DYDROGESTERONE-PRIMED OVARIAN STIMULATION VERSUS GNRH ANTAGONIST PROTOCOL IN IN-VITRO FERTILIZATION FOR POOR RESPONDERS: A PILOT STUDY

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PPOS (Progestin-Primed Ovarian Stimulation) has shown efficacy in preventing early luteinization and improving outcomes in infertile women. However, data among those with poor ovarian response (POR) are limited. This study aimed to evaluate the effect of PPOS protocol on the chance of success throughout the IVF process in patients with POR (POSEIDON group III & IV) undergoing IVF/ICSI in Vietnam. This was a randomized controlled trial involving 120 infertile women with POR. PPOS or GnRH-ant protocol was randomly applied to the participants of either group (n = 60 in each group). The primary outcome measures the chances of achieving oocyte maturation and retrieval, fertilization, and embryo formation. The effect of PPOS compared to GnRH-ant protocol was estimated as marginal risk-ratio (RR) from binomial regression analysis. We found that the chance of success in achieving MII oocytes, fertilization, and high-quality D3 and D5 embryos were equivalent between the two protocols; (RR (95% CI) were 0.97 (0.88-1.07), 1.00 (0.89-1.14), 1.04 (0.96-1.14), 1.14 (0.74-1.73), respectively). In conclusion, PPOS protocol using DYG slightly improves the IVF funnel in women with POR undergoing IVF/ICSI; however, these differences were not significant. Therefore, we concluded that the efficacy of the PPOS protocol is equivalent to that of the GnRH-ant stimulation protocol. Clinical Trial Registration Number: NCT06191809

Keywords: Progestin-primed ovarian stimulation, GnRH antagonist, controlled ovarian stimulation, poor ovarian response, dydrogesterone.

I. INTRODUCTION

Controlled Ovarian Stimulation (COS) plays a significant role in the cycle of In Vitro Fertilization (IVF) or Intra-Cytoplasmic Sperm Injection (ICSI). Premature luteinizing hormone (LH) surge is the primary cause of cycle cancelation in patients undergoing COS. Two principal methods of pituitary suppression could be employed to delay the onset of LH peak in the COS cycle:

(1) prolonging the administration of a gonadotropin-releasing hormone agonist (GnRH-a) before the implementation of COS in a long-acting agonist regimen

(2) immediate pituitary suppression with a gonadotropin-releasing hormone antagonist (GnRH-ant) in a short-acting antagonist regimen.[1]

Recently, with the successful development of embryo freezing and thawing techniques, a new COS protocol was proposed using progestin as a "primer" (Progestin-Primed Ovarian Stimulation – PPOS) in the act of inhibiting the LH peak and preventing ovulation

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Received: 16/04/2024
Accepted: 07/05/2024

occurrence. In 2015, Kuang et al. first reported this protocol and demonstrated efficacy in preventing premature luteinization.[2] High levels of progesterone from the early menstrual cycle have been shown to inhibit LH surge, initially inhibit the growth of follicles, and then inhibit the LH peak, which is the contraceptive base of progestin-only contraceptives. The PPOS protocol is preferred for its patient-friendly benefits: oral administration, convenience, and lower cost. Compared to the GnRH-ant protocol, PPOS protocol reduces OHSS rates, and the rates of miscarriages, multiple pregnancies, and ectopic pregnancy are similar between the two groups.[3] While previous studies have highlighted the potency of PPOS in prohibiting premature LH surge and promoting positive pregnancy outcomes compared to classical COS regimens in infertile women with normal ovarian reserve (NOR) or those with polycystic ovary syndrome (PCOS),[4-6] the efficacy of PPOS among infertile women with poor ovarian response (POR) remains unclear.

POR accounts for 15 – 16% of all United States IVF/ICSI cycles and poses ongoing challenges for reproductive endocrinologists.[7] Besides that, the definition of POR remains complex, with overlapping terminology, leading to difficulties and inconsistencies in clinical trials and analyses. A widely used instrument to determine women with POR is the Bologna criteria. Yet, persistent disagreements on its clinical application have led to introducing the POSEIDON criteria in 2016.[8] The POSEIDON demonstrates the concept of “low prognosis” and stratifies patients into four groups based on age, ovarian reserve biomarkers (anti-Müllerian hormone – AMH; antral follicle count ultrasound – AFC), and history of ovarian response to previous cycles of ovarian stimulation. This has assisted physicians in devising individually tailored treatment plans.

