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Effect of Switching from a Progestin-Primed Ovarian Stimulation Protocol to a Modified Ultra-Long Protocol Among Women Who Had 1 Progestin-Primed Ovarian Stimulation (PPOS) Failure Verses Those Who Had 2 PPOS Failures

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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: There is little research on whether normoresponsive patients who produced poor-quality embryos once verses those who produced poor-quality embryos twice when using a single COH protocol should change to a different controlled ovarian hyperstimulation (COH) protocol.

Material/Methods: In this retrospective study, we enrolled 108 patients with 1 PPOS failure who chose to continue receiving the progestin-primed ovarian stimulation (PPOS) protocol (*n*=61) versus those who decided to switch to the modified ultra-long protocol (*n*=47). We also enrolled 131 normoresponsive patients with 2 PPOS failures who chose to continue receiving the PPOS protocol (*n*=60) versus those who decided to switch to the modified ultra-long protocol (*n*=71) in the third cycle.

Results: We found no significant difference in clinical outcomes of patients with 1 PPOS failure who continued using the PPOS protocol verses those who switched to the modified ultra-long protocol in the second cycle, expect for a lower cancelation rate (4.3% *vs.* 16.4%). However, the patients with 2 PPOS failures had significantly more good-quality embryos (0.9 *vs.* 0.4), more viable embryos (1.8 *vs.* 0.9), lower cancelation rates (18.3% *vs.* 53.3%), and higher pregnancy rates per aspirated cycle (26.8% *vs.* 10.0%) when switching to the modified ultra-long protocol compared to those who decided to continue receiving the PPOS protocol ($P<0.05$). Furthermore, the odds of clinical pregnancy (odds ratio [OR] 5.997, 95% confidence interval [CI] 1.476–24.361, $P=0.01$) were positively associated with switching to the COH protocol in the third cycle.

Conclusions: For normoresponsive patients with poor-quality embryos when using the PPOS protocol, switching to the modified ultra-long protocol after having 2 PPOS failures was associated with better ART outcomes.

MeSH Keywords: Embryonic Development • Fertilization *In Vitro* • Medroxyprogesterone • Ovulation Induction

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Background

The progestin-primed ovarian stimulation (PPOS) protocol is considered to be an effective method to prevent premature production of luteinizing hormone (LH) in patients, mainly by using medroxyprogesterone acetate (MPA) [1]. Satisfying results have been achieved, which are not significantly different from those achieved using the conventional protocol. Furthermore, this protocol is suitable for specific patients, such as patients with diminished ovarian reserve who need to increase the number of viable embryos, prompting better pregnancy outcomes in obese patients, and decreasing the ovarian hyperstimulation syndrome rate in polycystic ovarian syndrome patients [2–5]. However, compared with the cancelation rate (6.3%) of the conventional protocol [6], PPOS had a non-negligible cancelation rate (9.3%). Moreover, we found that approximately 3% of normoresponsive patients experienced repeated failures when using the PPOS protocol in clinical treatment, and their oocytes were characterized by high immaturity rates, low fertilization rates, slow division rates, and poor cell morphology [6].

Currently, a variety of regimens are applied for ovarian stimulation, and there are no obvious differences in the quality of embryos between protocols in most meta-analyses [7, 8]. However, for patients who have failed once, it is controversial whether the first cycle can predict the next cycle [9–11]. Moreover, few studies have explored how patients should be treated after repeated *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) failures after undergoing a single protocol. Some studies demonstrated that the gonadotrophin-releasing hormone (GnRH)-antagonist protocol may be beneficial after GnRH agonist protocol failures [12]. A novel ultra-short GnRH agonist combined with GnRH antagonist protocol was proven to be effective for treating patients who were repeatedly found to have poor-quality embryos [13]. These studies illustrate that an alternative controlled ovarian stimulation (COH) regimen can be beneficial in these patients.

Human menopausal gonadotropin (hMG) promotes follicular development from days 2 to 5, and MPA prohibits the premature LH surge before oocyte retrieval by inhibiting the positive feedback of estrogen via the hypothalamus, but not the pituitary; mainly through regulating the activity of GnRH upstream neurons (e.g., kisspeptin, neurokinin B, and dynorphin neurons) in the PPOS protocol [14,15]. Because of the effect of premature production of progesterone on the endometrium, the PPOS protocol requires a freeze-all strategy [5], which can damage poor-quality embryos. Therefore, for patients with PPOS who have had single or repeated poor-quality embryos, we reviewed previous studies and considered the effect of switching to another protocol with different action mechanisms and transfer strategies, such as the ultra-long protocol. The modified ultra-long protocol involves GnRH-induced pituitary desensitization, resulting in deep inhibition of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), and it is usually associated with fresh embryo transfer (ET), avoiding the use of freeze-thawed embryos [16]. The ultra-long protocol is usually used in young patients and in normogonadotropic patients, and it results in a higher live birth rate than with the classic mid-luteal long-agonist protocol [17]. Therefore, we hypothesized that the ultra-long protocol would benefit patients who had experienced PPOS protocol failures.

