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Original Research

Comparative Analysis of Progestin-Primed and Luteal-Phase Ovarian Stimulation Protocols in Patients With Diminished Ovarian Reserve Undergoing *In Vitro* Fertilization–Embryo Transfer

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Abstract

Background: Patients with diminished ovarian reserve (DOR) face challenges such as inadequate follicular recruitment and decreased oocyte quality when subjected to *in vitro* fertilization and embryo transfer (IVF-ET) treatment. **Methods**: This retrospective self-controlled study included 130 patients with DOR who underwent IVF-ET using either the progestin-primed ovarian stimulation (PPOS) or luteal-phase ovarian stimulation (LPOS) protocol. In the PPOS protocol, ovarian stimulation was initiated in the early follicular phase with medroxyprogesterone acetate (MPA) combined with gonadotropins. In the LPOS protocol, ovarian stimulation began in the luteal phase with letrozole and gonadotropins, followed by dydrogesterone. Final oocyte triggering, retrieval, and embryo culture were performed using standardized procedures. The primary outcomes included gonadotropin consumption, oocyte maturation and fertilization rates, as well as pregnancy-related outcomes. **Results**: Compared to the LPOS group, the PPOS protocol was associated with a significantly shorter duration of gonadotropin stimulation and a lower total gonadotropin dose ($p < 0.05$). The LPOS group did not have significantly higher metaphase II (MII) oocyte and normal fertilization rates ($p > 0.05$). The abnormal fertilization rate was numerically lower in the LPOS group, but the difference was not statistically significant. Multivariate logistic regression analysis revealed that the LPOS protocol remained independently associated with a higher MII oocyte rate (adjusted odds ratio [aOR]: 1.42, 95% confidence interval [CI]: 1.07–1.91, $p = 0.017$), even after adjusting for age, body mass index (BMI), and antral follicle count (AFC). No significant association was observed between stimulation protocol and clinical pregnancy after adjustment. **Conclusion**: Both PPOS and LPOS protocols effectively prevent premature luteinizing hormone (LH) surges and support the development of viable embryos in patients with DOR. Multivariate analysis further confirmed LPOS as an independent predictor of improved oocyte maturity, suggesting its potential utility in individualized stimulation strategies for this patient population.

Keywords: progestin primed ovarian stimulation; luteal-phase ovarian stimulation; diminished ovarian reserve; self-controlled study

1. Introduction

Diminished ovarian reserve (DOR) poses a significant challenge in the field of reproductive medicine [1]. Characterized by a reduction in both the quantity and quality of ovarian follicles, DOR leads to decreased fertility potential and is a major cause of infertility in women [2,3]. Epidemiological data indicate a rising prevalence of DOR, reflecting a concerning trend not only in aging populations but also among younger women of reproductive age [4]. Although current evidence implicates multifactorial etiologies, including genetic predisposition, environmental exposures, and lifestyle influences, the precise molecular mechanisms underlying DOR pathogenesis remain elusive, thereby hindering the development of mechanism-based therapeutic interventions [5,6].

With the advancement of assisted reproductive technologies (ART), an increasing number of DOR patients have achieved successful pregnancies through *in vitro* fertilization and embryo transfer (IVF-ET) [7,8]. Nevertheless, optimizing ovarian response in patients with DOR undergoing controlled ovarian hyperstimulation (COS) remains a significant clinical challenge, often resulting in suboptimal follicular recruitment, cycle cancellations, and reduced live birth rates [9,10]. Premature ovulation, poor ovarian response to stimulation, low oocyte yield, and a higher incidence of aneuploid embryos are common challenges that can significantly impact the success rates of IVF-ET. These challenges adversely impact not only clinical success rates but also impose profound psychological distress and significant economic burdens on affected individuals and their families. Given the limited efficacy of conventional therapeutic approaches, the development of novel, cost-effective, and patient-tailored ovulation induction strategies for DOR management represents a critical unmet need in reproductive medicine.

Recent advances in COS have led to the development of innovative protocols specifically designed to enhance treatment outcomes for patients with DOR [11]. Notably, progestin-primed ovarian stimulation (PPOS) and luteal-phase ovarian stimulation (LPOS) have emerged as promising alternatives to conventional COS approaches [12,13].

These novel protocols were designed to overcome the inherent limitations of traditional stimulation methods by providing better regulation of the ovarian environment and effective prevention of premature luteinizing hormone (LH) surges. The PPOS protocol employs progestins to suppress the endogenous LH surge, thereby enabling more controlled follicular development [14], whereas LPOS leverages on the physiological characteristics of the luteal phase to initiate ovarian stimulation [15]. Although both protocols offer theoretical benefits, comparative data on their relative efficacy, cost-effectiveness, and clinical outcomes in patients with DOR remain limited. To address this critical knowledge gap, the present study employs a self-controlled design to conduct a comprehensive evaluation of PPOS versus LPOS, aiming to provide evidence-based recommendations for optimizing treatment strategies in this clinically challenging population.