A recent retrospective study was conducted on 1329 women, who were accordingly categorized into four POSEIDON groups; findings indicated that the cumulative live birth rate was not different between the two groups. Notably, among the POSEIDON groups III and IV, no significant distinction was reported from treatment initiation to the arrival of live birth. Nonetheless, in group II, more embryos were obtained in the PPOS protocol than in the control group.[9]

Since characteristics are not homogeneous among different populations and GnRH-ant is practically exclusive in some pharmaceutical companies, treatment strategies and clinical decisions of doctors, as well as health policies of various hospitals, are affected rigorously by drug shortages in the market. Progestin, on the contrary, is marketable in large quantities and relatively easy to obtain. Therefore, we conducted this randomized controlled trial to compare the efficacy of the PPOS protocol versus GnRH-ant ovarian stimulation protocol on the chance of success throughout the IVF process in patients with POR (POSEIDON group III & IV) in Vietnam.

II. METHODS

1. Study Settings and Patients

This open-label randomized controlled trial (registration number: NCT06191809) was conducted at the Assisted Reproduction Center of Tam Anh General Hospital between February 2023 and January 2024. The study was approved by the Institutional Ethical Review Board of Hanoi University of Medicine (Decision N°842, reference: IRB-VN01.001/IRB00003121/FWA 00004148).

The criteria for female participants in the study were as follows:

1) between 20 and 45 years;

2) experiencing infertility due to male factors, fallopian tube factors, or unknown causes;

3) undergoing in vitro fertilization (IVF) in a single cycle and planning to use either the GnRH-ant protocol or the PPOS protocol, and

4) agreed to participate in the study voluntarily.

Women were excluded from the study if they met any of the following criteria:

1) history of contraindications to ovarian stimulation and IVF or ICSI treatment;

2) hyperprolactinemia or other endocrine disorders;

3) use of hormonal drugs within the past three months;

4) systemic diseases such as kidney failure, lupus erythematosus, and depression;

5) abnormal uterine cavity structure;

6) endometriosis or cancer;

7) random-start cycles;

8) oocyte donation cycles; or

9) embryo biopsy.

2. Randomization

We apply a stratified randomization process to ensure unbiased allocation of participants to GnRH-ant and PPOS treatment arms. The process was designed and implemented in the Python platform. Initially, each patient was assigned a distinct identification (ID) number. Simultaneously, the participants were classified according to their "POSEIDON" groups. Within each "POSEIDON" stratum (III and IV), participants were evenly allocated to the two treatment groups (GnRH-ant or PPOS regimens). The process ensures an equitable allocation, especially in cases where a category has an odd number of participants, by randomizing the additional unit within its category rather than across the entire study population.

3. Controlled Ovarian Stimulation

Controlled ovarian hyperstimulation commenced on the second day of the cycle utilizing recombinant follicle-stimulating hormone (Follitrope, LG Chem, South Korea) at dosages between 150-300 international units (IU) per day.

Initial gonadotropin dosages were determined per patient based on baseline parameters, including age, AMH, baseline FSH, body mass index (BMI), antral follicle count (AFC), and titrated subsequently per folliculogenesis response.

In the gonadotropin-releasing hormone (GnRH) antagonist protocol group, pituitary suppression began on stimulation day six via daily 0.25 milligram GnRH antagonist administration (ganirelix or cetrorelix).

In the PPOS group, 30mg/day of dydrogesterone was initiated on cycle day 2 through to trigger day. From the 5th day of stimulation (S5), doses of exogenous gonadotropin were adjusted or remained unchanged depending on the ovarian response until ovulation stimulation.

Ovarian response was monitored by transvaginal ultrasound to measure follicle size, as well as (i) quantification of serum estradiol (E2), LH, and progesterone (P4) using competitive electrochemiluminescence immunoassays via Cobas analyzer on the morning of S5, S8, and ovulation induction day for the PPOS regimen; (ii) quantification of serum E2, LH, and P4 only once on the ovulation induction day for the GnRH-ant regimen.

Additionally, when there were more than two follicles with diameters larger than 17 mm, oocyte maturation would be stimulated with Triptoreline 0.2mg (Diphereline 0.1mg; Ipsen) or hCG 10,000 IU (IVF-C, LG Chem, South

Korea) or recombinant hCG DNA 250 mcg (Ovitrelle; Merck Serono).

Oocyte retrieval via transvaginal aspiration was performed approximately 36 - 38 hours later. The controlled ovarian stimulation protocols are presented in Figure 1.

