Progestin-primed Ovarian Stimulation With Clomiphene Citrate Compared With the Standard Progestin-primed Ovarian Stimulation in Various Aged Women With Diminished Ovarian Reserve: a Retrospective Study

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Research

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Abstract

Background: Clomiphene citrate (CC) supplementation in the progestin-primed ovarian stimulation (PPOS) protocol effectively alleviated profound pituitary suppression from progestin administration and reduced the gonadotropin (Gn) dose in the population of women with normal-ovarian-reserve or polycystic ovarian syndrome. Limited data are available about the role of CC in PPOS for women with diminished ovarian reserve (DOR). This study aimed to compare the efficiency of the PPOS protocol with CC supplementation and the standard PPOS protocol for various aged women with DOR.

Methods: A total of 364 patients with DOR were recruited for this retrospective cohort study. They were divided into subgroups based on female age, ≤35 years and ＞35 years. The clinical characteristics and outcome were compared between the groups in subgroups.

Results: In all patients with DOR, the PPOS protocol with CC supplementation was associated with a lower percentage of women with profound pituitary suppression (0.0% vs 18.6%, P ＜0.001 and 1.3% vs 11.0%, *P* ＜0.002) and a higher mean luteinizing hormone (LH) level during controlled ovarian stimulation (COS) than the standard PPOS protocol (*P* ＜0.05). In young women with DOR, more number of high-quality cleavage-stage embryos (1.96 vs. 1.38, *P* ＜0.018) was achieved in the PPOS protocol with CC supplementation. In elderly women with DOR, PPOS protocol with CC supplementation led to an increase in the incidence of LH levels above 10 IU/L on the trigger day (12.7% vs. 4.9%, *P* ＜0.028) and decrease in the rate of oocyte maturation (84.7% vs. 89.9%, *P* ＜0.034) compared to the standard PPOS protocol. No significant differences were observed in the Gn duration, total dosage of Gn, and pregnancy outcomes between the groups.

Conclusions: CC is an effective adjuvant to alleviate pituitary suppression in the PPOS protocol. For young women with DOR, CC supplementation has a positive impact on the number of high-quality embryos in PPOS protocol. However, elderly patients with DOR would be at risk of developing premature LH surge and poor oocyte maturation rate after the PPOS protocol with CC supplementation was applied.

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 age dependent or independent, are manifested mainly as decreased anti-Müllerian hormone (AMH) and low antral follicle count (AFC) [1]. Patients with DOR may be at risk of poor ovarian response, especially in women aged 35 to 40 [2]. A low or poor ovarian response often results in cycle cancellation, poor outcomes, and consequent stress and disappointment to the couple. Such patients could benefit from counseling regarding protocol selection to improve the clinical outcomes [3]. Therefore, clinicians and researchers worldwide are seeking a proper controlled ovarian stimulation (COS) protocol for patients with DOR.

Progestin-primed ovarian stimulation (PPOS) has been approved its effective in preventing an endogenous luteinizing hormone (LH) surge during COS [4–7]. But the continuous supply of progestin during PPOS can lead to profound pituitary suppression [4–6]. It was reported that profound pituitary suppression was positively correlated with the gonadotropin (Gn) duration [5]. Clomiphene citrate (CC) was used to block negative feedback trigged by estrogen, thereby resulting in elevated follicle-stimulating hormone and LH release from the hypothalamus [8, 9]. PPOS supplemented with CC significantly reduced the occurrence of profound pituitary suppression in women with normal ovarian reserve or polycystic ovarian syndrome and the combination of CC and PPOS is likely to reduce the Gn duration compared to those without CC [5, 6].

Currently, limited data are available about the role of CC in PPOS for women with DOR. In addition, age is considered a marker for oocyte quality, which is correlated with embryo euploid rates in ART [10]. Therefore, the present study was designed to compare the efficiency of the PPOS protocol with CC supplementation and the standard PPOS protocol for various aged 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 with two or more of the following items were diagnosed with DOR: (a) FSH ≥ 10IU; (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 a single stimulation cycle only. In women with multiple treatment cycles, the chronologically first cycle was chosen. For analysis, couples were grouped as follows: patients using PPOS protocol with CC supplementation were grouped into the “PPOS with CC group”, while patients using standard PPOS protocol were grouped into the “standard PPOS group”. Subpopulations of patients were characterized by different ranges of age, ≤35 years and ＞35 years. All data were retrospectively collected from computer databases and stored in a deidentified database. Permission for this study was given from the ethics committee of the First Affiliated Hospital of Wenzhou Medical University (no. 2020–02).

