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Original Article

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Comparison of Long-Term and Stimulated Cycle Protocols in Frozen Embryo Transfer Cycles in Women with Polycystic Ovary Syndrome: A Non-Randomized Clinical Trial

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Abstract

Background: In women with polycystic ovary syndrome (PCOS), the optimal endometrial preparation strategy for frozen embryo transfer (FET) remains unclear. This clinical trial aimed to compare a long-term protocol with a stimulated cycle protocol in FET cycles of PCOS women, with clinical pregnancy rate per embryo transfer as the primary outcome.

Methods: This non-randomized, open-label, two-arm clinical trial was conducted at Fatemieh Hamedan Hospital, Iran, involving 340 women with PCOS. Participants were allocated to the long-term or stimulated cycle protocols based on clinical scheduling and physician decision. The long-term protocol included low-dose combined oral contraceptives for 14 days, GnRH agonist downregulation, estradiol valerate (4–6 *mg/day* for ≥10 days), and progesterone before embryo transfer. The stimulated cycle protocol involved recombinant FSH with ultrasound monitoring, hCG trigger for oocyte maturation, and intramuscular progesterone (50 *mg/day*) for luteal phase support. For each participant one FET cycle was analyzed.

Results: Baseline characteristics were similar between groups (n=170 each). The long-term protocol showed higher clinical pregnancy rates (35.9%) compared to the stimulated cycle (22.9%) (OR=1.92; 95%CI: 1.15–3.19; p=0.011). Chemical pregnancy rates also favored the long-term protocol (34.7% *vs*. 21.8%) (OR=1.91; 95%CI: 1.17–3.09; p=0.008). Endometrial thickness was significantly greater in the long-term protocol (8.7±0.9 *mm vs*. 8.4±0.8 *mm*; p=0.004).

Conclusion: Although the long-term protocol showed higher clinical pregnancy rates in unadjusted analyses, after adjusting for confounders, protocol type was not independently associated with clinical pregnancy. Larger randomized trials are needed to confirm these findings, and adverse outcomes should be assessed in future studies.

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Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder, affecting 5–20% of women of reproductive age (1). It often leads to infertility due to anovulation and poor oocyte quality, complicating both natural conception and assisted reproductive technologies (ARTs) such as in vitro fertilization (IVF) (2). Additionally, women with PCOS are at increased risk of ovarian hyperstimulation syndrome (OHSS) during IVF cycles, which can further complicate their treatment

(3). FET has become a preferred strategy in IVF, offering higher live birth rates and a reduced risk of OHSS compared to fresh embryo transfer cycles (4).

Ovulation induction using agents such as human menopausal gonadotropin (hMG) or letrozole has been developed for endometrial preparation in FET cycles (5). Ovarian stimulation with exogenous gonadotropins aims to correct follicular and luteal phase defects, thereby improving endometrial receptivity for embryo implantation (6). The long-term protocol, which utilizes gonadotropin-releasing hormone agonists (GnRHa), is another method for endometrial preparation prior to FET. Some studies have reported improved pregnancy outcomes with this approach, although findings remain inconsistent (7).

Pilehvari et al. compared two endometrial preparation protocols, with and without GnRHa, in PCOS patients undergoing FET, and found that the use of GnRHa was associated with increased endometrial thickness and higher clinical pregnancy rates (8). Similarly, Tsai et al. investigated the use of GnRHa in hyperandrogenic PCOS women and suggested that reducing androgen levels before FET may improve ongoing pregnancy outcomes (9).

Despite these findings, direct comparisons of the long-term and stimulated cycle protocols in PCOS patients are limited, leaving uncertainty about the optimal approach (10). This study addressed this gap by comparing the efficacy of these protocols in women with PCOS undergoing FET, aiming to provide evidence to guide clinical practice.

Methods

Study design and registration: This was a non-randomized clinical trial with a control group, conducted at Fatemieh Hamedan Hospital, Iran, from May 2022 to January 2025. The study was approved by the Ethics Committee of Hamadan University of Medical Sciences (ID: R.UMSHA.REC.1401.326) and registered in the Iranian Registry of Clinical Trials (IRCT20120215009014-N434). Written informed consent was obtained from all participants. The study adhered to the Declaration of Helsinki. A non-randomized design was chosen due to ethical and logistical considerations, as treatment protocols were selected based on physician judgment and patient preference. While this may introduce selection bias, potential confounding variables were addressed through multivariate statistical analysis.