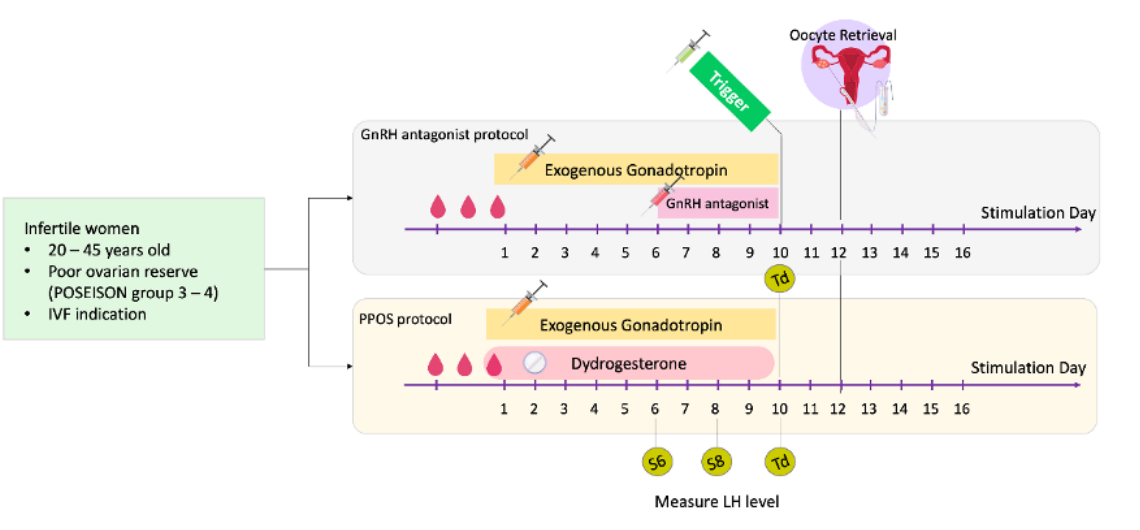

Figure 1 Ovarian stimulation protocols

IVF, In Vitro Fertilization; Td, Trigger day; S6, S8 stimulation day 6,8

4. Laboratory protocol

Oocyte-cumulus complexes were incubated for 2 hours in G-IVF medium (Vitrolife) using Origio benchtop incubators to complete nuclear maturation. After removing cumulus cells, denuded oocytes were evaluated under an inverted microscope to validate the achievement of metaphase II status, while degenerate, large, or severely dysmorphic oocytes were excluded. Intracytoplasmic sperm injection (ICSI) was executed 3-4 hours post-retrieval by experienced embryologists. Resultant zygotes were cultured in continuous single media (Fujifilm Irvine Scientific) within tri-gas incubators (37°C, 5% O2, 6% CO2) until day 3. Strict morphological criteria were enforced, only retaining normally fertilized two pronuclei zygotes while eliminating abnormal multinuclear embryos. Cleavage-stage quality was graded at 67-69 hours per Istanbul consensus based on cell number, fragmentation, multinucleation, and uniformity. On post-ICSI day 3, embryologists counseled patients on pursuing blastocyst culture versus cryopreservation. The morphology of the blastocysts was evaluated using the Gardner and Schoolcraft grading system, and embryos meeting the criteria of 3-6 AA/AB/BA blastocysts or 1-2 AA/AB embryos were classified as good quality.

5. Outcomes

The primary outcome consists of the probability of achieving mature, fertilized oocytes and the probability of achieving high-quality D3 and D5 embryos. Within the ovarian stimulation protocols, we considered the outcomes of either premature LH surge or cycle cancellation. The early LH surge was defined as LH levels exceeding 10 IU/L before the trigger days for ovulation induction. Cycle cancellation was determined if no follicle reached a size

greater than 12 mm after ten days of ovarian stimulation in COS cycles. The characteristics of canceled cycles (initial dose, total gonadotropin dose, duration of COS, LH quantification, E2, and P4 levels) were still collected as data. In addition, patients who experienced cycle cancellation would undergo COS again in the subsequent cycle.

6. Statistical analysis

Data analysis and visualization were done using the R programming language (ver 4.3.1). The primary outcomes consist of the chance of achieving MII oocytes, fertilization, and high-quality D3 and D5 embryos. The effect of the PPOS protocol compared with GnRH-ant on the success probabilities was evaluated as relative risk (RR) by a binomial regression analysis utilizing the GAMLSS package.[10] Confidence intervals for the marginal effects were determined using the delta method[11]. Statistical inference was based on null hypothesis testing at a significance level 0.05.