2. Materials and Methods

2.1 Study Subjects

This self-controlled study included 130 patients diagnosed with DOR who underwent IVF-ET at the First Affiliated Hospital of Fujian Medical University between March 2018 and March 2023. A self-controlled design was adopted in which each patient served as her own control by undergoing two separate ovarian stimulation cycles—one with the PPOS protocol and the other with the LPOS protocol—within the same clinical setting. This design enabled direct within-subject comparisons, effectively minimizing inter-individual variability in factors such as age, ovarian reserve, and response to stimulation.

To be eligible for inclusion, patients were required to have completed both PPOS and LPOS cycles within a six-month interval, minimizing the impact of age-related decline in ovarian function and other time-related confounders. Additionally, only those with comparable baseline characteristics (including day 2 follicle-stimulating hormone [FSH], anti-Müllerian hormone [AMH], and antral follicle count [AFC]) at the start of each protocol were included to ensure intra-patient consistency. The order of cycles (whether PPOS or LPOS was performed first) was not standardized but was balanced across the cohort, and no intervening surgeries, endocrine-modulating treatments, or major lifestyle changes occurred between the two cycles.

Each patient completed two stimulation cycles—one using the PPOS protocol and the other using the LPOS protocol—within a six-month interval. Initially, 133 patients were screened, but three cycles (two PPOS and one LPOS) were excluded due to premature ovulation, resulting in 130 complete paired cycles. This within-subject paired design enabled the application of matched statistical methods, increasing statistical power while minimizing inter-individual variability and potential confounding.

DOR was diagnosed based on a combination of clinical and biochemical parameters, including: (1) advanced maternal age ($\geq$40 years), (2) AFC $<$5–7, and/or (3) AMH $<$1.1 ng/mL. Although baseline FSH levels $\geq$10 mIU/mL or an FSH/LH ratio $>$3 were also recorded, these markers were considered supplementary due to their known intracycle variability.

Patients were excluded if either partner carried chromosomal abnormalities, or if the female patient had uterine anomalies (e.g., unicornuate uterus) or a history of chemotherapy or radiotherapy. Additional exclusion criteria were: severe male factor infertility defined as total motile sperm count $<$5 million, endocrine disorders (e.g., thyroid dysfunction, diabetes, adrenal disorders), and systemic diseases affecting hepatic, renal, cardiovascular, hematologic, or psychiatric function.

2.2 Study Methods

2.2.1 PPOS

PPOS Protocol: On the 2nd–3rd day of menstruation, patients underwent transvaginal ultrasound using the Mindray DC-80 system (Software Version 3.1.1, Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, Guangdong, China) to assess bilateral antral follicles. From that day until the day before oocyte retrieval, patients were administered medroxyprogesterone acetate (MPA, 2 mg/tablet, 4 mg/day; Pfizer Inc.; BATCH-MPA-202501; New York, NY, USA). Recombinant human gonadotropins (GONAL-f®, follitropin alfa, 150–225 IU/day; Merck Serono; BATCH-GONAL-202501; Darmstadt, Hesse, Germany) were initiated simultaneously. When two or more follicles reached an average diameter of 18 mm, final oocyte maturation was triggered with 0.05 mg of triptorelin (Decapeptyl®; Ferring Pharmaceuticals; BATCH-TRIP-202501; Saint-Prex, Vaud, Switzerland) and 2000 IU of human chorionic gonadotropin (Chorionic Gonadotropin for Injection; Livzon Pharmaceutical Group Inc.; BATCH-HCG-202501; Zhuhai, Guangdong, China). Oocyte retrieval was performed 35–36 hours post-trigger using ultrasound-guided aspiration.

2.2.2 LPOS

2.2.2.1 LPOS Protocol. Following either spontaneous ovulation or ovulation triggered by triptorelin, gonadotropins (GONAL-f®, 150–225 IU/day, maximum 375 IU/day; Merck Serono; BATCH-GONAL-202501; Darmstadt, Germany) were initiated. Patients also received oral letrozole (2.5 mg/day for 8 days; Jiangsu Hengrui Medicine Co., Ltd.; BATCH-LETRO-202501; Lianyungang, Jiangsu, China), followed by dydrogesterone (20 mg/day; Duphaston®; Abbott Biologicals B.V.; BATCH-DYDRO-202501; Olst, Overijssel, Netherlands). Triggering, oocyte retrieval, fertilization, and embryo culture procedures were conducted using identical procedures to those in the PPOS protocol.

Although letrozole is not strictly required in LPOS, it was included in this protocol to improve follicular recruitment efficiency and reduce supraphysiological estradiol levels during the luteal phase. Letrozole suppresses peripheral estrogen production, thereby enhancing endogenous gonadotropin release via negative feedback modulation of the hypothalamic-pituitary axis (HPA). This effect is particularly beneficial in the luteal phase, when elevated endogenous progesterone can induce relative gonadotropin resistance. Previous studies have shown that letrozole improves follicular synchronization and increases the number of recruitable antral follicles in non-traditional stimulation windows [16,17].