Stimulation protocols and pituitary suppression

In the PPOS with CC group, 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 day 2-3 (MC2-3) or after an episode of withdrawal bleeding. In standard PPOS group, HMG 150-225 IU and MPA 10 mg daily were initiated from menstrual cycle day 2-3 (MC2-3) or after an episode of withdrawal bleeding. The initial dose of Gn (150-225 IU) was based on patient’s age, basic FSH, AFC, and previous promotion plan. Follicle monitor and hormone assay (FSH, LH, E_2 and P) were performed 5 days later. The dose of Gn was adjusted according to follicle development and MPA dose was consistent up to the trigger day. When the dominant follicle diameter reached ＞17 mm and the majority of growing follicles, if there were any, reached 14mm or more, recombinant human chorionic gonadotropin (Ovitrelle, Serono, Bari, Italy) were administered to trigger ovulation. Oocytes were retrieved 36 to 38 hours later. All follicles larger than 10 mm in diameter were aspirated.

Fertilization was carried out in vitro after oocyte retrieval depending on the semen parameters and previous fertilization situation. 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 the 3rd day after oocyte retrieval, and remaining embryos were placed in extended culture [11, 12]. Subsequently, blastocysts with good morphological grades were frozen on day 5 or 6 of culture.

Hormone measurement

Serum FSH, LH, E_2, and P were measured on menstrual cycle day 2-3, day 7-10, and the trigger day. Hormone levels were determined with immunofluorescence assay (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 the trigger day was set as the cut-off for profound pituitary suppression [13].

Endometrium preparation and Frozen embryo transfer

All patients in the present study had received rozen-thawed embryo transfer (FET). Hormone replacement treatment was used for endometrial preparation. Briefly, the timing of embryo transfer was scheduled on the 3rd or the 5th day after progestin administration depending on the embryo stage. Embryo transfer was all conducted via the guidance of abdominal ultrasound in our center. Each patient received no more than two embryos at one time. Patients received luteal support after embryo transfer in the form of intravaginal progestin 200mg two times daily. Once pregnancy was achieved, the luteal support was continued until 10 weeks of gestation.

Outcome measures

We analyzed the pregnancy outcomes of the chronologically first cycle after oocyte retrieval that transferred at least one high-quality cleavage-stage embryos or good morphology blastocysts (blastocysts better than grade 322) [11]. Clinical pregnancy was defined as the presence of fetal cardiac activity confirmed by transvaginal ultrasound. Ongoing pregnancy rate was defined as the proportion of patients with ongoing pregnancy after the gestation 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

The data were evaluated by Student's t-test for continuous variables of normal distribution, the Mann-Whitney U-test for continuous variables of non-normal distribution, the χ^2-test or Fisher's exact for categorical variables, as appropriate. All tests were two-sided, and $P \leq 0.05$ was considered statistically significant. SPSS statistical software (version 25; SPSS Inc., USA) was used for data analysis. The dynamic changes in hormones during COS were presented with a broken line graph, created by Excel.

Results

Patient characteristics and cycle characteristics

In total, 364 women were enrolled. Table 1 shows the comparison of basic patient characteristics and the cycle 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 secondary infertility, body mass index (BMI), AMH, AFC, baseline hormones, Gn duration and total dosage of Gn ($P > 0.05$).

Table 1

Patient characteristics and controlled ovarian stimulation characteristics between groups stratified by age.

	≤35 years			＞35 years		
	PPOS with CC group (HMG/MPA/CC; n = 73)	Standard PPOS group (HMG/MPA; n = 59)	*P*-value	PPOS with CC group (HMG/MPA/CC; n = 150)	Standard PPOS group (HMG/MPA; 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)			ND[a]			ND[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
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

Percentage of women with profound pituitary suppression, % (n)	0.0 (0/73)	18.6 (11/59)	<0.001	1.3 (2/150)	11.0 (9/82)	0.002
Incidence of LH level ≥10 IU/L on the trigger day, % (n)	9.6 (7/73)	3.4 (2/59)	0.187	12.7 (22/150)	4.9 (4/82)	0.028

CC, clomiphene citrate; PPOS, progestin-primed ovarian stimulation; MPA, medroxyprogesterone acetate; BMI, body mass index; AMH, anti-Müllerian hormone; AFC, antral follicle count; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E_2, estradiol; P, progesterone; Gn, gonadotropin; ND, not defined.

