Received: 13 September 2021 | Accepted: 15 November 2021

DOI: 10.1002/rmb2.12429

Reproductive Medicine and Biology

WILEY

ORIGINAL ARTICLE

A modified GnRH antagonist method in combination with letrozole, cabergoline, and GnRH antagonist for PCOS: Safe and effective ovarian stimulation to treat PCOS and prevent OHSS

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Abstract

Purpose: To analyze the therapeutic efficacy of a modified controlled ovarian stimulation (COS) protocol for polycystic ovary syndrome (PCOS) that does not cause ovarian hyperstimulation syndrome (OHSS) while maintaining oocyte quality.

Method: This study is a retrospective cohort study of reproductive medicine at St. Mother Clinic. We analyzed ART clinical outcomes, embryonic development, and hormone levels in 175 PCOS patients treated with four COS (GnRH agonist based long protocol, Group A; GnRH antagonist protocol with HCG trigger, Group B; GnRH antagonist protocol with GnRH agonist trigger, Group C, and the modified COS group) between 2010 and 2021.

Results: Of 175 patients with PCOS, 45 and 130 patients underwent 47 and 136 oocyte retrieval cycles, 75 and 250 embryo transfer cycles with the modified COS, and with conventional methods, respectively. The cumulative pregnancy rate at one trial was a significantly higher result than in Group A and higher than in Groups B and C (cumulative pregnancy rate at one trial of Group A, B, C, and modified COS: 40.0%, 54.5%, 56.3%, and 72.3%, respectively). With this method, not clinically problematic OHSS and higher clinical outcomes than in conventional methods were observed.

Conclusion: This modified COS can significantly improve clinical outcomes and eliminate OHSS.

KEYWORDS
cabergoline, letrozole, ovarian hyperstimulation syndrome, polycystic ovarian syndrome

1 | INTRODUCTION

Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder associated with ovarian disorders and occurs in 4%–7% of all women of reproductive age, 50% of women affected by PCOS experience subfertility, and 15%–20% of them will also need assisted reproductive technology (ART).[1] The main challenges for PCOS patients are OHSS and the uneven, large number and low quality of the oocytes frequently produced after controlled ovarian stimulation (COS).[2] Almost all conventional treatments for PCOS have managed to avoid OHSS by reducing the number of growing follicles. In vitro maturation (IVM) and laparoscopic ovarian drilling (LOD) have been conducted for severe PCOS. IVM has been applied widely for the treatment of PCOS recently and its clinical outcome has been more successful.[2] However, general evaluation for IVM has some disadvantages to the COS with GnRH analogs (GnRH agonist and GnRH antagonist) due to the necessity of a special in vitro culture system which has not been established and it still needs to be improved to provide good clinical results.[1,3–5] Epigenetic risks after ART using in vitro matured oocytes are also controversial.[6]

On the other hand, laparoscopic ovarian drilling (LOD) has also been applied to the patients with PCOS. LOD results in an overall spontaneous ovulation rate of 30–90% and final pregnancy rates of 13–88%.[7] These benefits are more significant for women with CC-resistant PCOS. Disadvantages are short effective periods of only one year after LOD and risks associated with operative complications.[8,9] Considering the advantages and disadvantages following IVM or LOD and the spread use of cryopreservation of embryos, COS based on GnRH analogs are generally chosen for the treatment for PCOS.[10] We started the COS based on GnRH agonist long protocol in 2010. This method was superior to the stimulation methods used before the advent of GnRH analog because it increased the quality of oocytes.[11] However, it had the disadvantage that OHSS occurred frequently.[12] We then started to use COS based on GnRH antagonist with HCG trigger. This COS showed better clinical outcome with lower frequency of OHSS than the COS based on GnRH agonist long protocol.[13] The third method used was similar to the second one except for using GnRH agonist for trigger instead of HCG, leading to a dramatic decrease in the incidence of OHSS.[10,14]

We aimed to further improve these methods of inducing ovulation in patients with PCOS. We started the modified GnRH antagonist method (modified COS) for PCOS with aromatase inhibitor (Letrozole)[15] by administering the trigger to decrease high E2 level and administered a combination of letrozole, cabergoline,[16] and GnRH antagonist from right after the oocyte pickup (OPU) with the objective of overcoming the challenges associated with the conventional treatments. This modified COS greatly reduced OHSS and resulted in a higher accumulative pregnancy rate at one trial than previous published reports.

2 | MATERIALS AND METHOD

2.1 | Ethical aspect

This study was conducted with the informed consent of all participating patients. The institutional Review Board of the Saint Mother Obstetrics and Gynecology Clinic approved this study on December 20, 2017. UMIN Clinical Trial Registry was UMIN000045145.

2.2 | Patients

The embryonic development and the clinical outcome following this modified COS were studied for 45 patients and were defined as PCOS using the Rotterdam criteria[17] during the period between November 2018 and January 2021 at St. Mother Clinic. The Rotterdam criteria define PCOS patients as having two out of the three following conditions: (1) Oligo- and/or anovulation. (2) Clinical and/or biochemical signs of hyperandrogenism. (3) Polycystic ovaries.

2.3 | Method

Evaluation of the efficiency of this method was compared with the three conventional methods that we have used over the years, which were the best available treatment options at the time of their application. Group A: GnRH agonist based long protocol was used in 2010, Group B: GnRH antagonist protocol with HCG trigger was used between January 2010 and December 2018, Group C: GnRH antagonist protocol with GnRH agonist trigger was used between January 2018 and January 2021.