Participant selection: A total of 340 women diagnosed with PCOS were enrolled. Diagnosis was based on the Rotterdam criteria, which require at least two of the following features: oligo- or anovulation, clinical or biochemical hyperandrogenism, and polycystic ovarian morphology on ultrasound.

Participants were recruited consecutively from the infertility clinic. Screening included transvaginal ultrasound (TVS), hormonal profiling, and medical history review.

Inclusion and exclusion criteria: Participants were aged 18–45 years who underwent FET. Only those in their first or second FET cycle were included. Endometrial thickness had to be ≥7 *mm* after stimulation, based on evidence that this threshold is associated with improved implantation rates. Additionally, participants were required to have at least three high-quality embryos to ensure that embryo quality was not a limiting factor. Participants were excluded if endometrial thickness was <7 *mm* or if a triple-layer endometrium was absent, or if functional ovarian cysts were present. Individuals who refused or were non-compliant with medication, had uncontrolled systemic diseases, had undergone more than two previous FET cycles, or were older than 45 years were also excluded.

Baseline assessment: All participants underwent TVS on days 2–3 of menstruation to assess endometrial thickness and ovarian status. If the endometrium was thin (<7 *mm*) and ovaries were cyst-free, low-dose oral contraceptive pills (LD OCP) were prescribed for 14 days for cycle regulation.

Intervention protocols

Long protocol group: Patients received a GnRH agonist (5.5 *mg* buserelin acetate; Sinafact, Variopety, or Decapeptyl) either as a single depot dose or daily 0.5 *mg* injections for 7 days until menstruation occurred. Axis suppression was confirmed using both ultrasound and serum estradiol levels. Estradiol valerate (2 *mg* tablets; Aburaihan Pharmaceutical Co., Iran) was then administered at a dose of 4–6 *mg/day* for at least 10 days. Once the endometrial thickness reached ≥7 *mm*, intramuscular progesterone (50 *mg/day*) (Femogex-IH; Iran Hormone Pharmaceutical Co., Iran) was initiated. Embryo transfer was performed 3–5 days after progesterone initiation, depending on embryo age.

Ovulation stimulation group: Patients received recombinant FSH (300, 450, or 900 *IU* vials; Sin-

agen, Iran) at the minimum effective dose, determined based on age, BMI, and antral follicle count. TVS was repeated every 2–3 days to monitor follicular growth. When at least one follicle reached 16–18 *mm* and the endometrial thickness was ≥7 *mm*, HCG (5000–10000 *IU*) (Gonarex; Ronak Daru Co., Iran) was administered. After 36 hours, intramuscular progesterone (50 *mg/day*) was started. Embryo transfer was performed 3–5 days later.

Embryology procedures: ICSI was performed for all patients. Embryos were cultured in G-TL medium (Vitrolife, Sweden) using tri-gas incubators. Embryos were cryopreserved at the blastocyst stage (day 5) using vitrification (Kitazato protocol). Embryo grading followed the Gardner and Schoolcraft scoring system, and only embryos graded ≥3BB were considered high-quality (11). The warming protocol followed the manufacturer's instructions. Single embryo transfer (SET) was performed in 82% of cases and double embryo transfer (DET) in 18%, with no significant difference between groups (p=0.41). The primary outcome of the study was the clinical pregnancy rate. Secondary outcomes included endometrial thickness, implantation rate, and ongoing pregnancy rate.

Statistical analysis: Sample size was calculated using G*Power software based on a previous study by Pilehvari et al. (8), which reported clinical pregnancy rates of 20% and 33.3% in the two groups. A total of 340 participants were included to ensure adequate power. Data were analyzed using SPSS version 20 (IBM Corp., USA). Continuous variables were expressed as mean±SD and compared using independent t-tests. Categorical variables were compared using chi-square tests. Multivariate logistic regression was used to control for confounding variables such as age, BMI, endometrial thickness, and embryo quality. A $p< 0.05$ was considered statistically significant.

Results

Participant flow and baseline characteristics: A total of 340 women with PCOS were enrolled and non-randomly assigned to either the long protocol group (n=170) or the ovulation stimulation group (n=170). Assignment was based on clinical judgment and patient preference, as described in the Methods section.