III. RESULTS

1. Participant Characteristics

A total of 120 patients were enrolled in this pilot study. These patients were randomly assigned to the GnRH antagonist or PPOS group, with 60 participants in each group. A flowchart of the participant allocation is presented in Figure 2.

Characteristics of participants by treatment arm are shown in Table 1. The two groups showed no significant difference regarding age, body mass index (BMI), AMH level, and AFC.

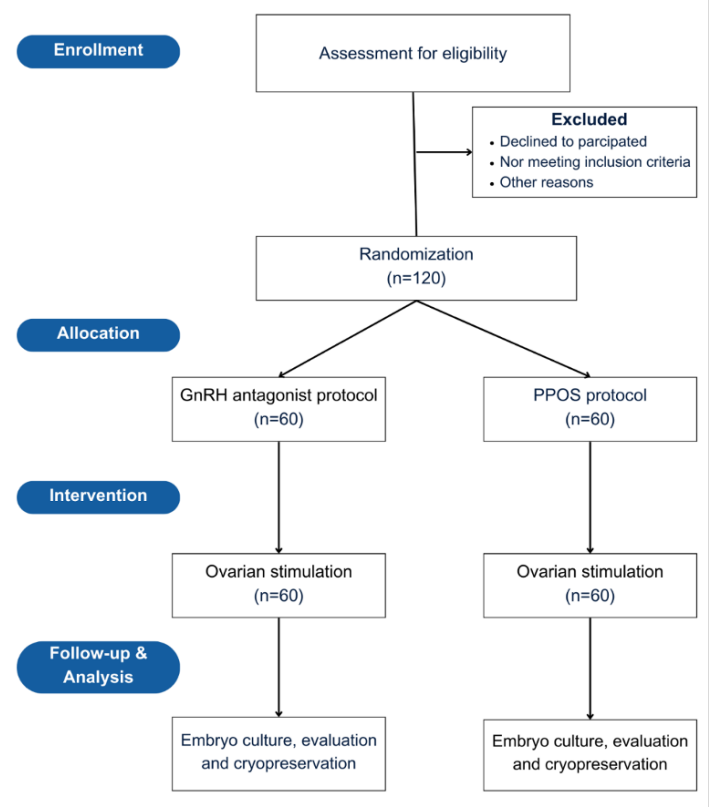

Figure 2 Flowchart of the participant allocation

Table 1. Characteristics of participants

Parameters	GnRH-ant group (n=60)		PPOS group (n=60)		P value*
	Median	5th-95th perc	Median	5th-95th perc	
Age (years)	37.0	26-45.0	37.0	27.0 – 44.0	0.977
BMI (kg/m^2)	21.1	18.7-26.2	21.0	19.1 – 27.2	0.659
AFC (follicles)	7.0	3-13	7.0	3-13.1	0.343
AMH (ng/ml)	0.68	0.26 – 1.22	0.76	0.29 – 1.11	0.328

AFC, antral follicle count; AMH, Anti-Müllerian hormone; BMI, body mass index; GnRH-ant, gonadotropin-releasing hormone antagonist; SD, standard deviation.

*P values were based on the Mann-Whitney U test

There was no significant difference in proportion of POSEIDON classification between two treatment arms. Within the GnRH antagonist group, 21 patients (35%) were classified as POSEIDON III and 39 (65%) as POSEIDON IV. For those in the PPOS group, 18 patients (30%) were classified as POSEIDON III, and 42 (70%) were classified under POSEIDON IV (Table 2).

Table 2. POSEIDON group by treatment arms

Protocol	POSEIDON	n
GnRH-ant	III	21 (35%)
GnRH-ant	IV	39 (65%)
PPOS	III	18 (30%)
PPOS	IV	42 (70%)

Values are the number of patients (%), GnRH-ant, gonadotropin-releasing hormone antagonist; PPOS, progestin–primed ovarian stimulation

2. Oocyte retrieval and embryo outcomes

All patients successfully completed the COS procedure and harvested at least one oocyte. As presented in Figure 3, the PPOS protocol was associated with slightly improved overall quantitative outcomes across four steps in the IVF funnel, compared with the GnRH-ant protocol.