2.4 | Procedure of conventional ovarian stimulation

2.4.1 | Group A: GnRH agonist based long protocol

Administration of nasal drops of GnRH agonist was started from the middle luteal phase of the previous cycle. From the 3rd day of menstruation, we started with FSH injections (ASKA uFSH; ASKA Pharmaceutical Corporation) and then continued with HMG injections (FERRING HMG; Ferring Pharmaceuticals Corporation) until the injection of the trigger. They continued until the largest follicle was about 20–22 mm. HCG 5000 IU (FUJI HCG; FUJI Pharma Corporation) was used as a trigger. All embryos were cryopreserved in blastocysts, and then the frozen-thawed embryo was transferred in the next hormone replacement cycle. In the hormone replacement cycle, the estrogen preparation is started from the 3rd day of menstruation, the luteal hormone preparation is started when the endometrium reaches 8 mm or more, and the embryo is transplanted 5 days later.

2.4.2 | Group B: GnRH antagonist protocol with HCG trigger

From the 3rd day of menstruation, we started with FSH injections and then continued with HMG injections until the injection of the trigger. GnRH antagonist 0.25 mg shots (Cetrotide®; Nippon Kayaku Corporation) were started when the leading follicle reached 18 mm, they continued until the largest follicle was about 20–22 mm. HCG 5000 IU was used as a trigger. After OPU, it is the same as Group A.

2.4.3 | Group C: GnRH antagonist protocol with GnRH agonist trigger

The procedure is the same as for Group B, except that the trigger was a GnRH agonist.

2.5 | Procedure of modified GnRH antagonist method (modified COS)

In the modified COS protocol (Figure 1), we started with FSH injections 150 IU/ml and then continued with HMG 150 IU/ml until the injection of the trigger. GnRH antagonist 0.25 mg shots were started when the leading follicle reached 18 mm, they continued until the largest follicle was about 24 mm. Then leuprorelin acetate 2 mg (Lucrin® injection; AbbVie) was injected as trigger.

One tablet of letrozole 2.5 mg (Femara tablets; Novartis Pharma) was used when E2 level was 4000–5000 pg/ml. Two tablets were used when E2 level was over 5000 pg/ml. When E2 level was still over 2000–3000 pg/ml the following day, one tablet of letrozole was added, and trigger was injected on the same day.

In addition, after OPU, two tablets each of letrozole and cabergoline and one tablet of GnRH antagonist (Relumina 40 mg: ASKA Pharmaceutical Corporation) were administered for five consecutive days. All the embryos were cryopreserved following the ultrarapid vitrification method[18] with the Cryotop device (Kitazato Corporation), and then the frozen-thawed embryo was transferred in the next hormone replaced cycle. Levels of E2, progesterone (P), and VEGF were measured at trigger day, OPU, and six day after OPU. The value of VEGF was measured by ELISA method. We measured VEGF for the first fifteen patients. We stopped measuring it after confirmation of no occurrence of VEGF acceleration.

2.6 | Evaluation of collected oocytes

The collected oocytes were classified into Grade 1 (G1), Grade 2 (G2), Grade 3 (G3), Germinal vesicle (GV), and Degeneration (D) morphically under a dissecting microscope (Figure 2).[19] G1 are completely matured oocytes, G2 are almost completely matured oocytes, G3 are the least matured oocytes, GV is for oocytes with germinal vesicle found before starting the first meiosis and D refers to degenerated oocytes.

2.7 | Classification of OHSS

The severity of OHSS was classified according to the three following criteria.[20–22] Mild symptoms include abdominal bloating, ascites in minor pelvic cavity, ovarian size <6–8 cm in a diameter, and normal