Different progestins were selected based on protocol-specific considerations. MPA was chosen for PPOS due to its potent suppression of premature LH surges during follicular-phase stimulation. In contrast, dydrogesterone was selected for LPOS as a supportive agent during the luteal phase, offering endometrial compatibility and safety without the pronounced systemic suppression associated with MPA. Thus, the progestins were appropriately matched to their physiological context and protocol phase.

2.2.2.2 Endometrial Preparation for Frozen–Thawed Embryo Transfer (FRET) Protocol. In natural-cycle FRET, ovulation was monitored using the Mindray DC-80 ultrasound system, and embryo transfer was scheduled according to the patient's spontaneous cycle. For patients undergoing hormone replacement therapy (HRT) protocols, oral estradiol valerate (2 mg/day from day 2 to day 14; Progynova®; Bayer AG; BATCH-ESTRA-202501; Berlin, Germany) was administered, followed by oral progesterone (20 mg/day starting on day 14; Utrogestan®, Micronized Progesterone Capsules; Besins Healthcare; BATCH-PROG-202501; Paris, France). Serial ultrasound was used to monitor endometrial development, and embryo transfer was performed when endometrial thickness reached $\geq$7 mm.

2.3 Laboratory and Embryo Standards

Fertilization was assessed 18–72 hours after insemination, and embryos exhibiting two pronuclei (2PN) were considered normally fertilized. On day 3, embryos were graded according to Alpha/European Society of Human Reproduction and Embryology (ESHRE) consensus criteria: Grade I embryos exhibited $<$10% fragmentation, equal-sized blastomeres, and no evidence of multinucleation; Grade II embryos showed 10%–25% fragmentation, slightly uneven blastomeres, and no multinucleation; and Grade III embryos displayed $>$25% fragmentation, severely uneven blastomeres, and visible multinucleation. Embryos graded as Grade I or II were classified as high-quality. All embryo cultures were performed using the EmbryoScope™ time-lapse incubator (Software Version 5.1, Vitrolife AB, BATCH-EMB-202501, Göteborg, Sweden).

2.4 Outcomes Measures

Indicators included total gonadotropin (Gn) days, total Gn dose, number of oocytes retrieved, metaphase II (MII) rate, normal fertilization rate, abnormal fertilization rate, day 3 high-quality embryo rate (defined as embryos with a cavity smaller than half the embryo, excellent inner cell mass, and excellent trophectoderm), implantation rate, clinical pregnancy rate, ectopic pregnancy rate, miscarriage rate, and live birth rate. Definitions were based on the consensus of the human ART embryo laboratory data quality control expert group [18].

Abnormal fertilization was defined as the occurrence of fertilization without the formation of 2PN, including cases of polyspermy, the presence of only one pronucleus, or the absence of visible pronuclei. Migration number refers to the number of embryos successfully transferred into the uterine cavity during the embryo transfer procedure. This term encompasses all cases where embryos were transferred to the uterus, regardless of the outcome.

Live birth rate was defined as the proportion of embryo transfer cycles resulting in at least one live-born infant beyond 24 weeks of gestation.

2.5 Statistical Analysis

All data analyses were performed using IBM SPSS Statistics, Version 22.0 (IBM Corporation, Armonk, NY, USA). Continuous variables were first tested for normality using the Shapiro-Wilk test and for variance homogeneity using Levene's test. For normally distributed paired data, paired *t*-tests were applied. For non-normally distributed data, Wilcoxon signed-rank tests were applied, and results were reported as medians (interquartile range, IQR).

Categorical variables were compared using McNemar's test for paired proportions or Fisher's exact test when expected cell counts were $<$5. Logistic regression analysis was considered in exploratory models for multivariable adjustment of potential confounders (e.g., age, body mass index [BMI], AFC). All statistical tests were two-sided, and $p < 0.05$ was considered statistically significant.

3. Results

3.1 Patient Characteristics

The average age of the patients was 38.06 $\pm$ 5.56 years, and the average BMI of 22.20 $\pm$ 3.13 kg/m^2. The average baseline FSH level was 9.56 $\pm$ 6.25 IU/L, and the AFC was 3.88 $\pm$ 2.30. The mean duration of infertility among the patients was 3.30 $\pm$ 2.63 years. These characteristics were comparable between the two groups, ensuring a balanced comparison of the PPOS and LPOS protocols (Table 1).

3.2 Comparison of Ovarian Stimulation and Laboratory Outcomes

Notably, the PPOS protocol was associated with a significantly shorter duration of Gn administration (9.79 $\pm$

Table 1. Baseline characteristicsof the patients.

Item	Mean ± SD
Age (years)	38.06 ± 5.56
BMI (kg/m^2)	22.20 ± 3.13
AMH (µg/L)	0.73 ± 0.36
Basic E2 (IU/L)	161.80 ± 133.37
Basic FSH (IU/L)	9.56 ± 6.25
Basic LH (IU/L)	4.80 ± 2.94
AFC (n)	3.88 ± 2.30

Note: SD, standard deviation; BMI, body mass index; E2, estradiol; FSH, follicle-stimulating hormone; LH, luteinizing hormone; AMH, anti-Müllerian hormone; AFC, antral follicle count.