The present study explored the effect of switching to another COH protocol (the modified ultra-long protocol) among normoresponsive patients receiving the PPOS protocol who produced poor-quality embryos once verses those who produced poor-quality embryos twice, to achieve better clinical outcomes. In addition, the optimal time for switching protocols needs to be confirmed.

Material and Methods

This retrospective study was conducted at the Ninth People's Hospital, Department of Assisted Reproduction, Shanghai Jiao Tong University School of Medicine between September 2015 and July 2017. The study procedure was authorized by the Ethics Committee (Institutional Review Board) of the hospital (approval no. 2017-320-T240). All individual participants signed informed written consent. We used the following inclusion criteria: women ages 24–42 years, antral follicle count (AFC) >5, basal FSH less than 10 mIU/ml, and previous PPOS failures in normoresponsive patients with poor-quality embryos (definition: more than 5 oocytes retrieved but no more than 1 good-quality embryo on day 3 in their first or initial 2 cycles with the PPOS protocol) [18]. The exclusion criteria were: 1) hyperprolactinemia or other endocrine diseases, 2) patients who took hormone drugs within the past 3 months, 3) any contraindications to IVF/ICSI treatment, and 4) patients with laboratory method change (e.g., assisted oocyte activation) that could improve embryo quality.

Among the 108 patients had a single PPOS cycle failure, in their second cycle, 61 chose to stay with the PPOS protocol and 47 switched to the modified ultra-long protocol. There were 131 patients who had 2 successive PPOS failures; in their third cycle, 60 of these patients decided to stay with their current protocol and 71 decided to switch to the modified ultra-long protocol.

The detailed regimen in the PPOS protocol was: hMG (150–225 IU/d; Fengyuan Pharmaceutical Co., China) was administered on menstrual cycle days 2–5. At the same time, MPA (10 mg/day, XianJu Pharmaceutical Co., China) was used. Oocytes were triggered by hCG (5000 IU; Lizhu Pharmaceutical Trading Co.)

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and triptorelin (0.1 mg; Decapeptyl, Ferring Pharmaceuticals). In the modified ultra-long protocol, a long-acting gonadotrophin-releasing agonist (leuprorelin acetate, 3.75 mg, Lizhu Pharmaceutical Trading Co.) was administered on cycle days 2–5. If there was downregulation (E_2 <50 pg/ml) after 35 days, hMG (150–225 IU/d) was administered and the oocytes were triggered by hCG (5000 IU). Doses were adjusted by the test outcome of patients receiving the 2 protocols. Serum levels of the hormones (FSH, LH, E_2 and P) were measured by chemiluminescence during the COH (Abbott Biologicals B.V.), as described elsewhere [1].

Fertilization was carried out either by conventional insemination (IVF) or ICSI, according to sperm concentration and motility [19]. The embryos were individually cultured in microdrops (continuous single-culture medium, Irvine Scientific, USA). Third-day embryos were accessed by number and regularity of embryos, according to Cummins' criteria [20]. Our center defined the top-quality embryos as grades 1 and 2 cleavage-stage embryos (≥6 blastomeres). Embryos that did not meet the good-quality criteria (Grade 3 and 4; or Grade 1 and 2 with less than 6 cells) were placed in the extended culture. Embryos with good-quality cleavage and blastocysts with good morphology (≥3BB) cultured from the non-top-quality cleavage embryos were then frozen, expect for the fresh-transfer embryos in the ultra-long protocol [1,21].

In the modified ultra-long protocol, the fresh embryos were transferred 3 days after oocytes were aspirated, but frozen-thawed embryo transfer (FET) was used for some patients who had failed or canceled ETs. In the PPOS protocol, all the patients received transferred cryopreserved-thawed embryos. Natural cycle, letrozole mild stimulation, or hormone replacement treatment was implemented according to the situations of patients. After embryo transfer, the progesterone supplementation method was implemented: soft vaginal progesterone capsules (Utrogestan, 0.4 g/day, Laboratories Besins-Iscovesco) and yellow Femoston tablets (4mg/day, Complex Packing Estradiol Tablets/Estradiol and Dydrogesterone Tablets, Abbott Healthcare Products B.V.) were used until 3 months if the patients were pregnant.