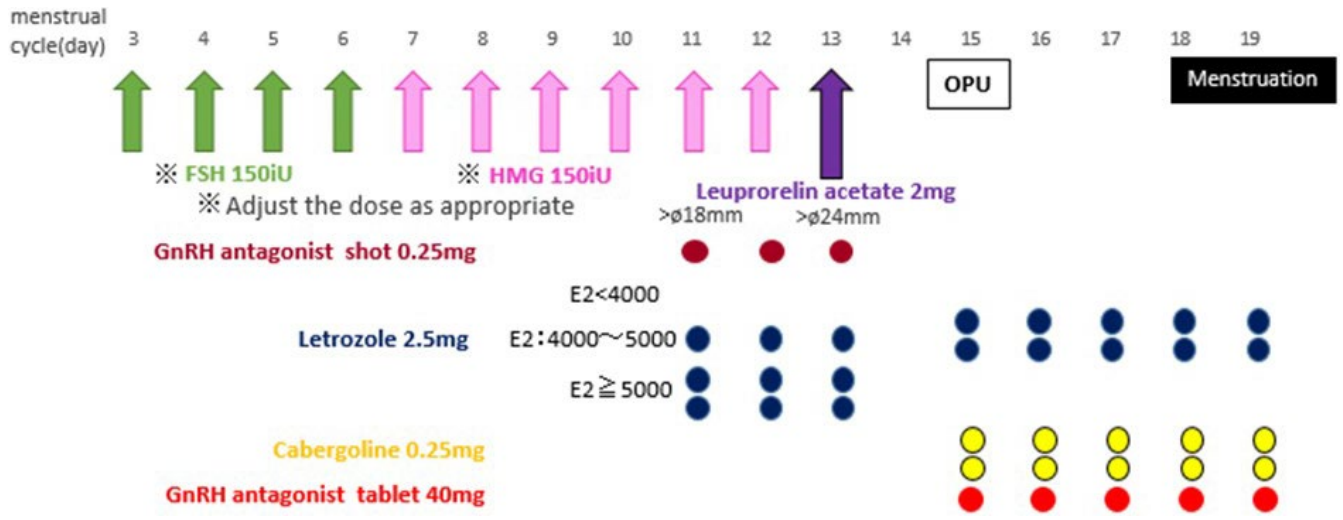

FIGURE 1 Simplified protocol of modified COS. We started with FSH injections (150 IU/ml) and then continued with HMG (150 IU/ml) until the injection of the trigger. GnRH antagonist shots (Cetrorelix acetate) were started when the leading follicle reached 18 mm. Then leuprorelin acetate (2 mg) was injected as trigger when the largest follicle was >24 mm. One tablet of letrozole was used when E2 level was 4000–5000 pg/ml, and two tablets were used when E2 level was over 5000 pg/ml before trigger administration. When E2 level was less than 4000 pg/ml no tablet of letrozole was given. After egg collection, 2 tablets each of letrozole and cabergoline, and 1 tablet of GnRH antagonist (Relugolix) were administered for 5 consecutive days

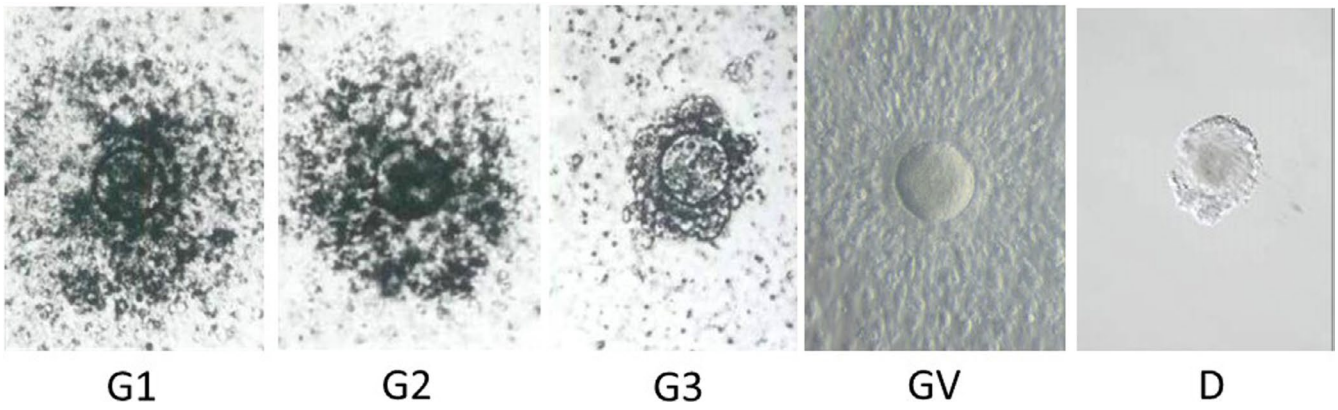

FIGURE 2 Classification criteria of collected oocytes. G1 are completely matured oocytes, G2 are almost completely matured oocytes, G3 are the least matured oocytes, GV is for oocytes with germinal vesicle found before starting the first meiosis, and D refers to degenerated oocytes

result in biochemical examination. In the case we found a small echo-free space in Douglas Porch, we diagnosed it as hemorrhage at OPU if ovary size was less than 6 cm and no abdominal bloating was reported. Moderate symptoms include nausea and vomiting, ascites reaching upper abdomen, diameter of ovary over 8 cm, and deteriorating trend of biochemical examination. Severe symptoms include abdominal pain, breathing difficulties, retention of ascites in total abdominal cavity and thoracic cavity, and abnormal data in biochemical examination.

2.8 | Statistical evaluation

Data were evaluated by chi-square test or Tukey-Kramer method and the difference was considered significant at the $p = 0.05$ level.

3 | RESULTS

The clinical data of modified COS was compared with conventional COS (Groups A, B, and C). The number of patients and cycles of modified COS were 45 and 47. Average age of patients was 32.57 ± 4.07 years, average BMI was 22.74 ± 3.89, number of type 2 diabetes patients was zero, and the average number of collected oocytes was 23.96 ± 7.88. No statistically significant differences were found in the above-mentioned parameters among the conventional control groups. Average maximum level of E2 during stimulation was 4635.83 ± 2029.10 pg/ml and this result was significantly higher than in the Groups A and B (p-value = 0.0112, 0.035, respectively) (Table 1). G1+G2 ratio used as the index of oocyte maturity was 61.6% (635/1031) and this rate was significantly higher than in the three control groups (Table 1). Cryopreservation rate (total number of cryopreserved cycles/ total number of treatment cycles) was 100% (47/47) (Table 1). This result was significantly higher than in Group A and B (p-value <0.0001, 0.0022, respectively), but almost the same as in groups C. Average number of cryopreserved blastocysts was 6.13 ± 3.54 (min-max: 1–16), and it was significantly higher than that of three control groups (Table 1). Average number of days between OPU and menstruation was 5.24 ± 2.54 (range: 5–7 days) which was significantly shorter than in the three control groups (Table 1). No incidences of OHSS (mild/ moderate/severe) were observed in the modified COS group while the conventional Groups A, B, and C had the following frequencies: A: 80.0% (48/60; 29/14/5), B: 50.0% (22/44; 15/5/2), and C: 21.9% (7/32; 6/1/0), respectively (p-values, Group A vs. modified COS: <0.0001, Group B vs. modified COS: <0.0001, Group C vs. modified COS: <0.00116) (Table 2).