2.23 days vs. 10.38 ± 2.23 days, $p < 0.05$) and a lower total Gn dose (2351.18 ± 1034.09 IU vs. 2887.16 ± 1062.33 IU, $p < 0.001$) compared with the LPOS protocol. Despite these differences in stimulation characteristics, both protocols yielded comparable oocyte retrieval outcomes (PPOS: 4.13 ± 2.81; LPOS: 4.31 ± 2.98; $p > 0.05$) (Table 2).

The LPOS protocol did not demonstrate superior oocyte maturation rates (MII oocytes rate: 92.38% ± 17.09%) compared to PPOS (88.46 % ± 23.28%; $p > 0.05$). The normal fertilization rate was higher in the LPOS group (median: 73.2 (IQR: 50–100) vs. median: 69.0 (IQR: 50–100)), but this difference was not statistically significant ($p > 0.05$) (Table 3).

3.3 Comparison of Clinical Outcomes

Both protocols yielded similar implantation rates (PPOS: 21.49%; LPOS: 30.43%; $p > 0.05$) and clinical pregnancy rates (PPOS: 21.13%; LPOS: 30.26%; $p > 0.05$). However, the LPOS group exhibited a higher miscarriage rate (43.47%) than the PPOS group (20.00%), although this difference was not statistically significant ($p > 0.05$). These findings suggest that, despite some laboratory advantages of the LPOS protocol, both protocols achieve comparable clinical pregnancy rates (Table 4).

3.4 Oocyte Retrieval and Embryo Transfer Rates

Among the 130 patients in each group, oocytes were successfully retrieved in 112 (86.2%) in the PPOS group and 116 (89.2%) in the LPOS group. Similarly, the migration number—defined as the number of patients who proceeded to embryo transfer—was 71 in the PPOS group and 76 in the LPOS group, corresponding to 54.6% and 58.5% of patients, respectively, reaching the embryo transfer stage (Table 5).

3.5 Live Birth Rate Comparison Between PPOS and LPOS Protocols

Live birth rate, defined as the proportion of embryo transfer cycles resulting in at least one live-born infant, was compared between the PPOS and LPOS groups. In the PPOS group, 12 out of 71 embryo transfer cycles resulted in live births, yielding a live birth rate of 16.90%. In the LPOS group, 13 out of 76 cycles resulted in live births, yielding a live birth rate of 17.11%. Although the LPOS group exhibiting a slightly higher rate, the difference was not statistically significant ($\chi^2 = 0.004$, $p = 0.948$). These results indicate that both protocols achieve comparable live birth outcomes (Table 6).

Although the LPOS group showed numerically higher implantation (30.43% vs. 21.49%) and clinical pregnancy rates (30.26% vs. 21.13%) compared with the PPOS group, these differences were not statistically significant. Given these observed trends, a post hoc power analysis was conducted to evaluate whether the sample size was sufficient to detect clinically meaningful differences. Assuming a two-sided α of 0.05 and an absolute 10% difference in clinical pregnancy rate (e.g., 20% vs. 30%), the estimated required sample size was approximately 370 patients per group. With only 130 patients per group, the current study had limited statistical power (~45%) to detect such a difference, highlighting the potential for a type II error.

3.6 Multivariate Logistic Regression

After adjusting for potential confounders using multivariable logistic regression, the LPOS protocol remained significantly associated with a higher likelihood of mature (MII) oocyte yield (adjusted odds ratio [aOR]: 1.42, 95% confidence interval [CI]: 1.07–1.91, $p = 0.017$). However, no significant differences were observed between LPOS and PPOS in terms of normal fertilization (aOR: 1.08, 95% CI: 0.79–1.48, $p = 0.627$), clinical pregnancy (aOR: 1.24, 95% CI: 0.69–2.23, $p = 0.472$), or live birth rate (aOR: 1.05, 95% CI: 0.51–2.17, $p = 0.887$).

These findings suggest that, although LPOS may improve oocyte maturation independently of age, BMI, and AFC, it does not provide a statistically significant advantage in downstream clinical outcomes after adjustment for covariates.

4. Discussion

Management of DOR poses substantial challenges in reproductive medicine, largely due to a reduced ovarian follicular pool and compromised oocyte quality, which collectively adversely affect ART outcomes [19–21]. In this comparative study, we systematically evaluated the efficacy of two ovarian stimulation protocols—PPOS and LPOS—in patients with DOR undergoing IVF-ET. Our findings yield clinically relevant insights into the comparative performance of these protocols, elucidating their respective advantages and limitations in this challenging patient population.

Notably, our data reveal that the PPOS protocol is associated with a significantly shorter stimulation duration and reduced total Gn requirements compared with the LPOS approach. This differential carries important clinical implications, directly affecting both treatment costs

Table 2. Comparison of outcomes between PPOS and LPOS regimens.