Statistical analysis

The primary outcome in this study was the number of good-quality embryos on day 3. The secondary outcomes were the oocyte utilization rate, cancelation rate, and clinical pregnancy rate per transfer cycle or aspirated cycle. We used the following formulas: the oocyte utilization rate=the number of viable embryos/the number of oocytes aspirated; the cancelation rate=the number of cycles without viable embryos/number of total retrieving cycles; clinical pregnancy rate per transfer cycle=(intrauterine+ectopic) pregnancies/the number of transfer cycles; the implantation rate=the number of gestational sacs/the number of embryos transferred; the ongoing pregnancy rate was considered as the presence of a gestational sac with fetal heart activity by ultrasound examination at 12 weeks of gestation; and the clinical pregnancy rate per retrieved cycle=(intrauterine+ectopic) pregnancies/the number of retrieved cycles.

Statistical analyses were carried out using the SPSS 22.0 statistical package (SPSS, Inc., Chicago, IL). Variables are expressed as means±SDs or means (95% confidence intervals (Cls). The *t* test was used for normal distributions of continuous variables and the Mann-Whitney U-test was used for non-normal distributions. Qualitative data are shown as numbers and percentages. The chi-squared test or Fisher's exact test was used, as appropriate. To further investigate the parameters associated with clinical pregnancy outcomes, logistic regression analysis was performed. $P<0.05$ was defined as a statistically significant difference.

Results

In both groups, although these patients had consistently normal responses in their failed PPOS cycles (Figure 1A, 1F), their common characteristic was that they rarely had good-quality embryos (Figure 1B, 1G) or even viable embryos (Figure 1C, 1H), thus resulting in a lower pregnancy rate per transfer cycle (Figure 1D, 1I) and per aspirated cycle (Figure 1E, 1J) in their failed PPOS cycles compared to their subsequent cycle.

Table 1 shows that the duration of hMG was shorter and that the dosage of hMG was lower in the PPOS group ($P<0.05$) than in the modified ultra-long group. Among women in their second cycle after a first PPOS failure, there were no significant difference between the PPOS group and modified ultra-long protocol in numbers of retrieved oocytes (12.2 *vs.* 11.1), mature oocytes (10.5 *vs.* 9.1), day 3 good-quality embryos (2.0 *vs.* 1.8), or viable embryos (2.6 *vs.* 2.8). However, a significant difference was found in the cycle cancelation rate (16.4% *vs.* 4.3%, $P=0.047$). Furthermore, the embryonic quality was significantly improved in the second PPOS and ultra-long cycles compared to the first failed PPOS cycle (Figure 1B, 1C).

In the third cycle after consecutive PPOS failures, the numbers of aspirated oocytes and mature oocytes were comparable ($P>0.05$), but the modified ultra-long group had more good-quality embryos (0.9 [0.6, 1.3] *vs.* 0.4 [0.2, 0.5]) and viable embryos (1.8 [1.5, 2.2] *vs.* 0.9 [0.6, 1.2]), as well as a higher rate of good-quality embryos (17.5% *vs.* 6.3%), a higher oocyte utilization rate (21.8% *vs.* 8.9%), and a lower cancelation rate (18.3% *vs.* 53.3%), compared to the PPOS group (all $P<0.05$). Furthermore, the embryonic quality was significantly