[a] Because someone may have more than 1 cause of infertility.

Controlled ovarian stimulation and embryological outcomes

The COS and embryological outcomes of both groups are presented in Table 2. There was no statistical difference found in 2PN rate and number of 2PN cleavage between the groups. No difference was observed in the cancellation rate prior to oocyte retrieval (P 0.05). For women aged above 35 years, the mean number of oocytes retrieved in PPOS with CC group was significantly higher than those in standard PPOS group (4.23 vs. 3.50, P 0.034). However, the oocyte maturation rate in PPOS with CC group was significantly lower than those in standard PPOS group (84.7% vs. 89.9%, P 0.034). Compared with women aged below 35 years in standard PPOS group, a significantly higher number of high-quality cleavage-stage embryos and a significantly lower no high-quality cleavage-stage embryos cycles rate were noted in PPOS with CC group (1.96 vs. 1.38, P 0.018 and 11.0% vs 33.9%, P 0.001, respectively), while no difference was found in the other subgroups.

Table 2
Clinical outcomes between groups stratified by age.

	≤ 35 years			> 35 years		
	PPOS with CC group (HMG/MPA/CC; n = 73)	Standard PPOS group (HMG/MPA; n = 59)	*P*-value	PPOS with CC group (HMG/MPA/CC; n = 150)	Standard PPOS group (HMG/MPA; n = 82)	*P*-value
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
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
No 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
FET cycles	56	35		92	57	
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 pregnant 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

CC, clomiphene citrate; PPOS, progestin-primed ovarian stimulation; MPA, medroxyprogesterone acetate; No., number; ICSI, Intracytoplasmic sperm injection; FET, frozen-thawed embryo transfer; ND, not defined.

[a] Because someone may have more than 1 cause of infertility.

	≤ 35 years			> 35 years		
Ectopic pregnancy rate, % (n)	0	0	ND[a]	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

CC, clomiphene citrate; PPOS, progestin-primed ovarian stimulation; MPA, medroxyprogesterone acetate; No., number; ICSI, Intracytoplasmic sperm injection; FET, frozen-thawed embryo transfer; ND, not defined.

[a] Because someone may have more than 1 cause of infertility.

Hormone profile during treatment

The serum concentrations of FSH, LH, E_2 and P in the two groups stratified by age are presented in Figure 1. In both groups, the FSH levels increased after Gn administration, and no difference was found between the groups (P 0.05). The serum E_2 levels and P levels increased gradually in both groups after Gn administration. The serum E_2 levels and P levels on day of trigger in the PPOS with CC group were significantly higher than those in all subgroups of the standard PPOS group (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, 0.99±0.87 ng/ml vs. 0.69±0.40 ng/ml, P 0.001, respectively).

The LH levels of the two groups showed different trends. The LH levels in the PPOS with CC group increased first and then remained steady at a range of 5.52–6.61 IU/L; in contrast, the LH levels in the standard PPOS group remained at a certain level initially and then slightly decreased. There was no significant difference in the value of basal LH between the groups (P 0.05), whereas the LH levels on day 7–10 and day of trigger in the PPOS with CC group were significantly higher than that in all subgroups of the standard PPOS group (P 0.05).

As shown in Table 1, the percentage of women with profound pituitary suppression was significantly lower in the PPOS with CC group than that in standard PPOS group (0.0% vs 18.6%, P 0.001 and 1.3% vs 11.0%, P =0.002). In addition, compared with the standard PPOS group, the percentage of patients with LH levels above 10 IU/L on the trigger day was higher in the PPOS with CC group, especially for patients aged above 35 years (12.7% vs. 4.9%, P 0.028). All patients with LH levels above 10 IU/L on the trigger day confirmed the sonolucent follicles are still there by transvaginal ultrasound just before the planned oocyte retrieval and underwent oocyte retrieval, among which only two patients had no oocytes retrieved and only one patient had no mature oocytes being retrieved.

Pregnancy outcomes

In this study, 29 women did not complete the FET cycles for no viable embryos and 62 women for personal reasons before the end of the study. In total, 273 women completed FET cycles during the 18 months of follow-up, among which 4 women transferred with embryos collected from twice oocyte retrieval and 29 women transferred with no high-quality cleavage-stage embryos or good morphology blastocysts. The discontinuation rate of the cycle, which achieved at least one high-quality cleavage-stage embryos, was comparable between the groups (10.3% [4/39] vs 13.8% [9/65]; 9.5% [6/63] vs 17.9% [20/112]; P 0.05). Only 240 women were transferred with at least one high-quality cleavage-stage embryos or good morphology blastocysts, which were included in the analysis of pregnancy outcomes (Table 2). The rate of blastocysts transfers was similar between the groups 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 pregnant rate, implantation rate, miscarriage rate, ectopic pregnancy rate and live birth rate were comparable between the groups in all subgroups (P 0.05).