Item	PPOS (n = 130)	LPOS (n = 130)	*p*-value	*t*-value
Duration of Gn (days)	9.79 ± 2.23	10.38 ± 2.23	0.036	–2.113
Dosage of Gn (IU)	2351.18 ± 1034.09	2887.16 ± 1062.33	<0.001	–4.114

Note: Gn, gonadotropin; PPOS, progestin-primed ovarian stimulation; LPOS, luteal-phase ovarian stimulation.

Table 3. Comparison of laboratory indices and embryo status between the PPOS and LPOS protocols.

Item	PPOS (n = 130)	LPOS (n = 130)	*p*-value	Statistical test
Number of oocytes retrieved (n)	3.94 ± 2.64	4.56 ± 2.99	0.131	Student's *t*-test
MII oocytes rate (%)	88.46 ± 23.28	92.38 ± 17.09	0.123	Student's *t*-test
Normal fertilization rate (%)	69.0 (50–100)	73.2 (50–100)	0.841	Wilcoxon signed-rank test
Abnormal fertilization rate (%)	0 (0–33.3)	0 (0–29.6)	0.220	Wilcoxon signed-rank test
D3 excellent embryo rate (%)	50 (0–75)	50 (0–100)	0.072	Wilcoxon signed-rank test

Note: MII, metaphase II.

Table 4. Comparison of pregnancy outcomes between the PPOS and LPOS regimens.

Item	PPOS (n = 130)	LPOS (n = 130)	*p*-value	Statistical test
Number of embryo transfers (n)	71	76	/	/
Number of transferred embryos (n)	1.56 ± 0.49	1.49 ± 0.50	0.251	Paired *t*-test
Implantation rate (%)	21.49 (23/107)	30.43 (35/115)	0.130	Chi-square test
Clinical pregnancy rate (%)	21.13 (15/71)	30.26 (23/76)	0.206	Chi-square test
Ectopic pregnancy rate (%)	0	0	/	/
Miscarriage rate (%)	20.00 (3/15)	43.47 (10/23)	0.176	Fisher's exact test

Table 5. Summary of patient progression through IVF-ET stages.

Stage	PPOS group (n = 130)	LPOS group (n = 130)
Patients with oocyte retrieval	112 (86.2%)	116 (89.2%)
Patients with no oocytes	18 (13.8%)	14 (10.8%)
Patients with embryo transfer	71 (54.6%)	76 (58.5%)
Patients without embryo transfer	59 (45.4%)	54 (41.5%)

Note: IVF-ET, *in vitro* fertilization-embryo transfer.

Table 6. Comparison of live birth outcomes between PPOS and LPOS protocols.

Group	Embryo transfer cycles (n)	Clinical pregnancies (n)	Miscarriages (n)	Live births (n)	Live birth rate (%)
PPOS	71	15	3	12	16.90
LPOS	76	23	10	13	17.11

Note: Live births = Clinical pregnancies – Miscarriages; Live birth rate = (Live births / Embryo transfer cycles) × 100%.

and patient tolerability. The reduced Gn consumption observed with PPOS may be explained by effective suppression of premature LH surges through MPA administration, thereby enabling more controlled follicular recruitment [14,22,23]. In contrast, the LPOS protocol's higher Gn requirements likely reflect luteal-phase initiation, where elevated endogenous progesterone levels may induce relative gonadotropin resistance.

Interestingly, although the LPOS protocol showed a trend toward a higher day-3 high-quality embryo rate, the difference did not reach statistical significance. This suggests that both protocols can generate embryos with comparable developmental potential, likely through different mechanisms of follicular recruitment and maturation. Clinically, both protocols yielded similar pregnancy outcomes, including implantation, clinical pregnancy, and miscarriage rates. This similarity in clinical outcomes is noteworthy, given the distinct differences in Gn dosage and stimulation duration between the two protocols. The comparable pregnancy rates indicate that embryos derived from both PPOS and LPOS protocols possess adequate developmental competence to achieve implantation and clinical pregnancy in DOR patients. Nevertheless, the higher miscarriage rate observed in the LPOS group, although not statistically significant, warrants further investigation. Although the LPOS group demonstrated a higher miscarriage rate numerically, the difference did not reach statistical significance. This suggests that the observed variation may reflect

random error or insufficient sample size rather than a genuine difference in protocol safety. Miscarriage in ART can be influenced by multiple factors, including embryo quality, endometrial receptivity, and patient-specific characteristics [24–26]. The trend toward a higher miscarriage rate in the LPOS group may reflect underlying differences in these factors that were not fully controlled for in this study.

Our findings indicate that both PPOS and LPOS are effective for women with DOR undergoing IVF, with PPOS associated with improved oocyte maturation and fertilization rates. These results are consistent with a recent meta-analysis reporting that PPOS improves clinical pregnancy rates and embryo quality while reducing gonadotropin consumption compared with other protocols. Nevertheless, while both studies suggest benefits of PPOS, large-scale, well-designed trials are still required to validate these findings and evaluate long-term outcomes [27].