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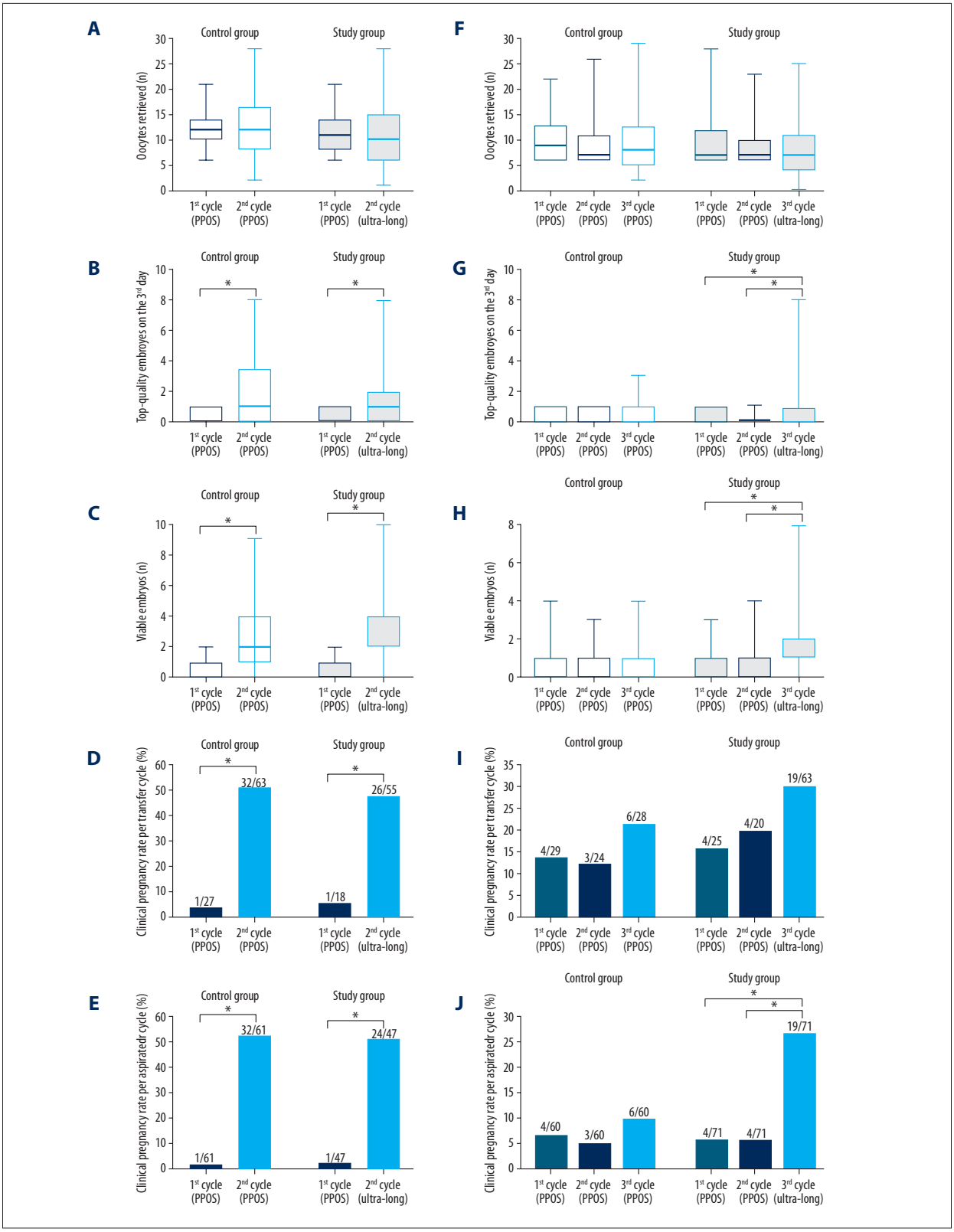

Figure 1. (**A–E**) Oocytes retrieved, top-quality embryos on third day, viable embryos, clinical pregnancy rate per transfer cycle, and clinical pregnancy rate per aspirated cycle in patients with first PPOS failure. (**F–J**) The above variables in patients with 2 PPOS failures. PPOS: progestin-primed ovarian stimulation.

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Table 1. Ovarian stimulation and oocyte performance between PPOS and the ultra-long protocol after once or twice PPOS failures.

Parameters	2nd cycle after 1st PPOS failure			3rd cycle after repeated PPOS failures		
	PPOS protocol	Modified ultra-long protocol	*P* value	PPOS protocol	Modified ultra-long protocol	*P* value
No. of aspirated cycles (n)	61	47		60	71	
Age (year)	31.1±3.3	31.8±4.1	0.33	33.5±3.5	33.9±3.7	0.47
Infertility duration (year)	3.1±2.3	2.7±2.5	0.41	4.1±2.8	3.9±2.8	0.70
BMI (kg/m2)	22.6±3.5	21.8±3.6	0.21	21.7±3.1	21.2±2.6	0.34
Antral follicle count (n)	14.3±4.9	14.6±4.9	0.76	12.3±5.8	11.1±4.3	0.19
Duration of Gn used (d)	9.6±1.8	11.6±3.1	<0.01	9.4±2.3	11.5±2.5	<0.01
Total dosage of Gn used (IU)	1963.5±538.1	2669.7±869.3	<0.01	1956.4±654.0	2572.2±588.7	<0.01
Oocytes retrieved (n)	12.2±6.1	11.1±5.8	0.36	9.7±6.1	8.4±5.0	0.16
MII oocytes (n)	10.5±5.5	9.1±4.9	0.17	7.3±5.5	6.8±4.5	0.59
Normal fertilized oocytes (n)	7.6±4.3	8.3±5.0	0.45	5.7±5.0	5.2±4.1	0.63
Top-quality embryos on the third day (n)	2.0 (1.5,2.5)	1.8 (1.3,2.3)	0.81	0.4 (0.2,0.5)	0.9 (0.6,1.3)	0.02
Viable embryos (n)	2.6 (2.0,3.1)	2.8 (2.3,3.3)	0.21	0.9 (0.6,1.2)	1.8 (1.5,2.2)	<0.01
MII oocyte rate (%)	86.8±16.0	81.9±16.2	0.12	74.8±25.2	83.5±19.1	0.06
Good-quality embryos per normal fertilized oocyte (n) (%)	119/504 (23.6)	83/356 (23.3)	0.92	21/334 (6.3)	64/366 (17.5)	<0.01
Oocyte utilization rate (n) (%)	156/743 (21.0)	130/522 (24.9)	0.10	51/575 (8.9)	129/593 (21.8)	<0.01
Non-viable embryo cancelation rate (n) (%)	10/61 (16.4)	2/47 (4.3)	0.047	32/60 (53.3)	13/71 (18.3)	<0.01

