on trigger day was higher in PPOS+CC, especially for women aged >35 y ($P < 0.05$). Guo et al reported that a higher percentage of premature LH surge (10.2%) is found in women aged >35 y after PPOS protocols using utrogestan were applied.[29] Three previous independent studies have found a similar correlation between diminished ovarian reserve and premature LH elevation.[30–32] These results suggest that, although CC supplementation alleviates profound LH suppression, older women with DOR would be at risk of developing a premature LH surge and poor oocyte maturation. The reason for this remarkable variability needs to be clarified. It is speculated that dysregulation of ovarian factors could be an underlying mechanism in women of advanced maternal age. According to animal and human studies, the transition towards menopause involves not only the loss of ovarian follicles but also a dysregulation of E_2 feedback mechanisms.[33]

With a relatively large sample size, the current study was able to verify the feasibility of CC co-administration in the PPOS protocol in women of different ages with DOR. These results from using a CC combination regimen may provide new insights to develop an individualized treatment regimen for women with DOR to improve clinical outcomes.

Our study also has some limitations. Firstly, data were derived from a post-hoc analysis. Secondly, the LH assays currently used do not always accurately reflect LH bioactivity.[34]

Conclusion

This retrospective cohort trial showed that CC supplementation could mitigate the profound LH suppression caused by progestin administration. For young women with DOR, the PPOS protocol with CC supplementation led to an increase in the number of high-quality cleavage-stage embryos and a decrease in the dosage of Gn per oocyte retrieved as compared to the standard PPOS protocol. However, older women with DOR would be at risk of developing a premature LH surge and experience poor oocyte maturation rate after the PPOS+CC protocol.

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Disclosure

The authors report no conflicts of interest in this work.

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