Multivariate analysis confirmed that the LPOS protocol was independently associated with a higher MII oocyte rate, after adjustment for age, BMI, and ovarian reserve indicators. This supports the inference that LPOS may enhance both cytoplasmic and nuclear maturation. However, no statistically significant associations were found between protocol type and clinical pregnancy or live birth after adjustment, indicating that other patient-level or embryo-endometrial factors may play a predominant role in determining final reproductive outcomes. Nevertheless, the limited sample size constrains the precision of these estimates, particularly for low-incidence outcomes (e.g., live birth). Future prospective studies with larger cohorts and stratified randomization are needed to further validate these associations.

Although the LPOS protocol showed numerically higher implantation and clinical pregnancy rates, these differences were not statistically significant. Nonetheless, the direction and magnitude of these trends may indicate potential clinical relevance. Our post hoc power analysis revealed that the current sample size (n = 130 per group) was insufficient to detect an absolute 8–10% difference in pregnancy-related outcomes with adequate statistical power. Specifically, detecting a 10% absolute difference in clinical pregnancy rates with 80% power would require approximately 370 patients per group. Therefore, the lack of statistical significance in the present study may reflect an underpowered design rather than the absence of a true effect. Future large-scale, multicenter trials with adequate sample sizes are needed to validate these findings and to more definitively compare the reproductive outcomes of PPOS and LPOS protocols.

Although the self-controlled design employed in this study effectively minimized inter-subject variability, a critical methodological limitation lies in the non-randomized sequence of protocol administration. Patients received PPOS and LPOS in a non-random order determined by clinical decisions or logistical considerations, which may introduce first-protocol effect bias. For example, patients undergoing PPOS first may have exhibited a stronger ovarian response simply due to being earlier in their treatment course, whereas those receiving LPOS as a subsequent cycle could have experienced physiological fatigue, diminished follicular recruitment, or psychological stress stemming from prior suboptimal outcomes.

The lack of randomization in protocol order compromises the internal validity of intra-individual comparisons, particularly for cycle-dependent outcomes such as oocyte yield, embryo quality, and implantation potential. Additionally, cumulative effects from prior stimulation, such as residual suppression or hormonal milieu carryover, may confound the observed differences in response between PPOS and LPOS. To mitigate this source of bias, future studies should adopt a randomized crossover design, with patients prospectively assigned to receive either PPOS or LPOS first, followed by crossover to the alternative regimen. Such a design would enable balanced sequencing and appropriate washout intervals, thereby enhancing the robustness of within-subject comparisons and strengthening causal inferences regarding protocol efficacy.

The self-controlled study design employed here offers distinct methodological advantages, particularly by minimizing inter-subject variability and enabling direct comparison of treatment efficacy within the same patient cohort. However, this approach entails several challenges. The non-randomized sequence of protocol administration may influence outcomes, as ovarian response could be affected by treatment order due to factors including cumulative hormonal exposure or psychological effects from prior cycle results. Furthermore, attributing clinical outcomes (e.g., implantation and pregnancy rates) to specific protocols becomes inherently complex when both interventions are applied sequentially within the same patient.

Future research should prioritize larger, multicenter trials to confirm these findings and explore the mechanisms underlying driving the differences between PPOS and LPOS protocols. Additionally, studies incorporating patient-reported outcomes, including treatment satisfaction and quality of life, will provide a more comprehensive understanding of the clinical utility and patient acceptability of these protocols.

Limitation

The self-controlled study design employed here offers distinct methodological advantages, particularly by minimizing inter-subject variability and enabling direct comparison of treatment efficacy within the same patient cohort. However, this approach entails several challenges. The non-randomized sequence of protocol administration may influence outcomes, as ovarian response could be affected by treatment order due to factors including cumulative hormonal exposure or psychological effects from prior cycle results. Furthermore, attributing clinical outcomes

(e.g., implantation and pregnancy rates) to specific protocols becomes inherently complex when both interventions are applied sequentially within the same patient.

5. Conclusion

In this study comparing the PPOS and LPOS protocols in patients with DOR, both regimens achieved comparable clinical outcomes in terms of implantation rate, clinical pregnancy rate, and live birth rate. Additionally, the PPOS protocol required a shorter duration and lower total gonadotropin dose, suggesting a more economical and patient-friendly stimulation strategy.

Importantly, despite the higher oocyte maturation rate in the LPOS group, live birth rates were nearly identical between the two protocols, underscoring that enhanced laboratory metrics do not necessarily translate into improved clinical outcomes. These findings highlight the need for individualized stimulation strategies that consider both laboratory efficiency and patient tolerance. Larger randomized controlled trials are warranted to validate these results and optimize protocol selection in this challenging patient population.

Availability of Data and Materials

The datasets generated or analyzed during the current study are not publicly available due to patient privacy and institutional data protection policies, but are available from the corresponding author upon reasonable request. Requests for access to anonymized data will be reviewed and granted for non-commercial academic purposes in compliance with ethical and legal guidelines.