The average values ± standard error of VEGF in 15 cases at trigger day, OPU day, and 6th day after OPU were 122.22 ± 23.34 pg/ml, 126.99 ± 23.34 pg/ml, and 108.33 ± 18.58 pg/ml, respectively (p-values, Trigger day vs. OPU day: 0.89, Trigger day vs. 6th day after OPU: 0.66, OPU day vs. 6th day after OPU: 0.55) (Figure 3).

These results show the superiority of this modified COS. Clinical pregnancy rate in the modified COS group was significantly higher than in Group A, but almost the same as in Groups B and C (clinical pregnancy rates of Groups A, B, C, and modified COS: 28.7%, 42.0%, 42.6%, and 48%, respectively; p-value of Group A vs. modified COS, Group B vs. modified COS, and Group C vs. modified COS: 0.0068, 0.4497, and 0.5431, respectively) (Table 3). There was no significant difference in miscarriage rate among the four groups (miscarriage rates of Group A, B, C, and modified COS: 24.2%, 29.4%, 30.4%, and 19.4%, respectively) (Table 3). The cumulative pregnancy rate at one trial in the modified COS group was significantly higher than in Group A and higher than in Groups B and C (cumulative pregnancy rates at one trial of Group A, B, C, and modified COS: 40.0%, 54.5%, 56.3%, and 72.3%, respectively; p-value of Group A vs. modified COS, Group B vs. modified COS, and Group C vs. modified COS: 0.0009, 0.0776, and 0.1388, respectively) (Table 3). The six remaining unsuccessful cases still have 10, 7, 6, 5, 3 and 3 frozen embryos. Therefore, there is a possibility that the cumulative pregnancy rate at one trial might increase further in the future. Live birth rate and the cumulative birth rate at one trial in the modified COS group were significantly higher

TABLE 1 Comparison of clinical outcome between modified COS and conventional treatments—1

	Group A[a] 60 women (60 cycles)	Group B[a] 38 women (44 cycles)	Group C[a] 32 women (32 cycles)	Modified COS 45 women (47 cycles)	*p*-value (Group A vs. Modified COS)	*p*-value (Group B vs. Modified COS)	*p*-value (Group C vs. Modified COS)
Age[b]	33.90 ± 3.81	33.66 ± 4.41	32.60 ± 2.42	32.57 ± 4.07	0.19	0.23	0.85
BMI[b]	23.11 ± 3.60	22.53 ± 2.14	23.52 ± 2.10	22.74 ± 3.89	0.57	0.77	0.31
Type 2 DM	1 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)	0.37	N/A	N/A
Maximum E2 level (pg/ml)[b]	3034.73 ± 1355.28	3719.36 ± 1399.58	4163.00 ± 1251.70	4635.83 ± 2029.10	0.0112	0.035	0.15
No. of collected oocytes[b]	20.10 ± 8.50	21.55 ± 4.82	27.00 ± 5.37	23.96 ± 7.88	0.057	0.0835	0.072
G1+G2 rate[c]	34.3% (510/1487)	36.7% (407/1108)	37.2% (348/936)	61.6% (635/1031)	<0.0001	<0.0001	<0.0001
Cryopreservation rate	70.0% (42/60)	81.8% (36/44)	100.0% (32/32)	100.0% (47/47)	<0.0001	0.0022	N/A
No. of frozen embryos[b]	3.00 ± 2.78	3.02 ± 2.62	4.09 ± 2.31	6.13 ± 3.54	<0.0001	<0.0001	0.0031
Days between OPU and menstruation[b,d]	16.30 ± 6.98	24.97 ± 17.69	17.80 ± 12.90	5.24 ± 2.54	<0.0001	<0.0001	<0.0001

[a]A: GnRH agonist-based long protocol, B: GnRH antagonist-based protocol with HCG trigger protocol, C: GnRH antagonist protocol with GnRH agonist trigger.

[b]Mean ± standard deviation.

[c]Total No. of G1+G2/Total No. of collected oocytes, G1: completely matured oocytes, G2: almost completely matured oocytes.

[d]Days between oocyte pickup and menstruation start.

TABLE 2 Comparison of OHSS incidence between modified COS and conventional treatments

OHSS	Group A[a] 60 women (60 cycles)	Group B[a] 38 women (44 cycles)	Group C[a] 32 women (32 cycles)	Modified COS 45 women (47 cycles)	*p*-value (Group A vs. Modified COS)	*p*-value (Group B vs. Modified COS)	*p*-value (Group C vs. Modified COS)
Mild	48.3% (29/60)	34.1% (15/44)	18.8% (6/32)	0.0% (0/47)	<0.0001	<0.0001	0.00326
Moderate	23.3% (14/60)	11.4% (5/44)	3.1% (1/32)	0.0% (0/47)	0.00021	0.02335	0.4051
Severe	8.3% (5/60)	4.5% (2/44)	0.0% (0/32)	0.0% (0/47)	0.0658	0.231	N/A
Total	80.0% (48/60)	50.0% (22/44)	21.9% (7/32)	0.0% (0/47)	<0.0001	<0.0001	0.00116

[a]A: GnRH agonist-based long protocol, B: GnRH antagonist-based protocol with HCG trigger protocol, C: GnRH antagonist protocol with GnRH agonist trigger