Baseline demographic and clinical characteristics, including age, BMI, duration of infertility, and type of infertility, were statistically comparable between the two groups. For example, the mean age was 31.9±6.1 years in the long protocol group and 31.1±5.3 years in the ovulation stimulation group (p=0.167). Full details are presented in table 1.

Pregnancy outcomes: The chemical pregnancy rate was 38.2% (65/170) in the long protocol group and 24.7% (42/170) in the ovulation stimulation group, with an odds ratio (OR) of 1.91 (95%CI: 1.20–3.05; p=0.006). Similarly, the clinical pregnancy rate was 35.9% (61/170) in the long protocol group and 22.9% (39/170) in the ovulation stimulation group (OR=1.92, 95%CI: 1.17–3.15; p=0.011). These results are summarized in table 2.

Embryo quality: High-quality (Grade 1) embryos were observed in 42.9% (73/170) of patients in the long protocol group and 33.1% (56/170) in the ovulation stimulation group. Although the frequency was higher in the long protocol group, the difference was not statistically significant (p=

Table 1. Baseline demographic and clinical characteristics of participants in the long protocol and ovulation stimulation groups

Variables	Stimulated cycle protocol (n=170)	Long-term protocol (n=170)	p-value
	Mean±SD/Frequency (%)	Mean±SD/Frequency (%)	
Type of infertility			
Primary	107 (62.9)	113 (66.5)	0.496 **
Secondary	63 (37.1)	57 (33.5)	
Age of mother (years), Mean±SD	31.1±5.3	31.9±6.1	0.167 *
Duration of infertility (years), Mean±SD	4.5±3.3	4.7±2.9	0.696 *
Body mass index (kg/m^2), Mean±SD	25.9±2.9	26.5±3.8	0.108 *
FSH (*IU/L*), Mean±SD	6.2±1.8	6.0±1.7	0.412 *
AMH (*ng/ml*), Mean±SD	7.8±2.5	7.5±2.3	0.297 *

* Independent t-test, ** Chi-square test

Table 2. Comparison of chemical and clinical pregnancy rates between the two groups, with odds ratios and significance levels

Pregnancy type	Stimulated cycle protocol (n=170)	Long-term protocol (n=170)	OR (95%CI)	p-value
	Frequency (%)	Frequency (%)		
Chemical pregnancy				
Yes	37 (21.8%)	59 (34.7%)		
No	133 (78.2%)	111 (65.3%)	1.91 (1.17-3.09)	0.008
Total	170 (100%)	170 (100%)		
Clinical pregnancy				
Yes	31 (18.2%)	51 (30.0%)		
No	139 (81.8%)	119 (70.0%)	1.92 (1.15-3.19)	0.011
Total	170 (100%)	170 (100%)		

0.063). This suggests that the protocol may influence endometrial receptivity more than embryo quality.

Endometrial thickness and predictors of pregnancy: The mean endometrial thickness was 8.7±0.9 *mm* in the long protocol group and 8.4±0.8 *mm* in the ovulation stimulation group, with a statistically significant difference (p=0.004). In univariate logistic regression analysis, several variables were significantly associated with clinical pregnancy. Belonging to the long protocol group was associated with increased odds of clinical pregnancy (OR=1.92, p=0.012). Additionally, each 1 *mm* increase in endometrial thickness was associated with higher odds of clinical pregnancy (OR=0.62, p=0.002). A shorter duration of infertility was also associated with increased likelihood of clinical pregnancy (OR=0.93, p=0.034). Furthermore, grade 1 embryo quality was significantly more likely to result in clinical pregnancy compared to grade 2 (OR=0.28, p=0.001).

In the multivariate model, after adjusting for confounders (p<0.2), endometrial thickness, embryo quality, and infertility duration remained significant predictors. However, the adjusted OR for the long protocol group was 1.68, which was not statistically significant (p=0.079), indicating that the protocol itself was not an independent predictor after controlling for these variables (Table 3).

Only the first embryo transfer cycle per patient was included in the analysis to avoid duplication and bias. This approach ensures that outcomes reflect the initial response to each protocol.