All newborns were examined with no congenital malformation except that oesophageal atresia was found in one baby of the PPOS with CC group and cleft lip and palate in one baby of the standard PPOS group.

Furthermore, we assess the clinical outcomes of patients with LH levels above 1 IU/L, but below 10 IU/L on the trigger day (Table 3). We found similar trend of the outcomes in these patients as in the whole population.

Table 3
Clinical outcomes patients with LH levels above 1 IU/L, but below 10 IU/L on the trigger day between groups stratified by age.

	≤ 35 years			> 35 years		
	PPOS with CC group (HMG/MPA/CC; n = 66)	Standard PPOS group (HMG/MPA; n = 46)	*P*-value	PPOS with CC group (HMG/MPA/CC; n = 126)	Standard PPOS group (HMG/MPA; n = 69)	*P*-value
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
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
No 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
FET cycles	52	25		79	51	
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 pregnant 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

CC, clomiphene citrate; PPOS, progestin-primed ovarian stimulation; MPA, medroxyprogesterone acetate; No., number; ICSI, intracytoplasmic sperm injection; FET, frozen-thawed embryo transfer; ND, not defined.

[a] Because someone may have more than 1 cause of infertility.

	≤ 35 years			> 35 years		
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	ND[a]	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

CC, clomiphene citrate; PPOS, progestin-primed ovarian stimulation; MPA, medroxyprogesterone acetate; No., number; ICSI, intracytoplasmic sperm injection; FET, frozen-thawed embryo transfer; ND, not defined.

[a] Because someone may have more than 1 cause of infertility.

Discussion

This retrospective cohort trial showed that the percentage of women with profound pituitary suppression was significantly lower in the PPOS with CC group than that in standard PPOS group in all subgroups. For young women with DOR, PPOS protocol with CC supplementation led to an increase in the number of high-quality cleavage-stage embryos compared to the standard PPOS protocol. For elderly patients with DOR, the efficiency of the two protocols in embryological outcomes was similar with each other, but significantly higher incidence of LH levels above 10 IU/L on the trigger day and significantly lower rate of oocyte maturation were found in the PPOS with CC group.

According to progesterone's antipositive feedback mechanism, concurrent administration of progestin with estrogen inhibited estrogen's positive feedback and therefore abolished the premature LH surge [14, 15]. Kuang et al [4] indicated that the application of progestin from the beginning of ovarian stimulation may lead to profound pituitary suppression and women in the standard PPOS protocol exhibited lower LH levels and had higher rates of profound pituitary suppression compared with the PPOS protocol with CC supplementation. According to our results, the proportion of women with profound pituitary suppression was also significantly higher in the standard PPOS protocol than in the PPOS protocol with CC supplementation in all subgroups (P 0.01). Some investigators believe that a low LH concentration results in unfavorable clinical outcomes for ovarian stimulation [16-18]. Fleming et al [16] observed that patients with very low LH concentrations (0.5 IU/L) tended to have a low oocyte yield and fertilization rate as well as less available embryos with the long protocol. A higher rate of pregnancy loss (45%) was documented by Westergaad et al [17] in patients with very low LH concentrations (0.5 IU/L). Low LH Level on the day of trigger (1.60 mIU/ml) was reported to be associated with reduced ongoing pregnancy and live birth rates and increased early miscarriage rates [19]. CC interacts with the hypothalamic estrogen receptors and increases endogenous FSH and LH secretion by blocking estrogen's negative feedback mechanism [8, 20, 21]. In this study, the LH levels during the COS process were higher in the PPOS with CC group in all subgroups (P 0.01). Therefore, CC supplementation alleviated profound LH suppression.