The data are presented as the means±SDs, means (95% CIs) or n (%).

improved in the third ultra-long cycle compared with the first and second failed PPOS cycles (Figure 1G, 1H). There were no significant differences associated with which insemination technique was used (data not shown). Overall, while similar numbers of mature oocytes were collected, more good-quality embryos were achieved in the patients who switched to the modified ultra-long protocol than those who stayed with the PPOS protocol after consecutive PPOS failures versus those who had only 1 PPOS failure.

Figure 2 presents the significantly different hormone profiles in the 2 groups in the third cycle, which could induce different embryological outcomes. Before triggering, FSH levels increased gradually in both protocols, but after triggering, FSH levels increased in the PPOS protocol and declined in the ultra-long group ($P<0.05$). LH levels decreased during COH and then clearly increased after triggering in the PPOS group, and were higher than in the modified ultra-long group ($P<0.05$). Estradiol (E_2) levels continuously increased and were significantly lower in the modified ultra-long protocol than in the PPOS protocol ($P<0.05$). In the ultra-long protocol, the progesterone (P) levels were different on the gonadotropin (Gn) start day because of downregulation, and in later stimulation days.

Table 2 shows pregnancy outcomes. In the second cycle after a first PPOS failure, no differences were observed in the clinical pregnancy rate per transfer (50.8% *vs.* 47.3%), ongoing pregnancy rate (46.0% *vs.* 43.6%), or clinical pregnancy rate per aspirated cycle (52.5% *vs.* 51.1%). There was also no significant difference between ET and FET in the modified ultra-long protocol (Supplementary Table 1). We thus concluded that after a first PPOS failure, the change of COH protocol did not lead to better pregnancy outcomes.

However, data from Table 2 and Supplementary Table 1 show that the highest clinical pregnancy rate per transfer was observed in modified ultra-long ET (u-ET, 32.0%), followed by modified ultra-long FET (u-FET, 23.1%) and PPOS-FET (21.4%). The same trends were observed in the ongoing pregnancy rate (u-ET, 28.0%; u-FET, 23.1%; PPOS-FET, 21.4%) and in the

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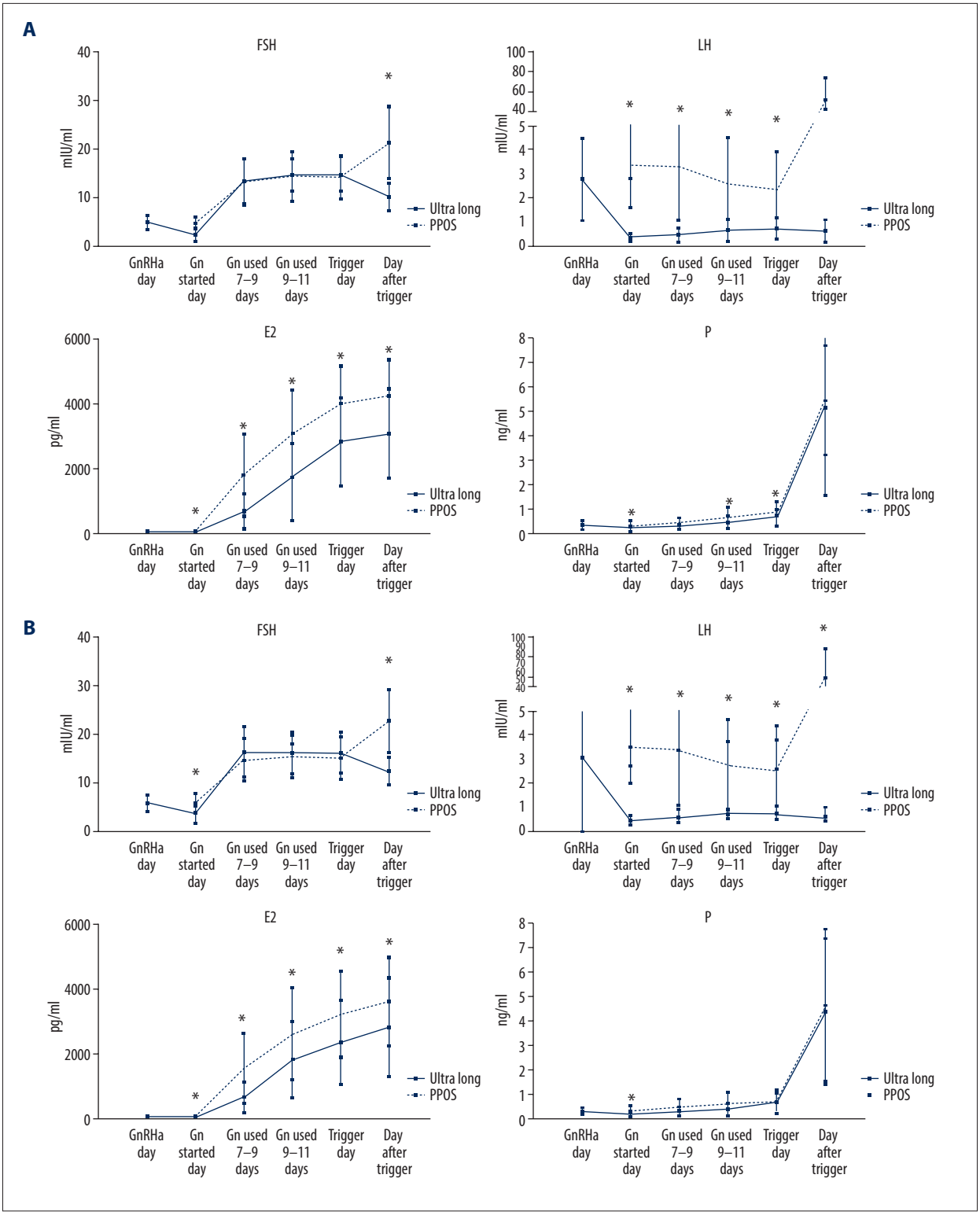