Discussion

This clinical trial compared two endometrial preparation protocols, the long-term protocol with GnRH agonists and the stimulated cycle protocol, in women with PCOS undergoing FET. The long-term protocol was associated with significantly higher chemical pregnancy rates (38.2% *vs.* 24.7%,

Table 3. Univariate and multivariate logistic regression analysis of factors associated with clinical pregnancy

Variables	OR [a]	p-value	95%CI	OR [b]	p-value	95%CI
Groups	1.92	0.012	1.15-3.19	1.08	0.079	0.99-1.19
Age of mother (years)	1.03	0.084	0.99-1.08	1.04	0.093	1.14-2.21
Age of husband (years)	1.03	0.208	0.99-1.09	-	-	-
Duration of infertility (years)	0.93	0.034	0.87-0.99	0.92	0.014	0.86-0.98
BMI (kg/m^2)	1.004	0.910	0.93-1.08	-	-	-
Cycle duration (days)	1.09	0.045	1.002-1.19	1.08	0.292	0.99-1.19
Endometrial thickness (*mm*)	1.62	0.002	1.20-2.19	1.59	0.006	1.14-2.21
Number of embryos	0.95	0.247	0.90-1.01	-	-	-
Embryo quality (grade 2 *vs.* grade 1)	0.28	0.001	0.16-0.14	0.30	0.001	0.17-0.52

OR: Odds Ratio, CI: Confidence Interal, a: Unadjusted, b: Adjusted

p=0.006) and clinical pregnancy rates (35.9% *vs*. 22.9%, p=0.011), greater endometrial thickness (8.7±0.9 *mm vs*. 8.4±0.8 *mm*, p=0.004), and a higher proportion of grade 1 embryos (42.9% *vs*. 33.1%, p=0.063), although the latter was not statistically significant.

The improved outcomes in the long-term protocol group may be attributed to effective suppression of the hypothalamic-pituitary-ovarian axis by GnRH agonists, enhancing endometrial receptivity. These findings align with Pilehvari et al.'s results, who reported increased endometrial thickness and clinical pregnancy rates with GnRH agonist-based preparation in PCOS patients (8). Similarly, Tsai et al. found that pre-treatment with GnRH agonists improved ongoing pregnancy rates in hyperandrogenic PCOS women, likely due to androgen suppression (9). However, contradictory findings exist in the literature. Siristatidis et al. (12), Zeng et al. (13), Rabiei et al. (14), Zhang et al. (15), and Zhu et al. (16) reported no significant differences in pregnancy outcomes across various endometrial preparation protocols (*e.g*., stimulated cycles, artificial cycles, or letrozole-based protocols) in PCOS patients or normal responders, suggesting that factors such as BMI, androgen levels, or embryo transfer strategies (*e.g*., single vs. double embryo transfer) may influence results.

Our multivariable analysis (Table 3) showed that the protocol type was not an independent predictor of clinical pregnancy after adjusting for endometrial thickness, embryo quality, and infertility duration (OR=1.68, p=0.079). This indicates that the long-term protocol's benefits are likely mediated through these variables, particularly endometrial thickness and embryo quality, rather than the protocol itself. This finding underscores the importance of optimizing endometrial preparation and embryo selection in PCOS patients, as supported by prior studies (10, 13). Our study contributed to the evidence by directly comparing two commonly used protocols in a well-defined PCOS population (10). It addressed a gap in direct comparisons of GnRH agonist and FSH-based protocols, providing insights into their efficacy in PCOS patients.

However, it is important to note that the current study was a non-randomized, single-center research, which may introduce selection and performance bias and limit generalizability. Embryo quality was only assessed morphologically, without genetic screening. Additionally, adverse outcomes (*e.g*., miscarriage rates, side effects, and preterm birth) were not collected, limiting the assessment of the protocols' long-term effectiveness, as noted in similar studies (15).

Conclusion

In women with PCOS undergoing frozen embryo transfer, the long protocol for endometrial preparation was associated with higher clinical pregnancy rates and greater endometrial thickness compared to the ovulation stimulation protocol. However, after adjusting for confounding variables, the protocol itself was not an independent predictor of pregnancy. These findings suggest a potential benefit of the long protocol, but further research is needed to confirm its impact on live birth rates and to establish standardized guidelines for endometrial preparation in this population. While the long-term protocol showed promising results for clinical pregnancy and endometrial thickness, its superiority requires further validation. Future randomized controlled trials, such as those by Zhu et al. with larger, multicenter cohorts and long-term outcomes (*e.g*., live birth and miscarriage rates) are essential to confirm these findings and guide clinical practice. Molecu-lar studies exploring endometrial receptivity mechanisms, such as androgen modulation or gene expression, could further elucidate the long-term protocol's advantages in PCOS patients.

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Conflict of Interest

The authors declare no competing interests.

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