Author Contributions

ML and QCL designed the study and performed literature research. ML conducted the experimental studies, clinical studies, and manuscript preparation. YLZ designed the research study. QCL defined the intellectual content. YJG conducted clinical studies. HLZ acquired data. JLW performed data analysis and statistical analysis. ML wrote the main manuscript text and reviewed the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Approval for the study was obtained from the Ethics Committee of The First Affiliated Hospital of Fujian Medical University [Date.2022.12; No.2022(657)]. All participants provided written informed consent prior to inclusion in the study, including authorization for clinical data collection and analysis.

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Conflict of Interest

The authors declare no conflict of interest.

Declaration of AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work the authors used ChatGpt-3.5 in order to check spell and grammar. After using this tool, the authors reviewed and edited the content as needed and takes full responsibility for the content of the publication.

References

[1] Devenutto L, Quintana R, Quintana T. *In vitro* activation of ovarian cortex and autologous transplantation: A novel approach to primary ovarian insufficiency and diminished ovarian reserve. Human Reproduction Open. 2020; 2020: hoaa046. https://doi.org/10.1093/hropen/hoaa046.

[2] Zarek SM, Hill MJ, Richter KS, Wu M, DeCherney AH, Osheroff JE, *et al*. Single-donor and double-donor sperm intrauterine insemination cycles: does double intrauterine insemination increase clinical pregnancy rates? Fertility and Sterility. 2014; 102: 739–743. https://doi.org/10.1016/j.fertnstert.2014.05.018.

[3] Luke B, Brown MB, Wantman E, Stern JE, Baker VL, Widra E, *et al*. A prediction model for live birth and multiple births within the first three cycles of assisted reproductive technology. Fertility and Sterility. 2014; 102: 744–752. https://doi.org/10.1016/j.fertnstert.2014.05.020.

[4] Pastore LM, Christianson MS, Stelling J, Kearns WG, Segars JH. Reproductive ovarian testing and the alphabet soup of diagnoses: DOR, POI, POF, POR, and FOR. Journal of Assisted Reproduction and Genetics. 2018; 35: 17–23. https://doi.org/10.1007/s10815-017-1058-4.

[5] Li Y, Xiao N, Liu M, Liu Y, He A, Wang L, *et al*. Dysregulation of steroid metabolome in follicular fluid links phthalate exposure to diminished ovarian reserve of childbearing-age women. Environmental Pollution. 2023; 330: 121730. https://doi.org/10.1016/j.envpol.2023.121730.

[6] Wu J, Liang C, Wang X, Huang Y, Liu W, Wang R, *et al*. Efficient Metabolic Fingerprinting of Follicular Fluid Encodes Ovarian Reserve and Fertility. Advanced Science. 2023; 10: e2302023. https://doi.org/10.1002/advs.202302023.

[7] Mitsui J, Ota K, Yamashita H, Sujino T, Hiraoka K, Katsumata S, *et al*. Predictive factors for empty follicle syndrome in infertile patients undergoing assisted reproductive technology treatment: a retrospective cohort study and brief literature review. Clinical and Experimental Obstetrics & Gynecology. 2023; 50: 80. https://doi.org/10.31083/j.ceog5004080.

[8] Zhu S, Jiang W, Liao X, Sun Y, Chen X, Zheng B. Effect of diminished ovarian reserve on the outcome of fresh embryo transfer in IVF/ICSI cycles among young women: A retrospective cohort study. BMC Women's Health. 2024; 24: 230. https://doi.org/10.1186/s12905-024-03039-6.

[9] Liu XH, Wu XH, Yang S. Changes and correlations of anti-Müllerian hormone and stem-cell factors in different ovarian re-

serve patients during GnRH-antagonist protocol and the effects on controlled ovarian hyperstimulation outcomes. Archives of Gynecology and Obstetrics. 2019; 300: 1773–1783. https://doi.org/10.1007/s00404-019-05332-4.

[10] Magnusson Å, Nilsson L, Oleröd G, Thurin-Kjellberg A, Bergh C. The addition of anti-Müllerian hormone in an algorithm for individualized hormone dosage did not improve the prediction of ovarian response-a randomized, controlled trial. Human Reproduction. 2017; 32: 811–819. https://doi.org/10.1093/humrep/dex012.

[11] Kuang Y, Chen Q, Fu Y, Wang Y, Hong Q, Lyu Q, *et al*. Medroxyprogesterone acetate is an effective oral alternative for preventing premature luteinizing hormone surges in women undergoing controlled ovarian hyperstimulation for in vitro fertilization. Fertility and Sterility. 2015; 104: 62–70.e3. https://doi.org/10.1016/j.fertnstert.2015.03.022.

[12] Llácer J, Moliner B, Luque L, Bernabéu A, Lledó B, Castillo JC, *et al*. Luteal phase stimulation versus follicular phase stimulation in poor ovarian responders: results of a randomized controlled trial. Reproductive Biology and Endocrinology. 2020; 18: 9. https://doi.org/10.1186/s12958-020-00570-7.

[13] Pai AHY, Sung YJ, Li CJ, Lin CY, Chang CL. Progestin Primed Ovarian Stimulation (PPOS) protocol yields lower euploidy rate in older patients undergoing IVF. Reproductive Biology and Endocrinology. 2023; 21: 72. https://doi.org/10.1186/s12958-023-01124-3.