Figure 2. (**A**) Serum FSH, LH, E2, and P levels in the second cycle after a first PPOS failure. (**B**) Serum FSH, LH, E2, and P levels in the third cycle after 2 consecutive PPOS failures. The solid lines represent the ultra-long group and the dotted lines represent the PPOS group. FSH – follicle-stimulating hormone; LH – luteinizing hormone; E2 – estradiol; P – progesterone; PPOS – progestin-primed ovarian stimulation. Asterisks (*) indicate significant differences between the PPOS protocol and the modified ultra-long protocol ($P<.05$).

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Table 2. Pregnancy outcomes in the two different protocols in the cycle after 1st or repeated PPOS failures.

Parameters	2nd cycle after 1st PPOS failure			3rd cycle after repeated PPOS failures		
	PPOS protocol	Modified ultra-long protocol	*P* value	PPOS protocol	Modified ultra-long protocol	*P* value
No. of oocyte aspirated cycles (n)	61	47		60	71	
No. of embryo transfer cycles (n)	63	55		28	63	
Ratio of fresh embryo transfer (n) (%)*	0 (0.0)	40 (72.7)		0 (0.0)	50 (79.4)	
No. of transferred embryos (n)	1.7±0.5	1.8±0.4	0.17	1.4±0.5	1.7±0.4	0.98
No. of transferred third-day good-quality embryos (n)	1.4±0.8	1.2±0.9	0.29	0.5±0.5	0.9±0.8	0.14
Biochemical pregnancy rate per transfer, cycle n (%)	33/63 (52.3)	27/55 (49.1)	0.72	7/28 (25.0)	21/63 (33.3)	0.43
Clinical pregnancy rate per transfer cycle, n (%)	32/63 (50.8)	26/55 (47.3)	0.70	6/28 (21.4)	19/63 (30.1)	0.39
Ectopic pregnancy rate, n (%)	0/32 (0.0)	0/26 (0.0)	/	0/6 (0.0)	0/19 (0.0)	/
Early abortion rate, n (%)	3/32 (9.4)	2/26 (7.7)	0.82	0/6 (0.0)	2/19 (10.5)	1.00
Twin pregnancy rate, n (%)	7/32 (21.9)	6/26 (23.1)	0.28	0/6 (0.0)	3/19 (15.8)	0.55
Ongoing pregnancy rate, n (%)	29/63 (46.0)	24/55 (43.6)	0.79	6/28 (21.4)	17/63 (27.0)	0.57
Implantation rate, n (%)	39/105 (37.1.)	32/99 (32.3)	0.47	6/38 (15.7)	22/109 (20.2)	0.55
Clinical pregnancy rate per aspirated cycle, n (%)	32/61 (52.5)	24/47 (51.1)	0.89	6/60 (10.0)	19/71 (26.8)	0.02

* Means fresh embryo transfer cycles/total embryo transfer cycles.

Table 3. Logistic regression analysis of factors associated with clinical pregnancy outcome.