Our data showed that the LH levels on day 7–10 and day of trigger in the PPOS with CC group were significantly higher than those in all subgroups of the standard PPOS group (P 0.05). The key role of LH in synthesizing and secreting androgens, which are required for further production of E_2 and P, is widely acknowledged [22]. In the present study, E_2 levels and P levels at the time of ovulation were significantly higher in the PPOS with CC group than in the standard PPOS group (P 0.01), indicating that a high level of LH in the PPOS with CC group may further promote E_2 and P synthesis. 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 [23]. In the present study, the number of high-quality

cleavage-stage embryos was higher among younger patients treated with the PPOS protocol with CC supplementation. Whereas, HMG was used in both treatments and we noted that Gn duration, total dosage of Gn, and the FSH level during COS between the groups were similar. It was not likely that the improved embryological outcomes could be attributed to the Gn use in the PPOS with CC group. These findings further support the notion that LH plays a critical role in normal follicular development [24]. However, for elderly patients with DOR, though the number of ≥14mm follicles on trigger day was comparable between the groups (P ≥0.05), PPOS protocol with CC supplementation led to a decrease in the rate of oocyte maturation compared to the standard PPOS protocol. In addition, the embryological outcomes between the groups were similar in elderly patients with DOR. We found similar trend of the outcomes in patients with LH levels above 1 IU/L, but below 10 IU/L on the trigger day as in the whole population. Furthermore, the pregnancy outcomes were comparable between the groups, demonstrating a similar development potential of the embryos despite the different LH levels. Thus, our study indicated that elevated serum LH during the COS process had no impact on embryological outcomes, but might be associated with decreases in the oocyte maturation rate for elderly patients with DOR. The possible explanation is that women with DOR of different ages may require different range of therapeutic window for LH [24]. On the basis of these results, we suggest that the early stage of embryo development may differ slightly between oocytes retrieved after the PPOS protocol with CC supplementation or standard PPOS protocol was applied. Additional fundamental researches should be performed to determine the alterations in the follicular microenvironment, which may help determine the optimal range of the LH level, elucidate the mechanism by which LH affects oocyte quality and provide evidence for the use of PPOS.

Our data showed that the incidence of profound pituitary suppression was lower but the incidence of LH levels above 10 IU/L on the trigger day was higher in the PPOS with CC group, especially for patients aged above 35 years (P ≥0.05). Guo et al reported that higher percentage of premature LH surge (10.2%) was found in patients aged more than 35 years after the PPOS protocols using utrogestan applied [25]. Three other independent previous studies have found a similar correlation between low ovarian reserve or older age group patients and premature LH elevation [26-28]. It suggested that, though CC supplementation alleviated profound LH suppression, elderly patients with DOR would be at risk of developing premature LH surge and poor oocyte maturation rate.

Our study had several strengths as well as a few limitations. This trial fills a gap in the literature because there are relatively few published studies verifying the feasibility of CC co-administration in the PPOS protocol in various aged women with DOR. These results from a CC combination regimen may provide a new insight for develop an individualized treatment regimen for women with DOR to improve clinical outcomes. Because we used the “freeze-all” strategy, there was a waiting period between oocyte retrieval and embryo transfer. Though there are 116 extra high-quality cleavage-stage embryos or good morphology blastocysts dose not get transferred, because the cycle discontinuation rate was similar between the groups, any possible bias should be minimal.

Conclusions

This retrospective cohort trial demonstrated that CC supplementation could mitigate the profound LH suppression caused by progestin administration. For young women with DOR, PPOS protocol with CC supplementation led to an increase in the number of high-quality cleavage-stage embryos compared to the standard PPOS protocol. However, elderly patients with DOR would be at risk of developing premature LH surge and poor oocyte maturation rate after the PPOS protocol with CC supplementation was applied.

Abbreviations

CC: Clomiphene citrate; PPOS: Progestin-primed ovarian stimulation; Gn: Gonadotropin; DOR: Diminished ovarian reserve; COS: Controlled ovarian stimulation; LH: Luteinizing hormone; ART: Assisted reproduction technologies; AMH: Anti-Müllerian hormone; FET: Frozen-thawed embryo transfer; AFC: Antral follicle count; FSH: Follicle-stimulating hormone; ICSI: Intracytoplasmic sperm injection; IVF: In vitro fertilization; MPA: Medroxyprogesterone acetate; E2: Estradiol; P: Progesterone.

Declarations

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Authors' contributions

Yue Lin, Qianqian Chen, Xuefeng Huang and Xia Chen were the chief investigators who completed the entire study, including procedures, conception, design and completion. Jing Zhu and Yili Teng were responsible for the collection of data. Yue Lin and Qianqian Chen analysed the data and drafted the manuscript together. All authors read and approved the final manuscript.