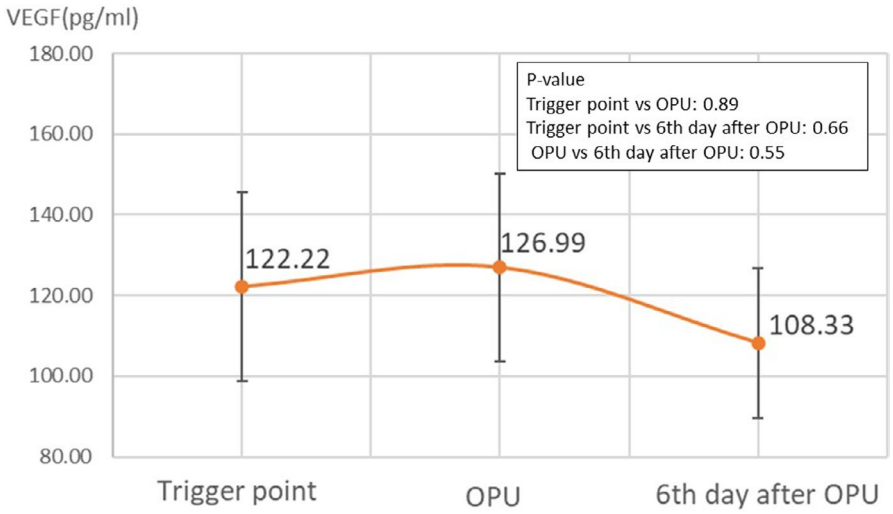

FIGURE 3 VEGF levels at three different points. The average values ± standard error of VEGF in 15 cases at trigger day, OPU, and six day after OPU were 122.22 ± 23.34 pg/ml, 126.99 ± 23.34 pg/ml, and 108.33 ± 18.58 pg/ml, respectively. VEGF concentrations did not increase significantly during the IVF cycle and decreased on 6th day after OPU. This result may lead to the prevention of the onset of OHSS

than in Group A and higher than in Groups B and C (live birth rates of Groups A, B, C, and modified COS: 18.3%, 27.2%, 25.9%, and 36%, respectively; *p*-value of Group A vs. modified COS, Group B vs. modified COS and Group C vs. modified COS: 0.0059, 0.2347, and 0.2254, respectively; cumulative birth rate of Groups A, B, C and modified COS: 30.0%, 38.6%, 37.5%, and 57.4%, respectively; *p*-value of Group A vs. modified COS, Group B vs. modified COS, and Group C vs. modified COS respectively: 0.0043, 0.0727, and 0.0817, respectively) (Table 3). This high clinical outcome is another advantage of this modified COS. There was no significant difference in average gestational duration and birth weight among the four groups (Average gestational duration of Groups A, B, C and modified COS: 39.33 ± 1.25 weeks, 39.62 ± 0.93 weeks, 38.90 ± 2.18 weeks, and 39.677 ± 1.48 weeks, respectively; average birth weight of Groups A, B, C and modified COS, respectively: 2949.05 ± 333.21 g, 3007.71 ± 511.38 g, 2982.78 ± 556.73 g, and 3014.13 ± 490.39 g, respectively) (Table 3). In the modified COS group, twenty-seven babies have been born (male: female = 13:14). All of them are healthy.

3.1 | A successful case report without development of OHSS

As an example, we have included the detailed case of one of the patients. The patient was 28-year-old. She had been suffering from PCOS with high levels of 12.24 mg/ml of Anti-Mullerian hormone (AMH) and antral follicles count with a large number of 20. We started FSH 150 IU from the 3rd day of the start of period, continued for four days and changed to HMG 150 IU for another four days. The diameter of leading follicle and E2 level on the 12th day were 22 mm and 3300 pg/ml, respectively. One more shot of HMG 150 IU was added to help the oocytes mature because of the heterogeneous size of follicles and the lack of tension of the leading follicles. Diameter of leading follicle was 26.8 mm and E2 level was 8865 pg/ml. Then two tablets of letrozole were administered in the morning and after confirmation of a decreased E2 level of 776 pg/ml before Leuprorelin acetate 2 mg was injected at night as trigger. Thirty-eight oocytes were retrieved, and nine high-quality blastocysts were cryopreserved. Three different drugs, letrozole, cabergoline, and GnRH antagonist tablet (Relugolix) were administered after OPU for five consecutive days. Menstruation started five days after OPU. E2 and P4 values three days after OPU were 5 pg/ml and 16.28 ng/ml, respectively. A healthy baby boy was born (3215 g, 39WD) after the frozen-thawed embryo transfer in the natural cycle (Figure 4). About 20 follicles each ovary was observed before OPU (Figure 5A) VEGF level, at the triggering point, OPU and 6 day after OPU were 145, 295, 95.2 pg/ml, respectively. Control level from women with an average age of 43 years was 134.3 pg/ml. All levels were within normal range (Figure 5B). Three days after OPU, both ovaries were not enlarged but hyperechoic angiogenesis in corpus luteum had hardly occurred (Figure 5C). Two days after menstruation both ovaries were normal in size (Figure 5D).

4 | DISCUSSION

PCOS is the typical cause of disordered ovarian function among young women and the biggest problems standing in the way are OHSS and the uneven and low-quality oocytes that follow COS.[2] Multiple treatments have been tried to overcome these difficulties. The advent of clomiphene has helped a lot of PCOS patients. However, for clomiphene-resistant patients, new alternative treatments were indispensable.[8] Low dose FSH administration, IVM,[1,3–5] LOD[6,8] and Coasting[23] have been performed to achieve relatively successful results. However, their actual clinical outcome is still unsatisfactory in comparison to that of patients with normal ovarian function.[1,6] Based on the above points, we started to look for alternatives and developed our modified COS. The GnRH antagonist protocol with GnRH agonist trigger for PCOS has been used widely around the world for PCOS and good results have been reported.[10,13] However, we wanted to develop a COS with more matured oocytes production without the problematic OHSS.