Parameter	2nd cycle after 1st PPOS failure		3rd cycle after repeated PPOS failures	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Age (year)	1.018 (0.904–1.147)	0.77	0.830 (0.699–0.986)	0.03
Infertility duration (year)	1.030 (0.848–1.250)	0.77	0.785 (0.573–1.075)	0.73
Gravidity (n)	0.974 (0.638–1.486)	0.90	1.004 (0.537–1.878)	0.99
Antral follicle count (n)	1.067 (0.759–1.500)	0.71	0.908 (0.784–1.053)	0.20
Basal FSH (mIU/ml)	0.873 (0.672–1.135)	0.31	0.773 (0.498–1.201)	0.25
Basal LH (mIU/ml)	1.004 (0.974–1.035)	0.80	1.412 (0.956–2.085)	0.08
Basal E2 (pg/ml)	1.029 (0.940–1.126)	0.54	0.984 (0.946–1.023)	0.41
No. of mature oocytes (n)	1.122 (1.023–1.232)	0.02	1.134 (0.991–1.298)	0.07
COH protocol				
PPOS protocol	Reference		Reference	
Modified ultra-long protocol	1.065 (0.459–2.469)	0.88	5.997 (1.476–24.361)	0.01

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implantation rate (u-ET, 20.7%; u-FET, 17.6%; PPOS-FET, 15.7%), but these differences were not significant ($P>0.05$). Notably, we found a higher clinical pregnancy rate per aspirated cycle (26.8% *vs.* 10.0%) in the modified ultra-long protocol than in the PPOS protocol ($P<0.05$). Overall, for pregnancy outcomes, we observed a higher trend in the u-ET group than in the u-FET group, followed by the PPOS-FET group, and the clinical pregnancy rate per aspirated cycles was significantly higher in the modified ultra-long protocol than in the PPOS protocol.

Logistic regression analysis was conducted to analyze the parameters associated with clinical pregnancy (Table 3). In the second cycle and third cycle after PPOS failures, the clinical pregnancy rate was not significantly associated with basic characteristics, including infertility duration, gravidity, or number of antral follicles ($P>0.05$), nor with the basal endocrine levels (FSH, LH, and E_2, $P>0.05$), but did have a significant relationship with the number of mature oocytes in the second cycle (OR 1.122, 95% CI 1.023–1.232, $P=0.02$), and logic regression analysis showed a weak effect of this variable in the third cycle (OR 1.134, 95% CI 0.991–1.298, $P=0.07$). The odds of clinical pregnancy significantly decreased with increasing age, which is consistent with our experience and previous research [22]. Compared with remaining on the PPOS protocol, switching to the modified ultra-long protocol in the second cycle after only 1 PPOS failure demonstrated no significant difference (OR 1.065, 95% CI 0.459–2.469, $P=0.88$). Notably, compared with continuing to use the PPOS protocol, switching to the modified ultra-long protocol in the third cycle significantly increased the odds of a clinical pregnancy (OR 5.997, 95% CI 1.476–24.361, $P=0.01$) (Table 3).

Discussion

Based on our data, for the normoresponsive patients using the PPOS protocol who had poor-quality embryos only 1 time, there was no significant difference between those changing to the modified ultra-long protocol versus those who continued using the PPOS protocol. However, after having poor-quality embryos 2 times, it was essential to change to the modified ultra-long protocol to produce more good-quality embryos, to increase the number of viable embryos, to lower cancelation rates (18.3% *vs.* 53.3%), and to increase pregnancy rates per retrieved cycle. Logistic regression analysis further supported that this increasing clinical pregnancy rate was induced by protocol change after having 2 PPOS failures.

The number of failures is an important index to use in deciding whether to change to another COH protocol. Our research indicates that the group of women with only 1 PPOS failure is not entirely representative of the subsequent IVF/ICSI cycles. This is consistent with previous studies [10,23], but different from the results of Stern et al. [9], probably because their first cycles, such as no retrieval, were induced by relatively specific reasons and therefore had better predictability. Some research also found that cycle 1 could predict the ovarian response of the next cycle [11], which was also verified by our finding that the second cycle retrieved a similar number of oocytes as the first cycle. However, our retrospective study suggests that changing to a different protocol, such as the ultra-long protocol, can result in better clinical outcomes after 2 consecutive PPOS failures rather than staying with the PPOS protocol. Although the specific mechanism remains unclear, our data prompts us to speculate that the changes made by switching to the ultra-long protocol may due to the decreased LH concentrations, increased Gn doses, and use of a fresh ET strategy.

Differences in LH levels may result in variations in the intrafollicular microenvironment. First, an optimal level of LH is required during COH. Figure 2 demonstrates that the lowest LH level was 2.6 mIU/ml in the PPOS regimen. However, the average LH level in the modified ultra-long group was less than 1 mIU/ml at all timepoints during COH. Basic and clinical studies demonstrated that ovarian follicle development needs a critical level of LH. No oocytes fully mature if LH levels are below this threshold, whereas follicular atresia occurs when LH levels are too high [24]. Low serum LH levels can increase LH receptor sensitivity in mural granulosa cells, so the same dose of HCG triggers higher amphiregulin (AR) concentrations in the follicular fluid, which can promote oocyte maturation [25]. Therefore, we hypothesized that the differences in endogenous LH levels during COH were one reason why the patients experienced repeated IVF failure when using the PPOS protocol but then obtained good embryos when changing to the ultra-long protocol.