Further analysis revealed that the PPOS protocol slightly improved the number of retrieved oocytes by 0.68 units, though this difference was insignificant (95% CI: -0.18 to 1.53). The probabilities of successfully achieving MII oocytes, fertilization, and high-quality D3 & D5 embryos were equivalent between the two protocols, with RR values of 0.97 (0.88 to 1.07), 1.005 (0.89 to 1.14), 1.04 (0.96 to 1.14), respectively (Table 3).

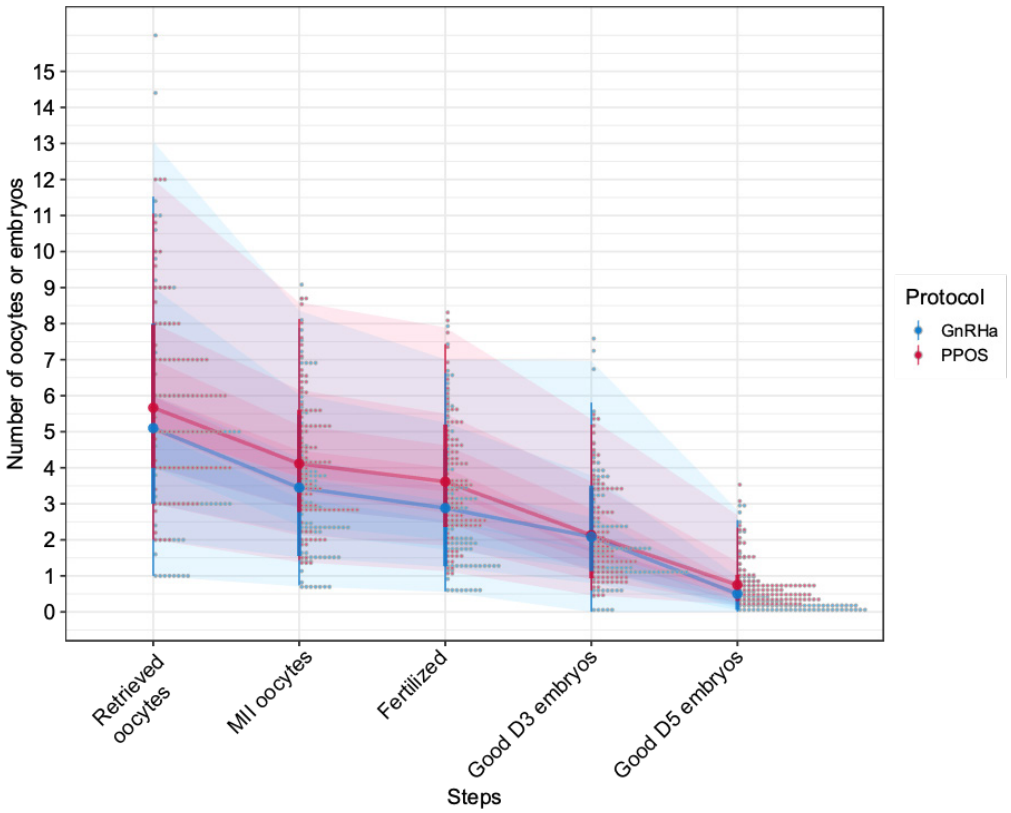

Figure 3. Overall effectiveness of the PPOS protocol versus GnRH-ant protocol on the IVF funnel

GnRHa, Gonadotropin-releasing hormone antagonist; PPOS, Progestin–primed ovarian stimulation; MII, metaphase II; D3, day 3; D5, day 5

Table 3 Effect of PPOS Protocol on the Number of retrieved oocytes and the chance of achieving MII oocytes, successful fertilization, achieving high-quality D3 and D5 embryos

Effect	Estimate	95% CI		p value#
Difference in number of retrieved oocytes (PPOS – GnRH-ant)	0.677*	-0.176	1.531	0.120
RR for achieving MII oocytes	0.967	0.876	1.067	0.504
RR for successful fertilization	1.005	0.886	1.141	0.933
RR for achieving high-quality D3 embryos	1.042	0.956	1.138	0.347
RR for achieving high-quality D5 embryos	1.135	0.745	1.729	0.555

MII, metaphase II; D3, day 3; D5, day 5; RR, relative risk, estimated as , where p indicates the probability of success for each unit of retrieved oocytes. A value above 1 suggests the superiority of the PPOS protocol relative to the GnRH-ant protocol.

*marginal effect measures the average difference in oocyte count between PPOS and

GnRH-ant protocols, using negative binomial regression analysis. A positive marginal effect value suggests PPOS's superiority.