TABLE 3 Comparison of clinical outcome between modified COS and conventional treatments—2

	Group A[a] 60 women (60 cycles)	Group B[a] 38 women (44 cycles)	Group C[a] 32 women (32 cycles)	Modified COS 45 women (47 cycles)	*p*-value (Group A vs. Modified COS)	*p*-value (Group B vs. Modified COS)	*p*-value (Group C vs. Modified COS)
Clinical pregnancy rate	28.7% (33/115)	42.0% (34/81)	42.6% (23/54)	48.0% (36/75)	0.0068	0.4497	0.5431
Clinical pregnancy (*n*)[b]	24	23	18	34	NA	NA	NA
Cumulative pregnancy rate	40.0% (24/60)	54.5% (24/44)	56.3% (18/32)	72.3% (34/47)	0.0009	0.0776	0.1388
Miscarriage rate	24.2% (8/33)	29.4% (10/34)	30.4% (7/23)	19.4% (7/36)	0.6293	0.3311	0.3331
Live birth rate	18.3% (21/115)	27.2% (22/81)	25.9% (14/54)	36.0% (27/75)	0.0059	0.2347	0.2254
Cumulative live birth rate	30.0% (18/60)	38.6% (17/44)	37.5% (12/32)	57.4% (27/47)	0.0043	0.0727	0.0817
Gestational duration (wks)[c]	39.33 ± 1.25	39.62 ± 0.93	38.90 ± 2.18	39.67 ± 1.48	0.748	0.892	0.590
Birth weight (g)[c]	2949.05 ± 333.21	3007.71 ± 511.38	2982.78 ± 556.73	3014.13 ± 490.39	0.803	0.951	0.863

[a]A: GnRH agonist-based long protocol, B: GnRH antagonist-based protocol with HCG trigger protocol, C: GnRH antagonist protocol with GnRH agonist trigger.

[b]Patients number.

[c]Mean ± standard deviation.