Another reason why changing to the ultra-long protocol can result in better clinical outcomes than staying with the PPOS protocol after 2 consecutive PPOS failures may be differences in the hMG doses. A higher dosage of Gn was used in the ultra-long protocol, consistent with past studies [26]. There were significant differences in the Gn dose per oocyte and per mature oocyte between the 2 groups. In some studies, a higher dose of gonadotropins resulted in more aneuploid embryos [27]. However, other studies found that high doses of gonadotropins in ovarian stimulation are not detrimental to embryo quality or the subsequent implantation rate, and may actually benefit the maturation of poor-quality oocytes [28]. More gonadotropins can stimulate AR expression via the protein kinase C (PKC) signal pathway, resulting in more good-quality embryos [29]. Therefore, the increasing dosage of Gn in the ultra-long protocol may contribute to follicular maturation.

Transfer strategies are also crucial in the types of patients described in this study. FET is becoming increasingly popular in

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IVF/ICSI procedures, but the freeze-thaw process can damage poor-quality embryos to some extent; these embryos have a lower survival rate and lower potential to retain morphological characteristics [30]. In addition, the vitrification-thaw process has a more dramatic influence on the cytoplasmic microstructure and oxygen consumption of poor-quality embryos than those of good-quality embryos [31]. In the modified ultra-long protocol, although more good-quality and viable embryos were obtained, the oocyte utilization rate (21.8%) was significantly lower than the rate (43.3%) reported in a previous study [1], indicating that the embryos were still not as good as those produced in normal patients. Hence, fresh ET may be beneficial for these patients with poor-quality embryos.

The present study has certain limitations. First, it was retrospective, and the conclusions need to be validated by a randomized controlled trial. However, it is worth mentioning that the patients themselves chose to continue using the PPOS protocol or to change to the ultra-long protocol after they had 1 or repeated PPOS failures, which, to some extent, may result in lower bias than if the decisions were made by doctors. Second, we did not evaluate whether other protocols would be effective, but we chose a different protocol in terms of the mechanisms of action and transfer strategy, which most likely changed the endocrine status. Additionally, the mechanism underlying the changes remains unclear and requires further exploration.

Conclusions

This retrospective study shows that when normoresponsive patients underwent 2 successive, but not 1, PPOS cycles with poor-quality embryos, changing to the modified ultra-long protocol resulted in more good-quality embryos and a higher pregnancy rate per aspirated cycle. Different LH levels, gonadotropin doses, and embryo transfer strategies may contribute to these improvements. Because similar situations frequently occur during clinical treatment, our study provides guidance by showing that changing to a different COH protocol can contribute to breaking the cycle of failure, resulting in better outcomes.

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Conflicts of interests

None.

Supplementary Data

Supplementary Table 1. ET and FET outcomes in the modified ultra-long protocol in the cycle after 1st or repeated PPOS failures.

Variable	2nd modified ultra-long protocol cycle after 1st PPOS failure		3rd modified ultra-long protocol cycle after repeated PPOS failures	
	ET	FET	ET	FET
No. of embryo transfer cycles (n)	40	15	50	13
No. of transferred embryos (n)	1.9±0.3*	1.3±0.5	1.8±0.4**	1.3±0.5
No. of transferred third-day good-quality embryos (n)	1.3±0.8	0.9±0.9	0.8±0.8	1.1±0.8
Biochemical pregnancy rate per transfer cycle, n (%)	22/40 (55.0)	5/15 (33.3)	17/50 (34.0)	4/13 (30.8)
Clinical pregnancy rate per transfer cycle, n(%)	21/40 (52.5)	5/15 (33.3)	16/50 (32.0)	3/13 (23.1)
Ectopic pregnancy rate, n(%)	0/21 (0.0)	0/5 (0.0)	0/16 (0.0)	0/3 (0.0)
Early abortion rate, n(%)	2/21 (9.5)	0/5 (0.0)	2/16 (12.5)	0/3 (0.0)
Twin pregnancy rate, n(%)	3/21 (14.3)	3/5 (60.0)	3/16 (18.8)	0/3 (0.0)
Ongoing pregnancy rate, n(%)	19/40 (47.5)	5/15 (33.3)	14/50 (28.0)	3/13 (23.1)
Implantation rate, n(%)	24/77 (31.2)	8/22 (36.4)	19/92 (20.7)	3/17 (17.6)

* Means significant difference between ET and FET in the 2nd cycle modified ultra-long protocol; ** means significant difference between ET and FET in the 3rd cycle modified ultra-long protocol.

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