#: Statistical testing to determine whether the marginal effects significantly differed from zero at a significance threshold 0.05.

IV. DISCUSSION

In our pilot study, no significant difference was found regarding chances of success in achieving MII oocytes, fertilization, and high-quality D3 and D5 embryos of PPOS protocol using DYG compared with GnRH-ant protocol in POR patients going IVF/ISCI. Progestin's pituitary suppression during COS is still being explored in the literature. In a meta-analysis, when compared to down-regulation or GnRH-ant protocols, clinical pregnancy rates, live birth, or ongoing pregnancy rates with the PPOS protocol were comparable with the control group; however, the rate of premature LH surge (RR=0.03, 95% CI=0.01-0.13, P < 0.001), and the rate of OHSS (RR=0.52, 95% CI=0.36-0.76, P<0.001) were significantly lower in the PPOS protocol.[12] In the study of Yildiz et al., the PPOS protocol using MPA had a similar outcome regarding premature ovulation and oocyte quality compared with GnRH-ant cycles in patients with and without PCOS.[13] An RCT of Sha Ya et al. (2018) found no significant difference in premature LH surge rate and the clinical pregnancy rate of the first FET cycle between the PPOS using DYG and GnRH-ant.[14] DYG is a potential alternative progestin for the PPOS protocol in ART;[15] it has a high selectivity for progesterone receptors with potent progestogenic activity. In contrast to other progestins, DYG has no clinically relevant agonistic or antagonistic action on the androgen, estrogen, and glucocorticoid receptors and only mild antimineralocorticoid features. Unlike natural progesterone, dydrogesterone has good oral bioavailability; therefore, it may reduce the side effects of progestins.[16]

These results begin the discussion on whether poor ovarian responders benefit most from PPOS or GnRH antagonists. Certain studies found that the PPOS did not statistically significantly increase the cumulative pregnancy rate or decrease the cycle cancellation rate compared to the GnRH antagonist protocol in the PORs.[17,18] A recent RCT by Chen et al. (2019) showed that in 340 poor responders defined by Bologna criteria, PPOS had more control for preventing premature LH rise than GnRH antagonists[6]. In contrast, similar to our results, in the cohort study of Lin et al. (2022), PORs were defined with POSEIDON IV criteria, and no differences in oocyte pick-up number and fertilization rate were found. However, patients who received ovarian stimulation with the PPOS protocol had a higher-high-quality MII oocyte ratio than those with the GnRH antagonist protocol (66.36% versus 54.46%, p < 0.05).[19] In addition, the proportion of good-quality blastocysts was higher (66.7% versus 56.3%; p = 0.182), and the cumulative LBR of PPOS was found to be similar (19.2% vs. 16.7%; p = 0.772) in the PPOS protocol compared to GnRH-ant protocol.[20] Besides that, the clinical outcomes in patients with PORs diagnosed by Bologna criteria were comparable when compared PPOS protocols using DYG and MPA regarding the number of oocytes retrieved, the oocyte retrieval, fertilization, viable embryo per oocyte retrieved, cancellation, and clinical pregnancy rates (36.4% versus 31.0%, p = 0.49).[21]

The present study has some specific limitations: firstly, because this was a pilot study, the number of patients enrolled was relatively small; secondly, the single-ethnicity

population included in this study could limit the external validity of our findings; finally, the pregnancy outcomes were not presented in our study. Regarding future research directions, the following points are worth considering:

(1) a larger sample or a large, well-designed multicenter RCT study is needed;

(2) the economic potency ratio must be considered. DYG drugs in the PPOS protocol are more convenient and cheaper to take orally; However, due to the impact of DYG on the receptivity of the endometrium, fresh cycle transfer cannot be performed, resulting in embryo cryopreservation costs. Last but not least, due to the short application time of the PPOS protocol and the limited amount of data, the offspring's safety of the PPOS protocol deserves further study.

V. CONCLUSION

In conclusion, the PPOS protocol presents a potential alternative to GnRH-ant in ovarian simulation procedures by offering equivalent effectiveness on the success rates of maturation, fertilization, and generation of good D3 or D5 embryos. However, to solidify these findings, further investigation through a larger sample size and well-designed multicenter RCT study is expected while also considering the cost-effectiveness ratio of this novel method.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Conflict of interest

All authors state that they have no conflicts of interest to disclose.

Data Availability

All data generated or analyzed during this study are included in this published article.

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