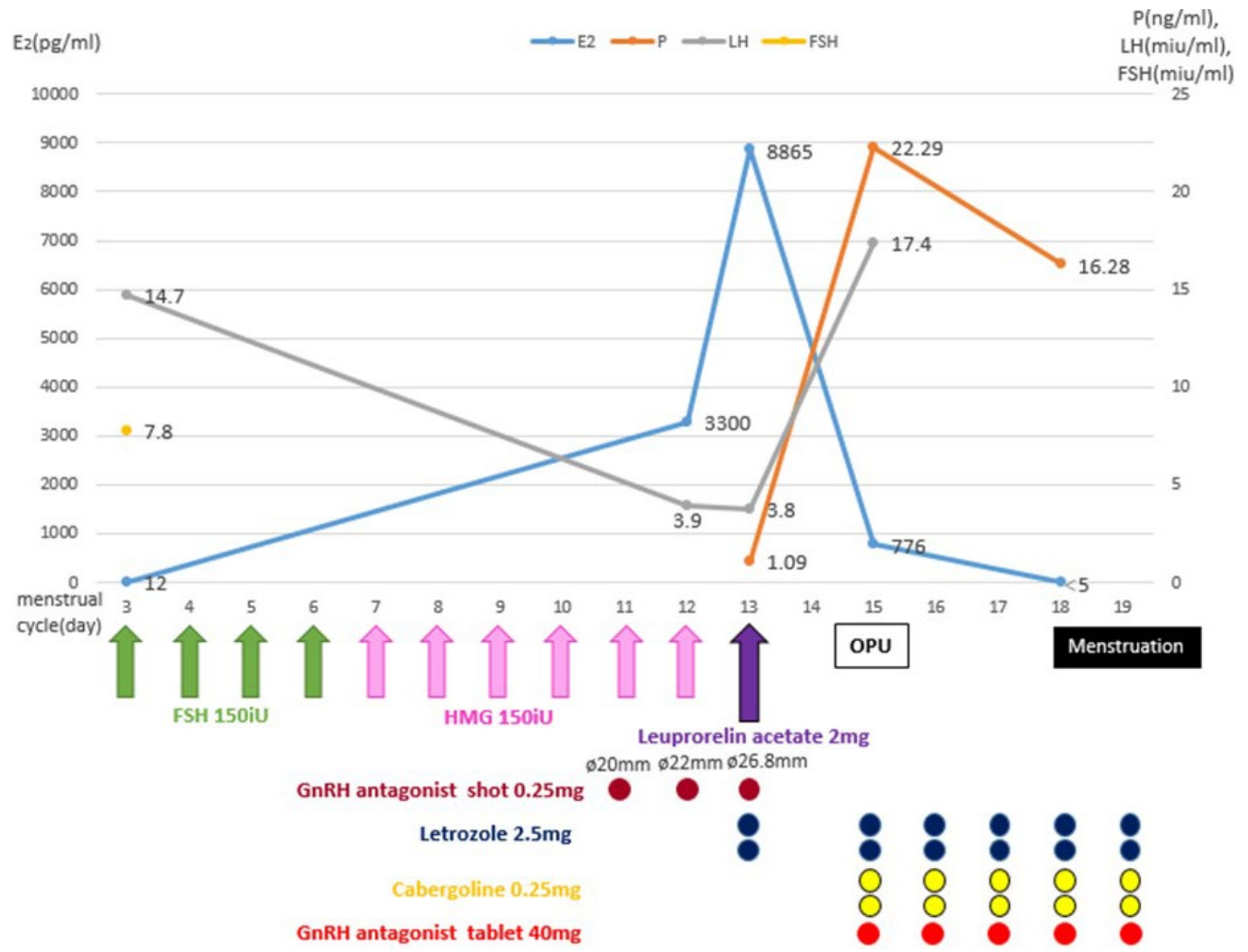

FIGURE 4 A successful case report without development of OHSS. We started FSH 150 IU from the 3rd day of the start of period for 4 day and changed to HMG 150 IU for 4 day. GnRH antagonist shots (Cetrorelix acetate) were started when the leading follicle reached 20 mm in diameter. The diameter of leading follicle and E2 level on the 12th day were 22 mm and 3300 pg/ml. One more shot of HMG 150 IU was added to help the oocytes mature. Diameter of leading follicle was 26.8 mm and E2 level was 8865 pg/ml. Then 2 tablets of letrozole were administered in the morning, and leuprorelin acetate 2 mg was injected at night as trigger. The E2 level at OPU was down to 776 pg/ml. Thirty-eight oocytes were retrieved, and 9 high-quality blastocysts were cryopreserved. Menstruation started 5 day after OPU. No problematic OHSS was observed

There are significant differences between our modified COS and the GnRH antagonist protocol with GnRH agonist trigger. The first difference in the modified COS is the use of aromatase inhibitor (Letrozole) to reduce the high E2 level caused by injection of gonadotrophin to less than 1000 pg/ml by the administration of the trigger. It has been believed that the high level of E2 level was dangerous to induce vessel permeability, but this is questionable.[24] High-level E2 itself does not induce OHSS. Thus, we waited until the follicles are fully grown (22–24 mm in diameter) and in some cases up to 26–28 mm in diameter. We also wait until the follicles other than the chief follicle mature uniformly. At this time, the E2 level might be higher (over 6000–8000 pg/ml). Some physicians may criticize this COS because of the risk of OHSS accompanied with low quality oocytes. However, due to the high ratio of G1 + G2/number of collected oocytes and the large number of cryopreserved embryos, this modified COS produced more highly matured oocytes than conventional COS (Table 1). It also showed that it improved clinical outcomes (Table 3). We believe that poor clinical outcome with conventional methods might be due to the use of trigger before the follicle matures enough to avoid OHSS. We believe that the reason we achieved a high number of high-quality oocytes was letting the lead follicle grow larger than usual, which led to uniform maturation of follicles other than the lead follicle.

The second difference is the administration of three different drugs, letrozole, cabergoline, and GnRH-antagonist tablet (Relugolix) from just after the oocytes collection continued for 5days to inhibit a formation of corpus luteum, resulting in no occurrences of clinically problematic OHSS.

The idea of this combination of drugs aiming at a synergetic effect for preventing OHSS was derived from four reports.[15,16,25,26] In 2013, H.S. Lee reported that aromatase inhibitor co-treatment with gonadotropin ovarian stimulation reduces peak E2 level without