[14] Cui L, Lin Y, Wang F, Chen C. Effectiveness of progesterone-primed ovarian stimulation in assisted reproductive technology: a systematic review and meta-analysis. Archives of Gynecology and Obstetrics. 2021; 303: 615–630. https://doi.org/10.1007/s00404-020-05939-y.

[15] Lin LT, Vitale SG, Chen SN, Wen ZH, Tsai HW, Chern CU, *et al*. Luteal Phase Ovarian Stimulation May Improve Oocyte Retrieval and Oocyte Quality in Poor Ovarian Responders Undergoing In Vitro Fertilization: Preliminary Results from a Single-Center Prospective Pilot Study. Advances in Therapy. 2018; 35: 847–856. https://doi.org/10.1007/s12325-018-0713-1.

[16] Olgan S, Humaidan P. GnRH antagonist and letrozole co-treatment in diminished ovarian reserve patients: a proof-of-concept study. Reproductive Biology. 2017; 17: 105–110. https://doi.org/10.1016/j.repbio.2017.01.006.

[17] Bülow NS, Warzecha AK, Nielsen MV, Andersen CY, Holt MD, Petersen MR, *et al*. Impact of letrozole co-treatment during ovarian stimulation on oocyte yield, embryo development, and live birth rate in women with normal ovarian reserve: secondary outcomes from the RIOT trial. Human Reproduction. 2023; 38: 2154–2165. https://doi.org/10.1093/humrep/dead182.

[18] Lee JA, Barritt J, Moschini RM, Slifkin RE, Copperman AB. Optimizing human oocyte cryopreservation for fertility preservation patients: should we mature then freeze or freeze then mature? Fertility and Sterility. 2013; 99: 1356–1362. https://doi.org/10.1016/j.fertnstert.2012.11.042.

[19] Li L, Sun B, Wang F, Zhang Y, Sun Y. Which Factors Are Associated With Reproductive Outcomes of DOR Patients in ART Cycles: An Eight-Year Retrospective Study. Frontiers in Endocrinology. 2022; 13: 796199. https://doi.org/10.3389/fendo.2022.796199.

[20] Zhang C, Song S, Yang M, Yan L, Qiao J. Diminished ovarian reserve causes adverse ART outcomes attributed to effects on oxygen metabolism function in cumulus cells. BMC Genomics. 2023; 24: 655. https://doi.org/10.1186/s12864-023-09728-0.

[21] Devine K, Mumford SL, Wu M, DeCherney AH, Hill MJ, Propst A. Diminished ovarian reserve in the United States assisted reproductive technology population: diagnostic trends among 181,536 cycles from the Society for Assisted Reproductive Technology Clinic Outcomes Reporting System. Fertility and Sterility. 2015; 104: 612–619.e3. https://doi.org/10.1016/j.fertnstert.2015.05.017.

[22] Guan S, Feng Y, Huang Y, Huang J. Progestin-Primed Ovarian Stimulation Protocol for Patients in Assisted Reproductive Technology: A Meta-Analysis of Randomized Controlled Trials. Frontiers in Endocrinology. 2021; 12: 702558. https://doi.org/10.3389/fendo.2021.702558.

[23] Dong J, Wang Y, Chai WR, Hong QQ, Wang NL, Sun LH, *et al*. The pregnancy outcome of progestin-primed ovarian stimulation using 4 versus 10 mg of medroxyprogesterone acetate per day in infertile women undergoing in vitro fertilisation: a randomised controlled trial. BJOG: an International Journal of Obstetrics and Gynaecology. 2017; 124: 1048–1055. https://doi.org/10.1111/1471-0528.14622.

[24] Linehan LA, San Lazaro Campillo I, Hennessy M, Flannery C, O'Donoghue K. Reproductive outcomes following recurrent first-trimester miscarriage: a retrospective cohort study. Human Reproduction Open. 2022; 2022: hoac045. https://doi.org/10.1093/hropen/hoac045.

[25] Busnelli A, Somigliana E, Cirillo F, Levi-Setti PE. Is diminished ovarian reserve a risk factor for miscarriage? Results of a systematic review and meta-analysis. Human Reproduction Update. 2021; 27: 973–988. https://doi.org/10.1093/humupd/dmab018.

[26] Emami F, Eftekhar M, Jalaliani S. Correlation between clinical and laboratory parameters and early pregnancy loss in assisted reproductive technology cycles: A cross-sectional study. International Journal of Reproductive Biomedicine. 2022; 20: 683–690. https://doi.org/10.18502/ijrm.v20i8.11757.

[27] Lin G, Zhong X, Li S, Liu X, Xu L. The clinical value of progestin-primed ovarian stimulation protocol for women with diminished ovarian reserve undergoing IVF/ICSI: a systematic review and meta-analysis. Frontiers in Endocrinology. 2023; 14: 1232935. https://doi.org/10.3389/fendo.2023.1232935.