Authors' information

The findings and conclusions of this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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Availability of data and materials

Data are not publicly shared; please contact the authors for data requests.

Ethics approval and consent to participate

Permission for this study was given from the ethics committee of the First Affiliated Hospital of Wenzhou Medical University (no. 2020–02). No patient consent was required for this retrospective cohort study.

Consent for publication

All authors have reviewed the manuscript and consent for publication.

Competing interests

The authors declare that they have no competing interests.

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Figures

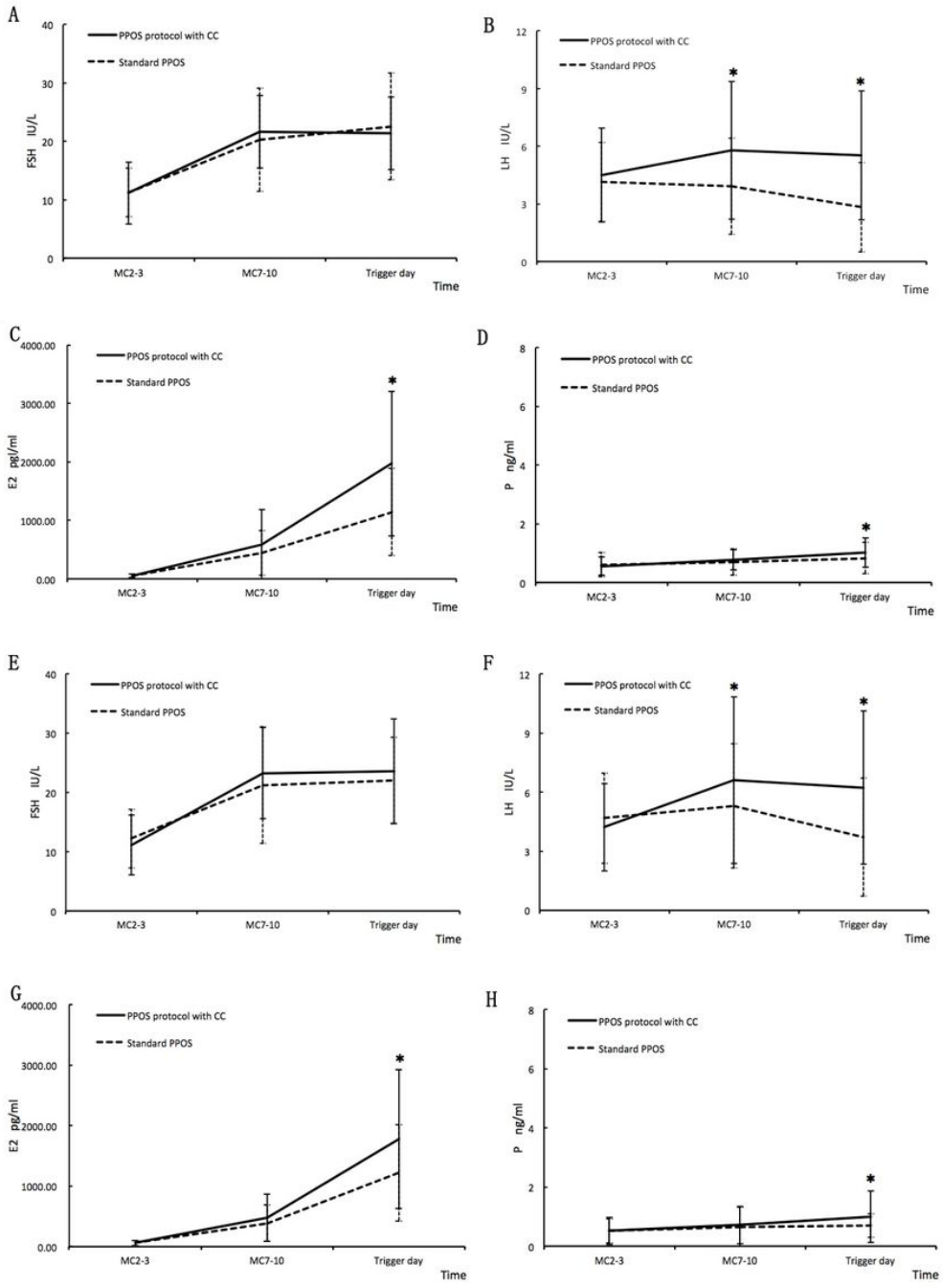

Figure 1

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. *P values < 0.05.

Supplementary Files

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