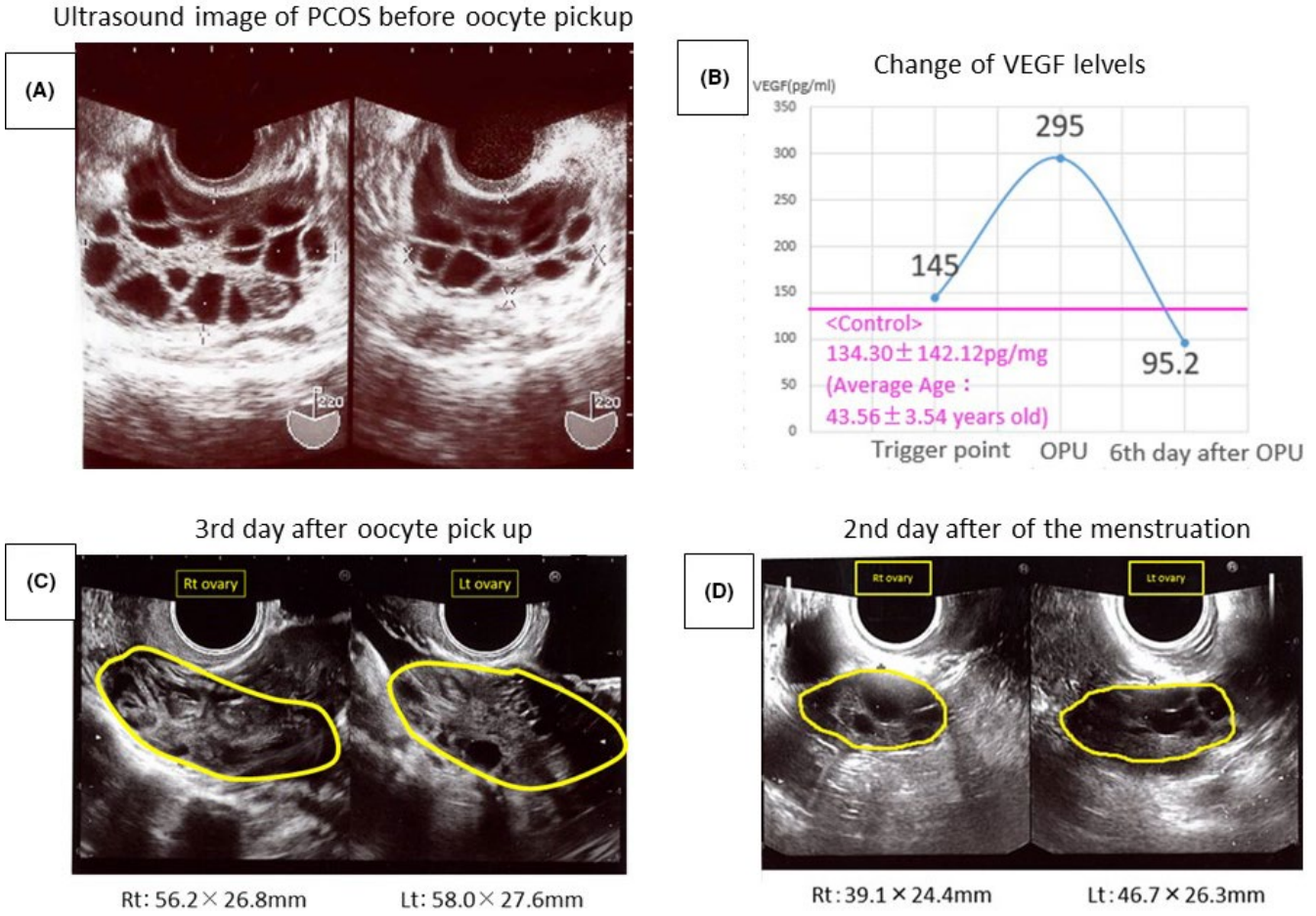

FIGURE 5 Clinical characteristics of a successful PCOS case using modified COS. (A) Ultrasound image of PCOS before oocyte pickup. About 20 follicles in both ovaries before OPU. (B) Change of VEGF levels at three different points. All VEGF levels were within normal range. (C) 3rd day after OPU, both ovaries were not enlarged and active corpus luteum was not be found. (D) 2nd day after the start of menstruation. They were normal in size

compromising IVF outcomes in high responder patients. In addition, the incidence of severe OHSS was apparently lower when letrozole was used.[25] Y. Chen reported in 2018 a retrospective study of letrozole treatment prior to human chorionic gonadotropin in PCOS patients and explained the mechanism of lowering the E2 level due to luteolysis.[15] S.R. Soares reported that dopamine agonist inhibits phosphorylation of VEGF receptor-2 resulted in prevention of OHSS in 2008.[16] W.M. Ataallar and T.A. Elhamid compared the efficacy for prevention of OHSS and concluded cabergoline could effectively prevent the development of early OHSS in 2017.[26] We started the new treatment considering the effect of the combination of the three different drugs aiming at their synergetic benefit for complete prevention of OHSS.

The suppression of the activation of VEGF and angiogenesis in corpus luteum consecutively following the use of the three different drugs is predicted by the absence of elevated serum VEGF and the reduction of E2 and progesterone level to the normal range six day after OPU and solid pattern inside of both ovaries evaluated by ultrasound. Hypoechoic image and not-enlarged ovaries seem to suppress the angiogenesis in corpus luteum. It has now been recognized that the VEGF system, composed of ligands and receptors, plays a pivotal role in the pathophysiology of OHSS.[27–30] R. Agrawal reported that serum VEGF concentrations rose during the phase of ovarian stimulation were significantly higher on the day of hCG administration and rose further thereafter. Furthermore, they reported that serum VEGF concentrations were higher in women in whom OHSS developed than in women in whom it did not.[27] In our study, serum VEGF concentrations did not increase significantly during the IVF cycle, and serum VEGF concentrations on day 6 after OPU tended to decrease. Therefore, suppressing the increase in serum VEGF concentrations caused by three different drugs may help prevent the onset of OHSS. Shortened number of days until menstruation (5.2 days in average), minimal swelling of the ovaries, and no ascites also prove the superiority of this modified COS.

Although highly promising, this method has some limitations, and it is premature to conclude that this method has been established. The number of cases is still small, and it is necessary to accumulate cases. Since there is no long-term observation, safety is not yet completely guaranteed. Study limitations, inherent of retrospective chart reviews, include the inability to collect data on potentially important confounders and avoid missing data. In addition, the treated cases

were diagnosed as PCOS using the Rotterdam criteria. However, the heterogeneity in the state of PCOS should be considered. Further investigation is still necessary to prove the superiority of this method for various conditions of PCOS compared to other treatments, including IVM, LOD and Minimal stimulation.

In conclusion, OHSS is potentially fatal complication associated with ART procedures; therefore, it must be avoided for safe treatment. Our novel COS, modified GnRH antagonist method using letrozole, cabergoline, and relugolix could completely prevent developing OHSS and collect a large number of mature oocytes following OPU in the women with PCOS, leading to a high clinical pregnancy and live birth rates in our study. However, further clinical studies including prospective controlled trials in the modified COS are warranted in the future.

ACKNOWLEDGMENTS

We thank Dr. Sumihide Okamoto for his devotion to the development of this method and Mr. Roberto Rodriguez for his advice in the preparation of this manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

HUMAN RIGHTS STATEMENTS AND INFORMED CONSENT

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and its later amendments. Informed consent was obtained from all patients for being included in the study.

ANIMAL STUDIES

This study does not involve any animals.

APPROVAL BY ETHICS COMMITTEE

This study was approved by the Institutional Review Board of the Saint Mother Obstetrics and Gynecology Clinic on December 20, 2017.

CLINICAL TRIAL REGISTRY

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How to cite this article: Yanagihara Y, Tanaka A, Nagayoshi M, et al. A modified GnRH antagonist method in combination with letrozole, cabergoline, and GnRH antagonist for PCOS: Safe and effective ovarian stimulation to treat PCOS and prevent OHSS. *Reprod Med Biol*. 2022;21:e12429. doi:10.1002/rmb2.12429