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Letrozole-induced proliferative phase frozen embryo transfer with pre-ovulatory progesterone initiation without ovulation triggering in women with polycystic ovary syndrome or oligo-anovulation: a proof-of-concept cohort study

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Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the principles of the Declaration of Helsinki. This study was approved by the Institutional Review Board of Tri-Service General Hospital, National Defense Medical University, Taipei, Taiwan (IRB No. B202505121). All patients who visited our center had consented to the anonymous use of their medical data for scientific research and publication, and informed consent was signed at the beginning of treatment. Because the study retrospectively analyzed the data of patients who had completed treatment, further consent was not necessary.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no competing interests.

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Authors' contributions

CC Huang and TC Huang conceived and designed the study. FW Chang, CY Huang, and GJ Wu were responsible for data acquisition and analysis. JH Yang and JL Hwang analyzed and interpreted the data. MJ Chen performed the statistical analysis. TC Huang and JL Hwang drafted the article. CC Huang, H Yarali, and KH Tsui reviewed and revised the manuscript. All authors approved the submitted version of the manuscript and agree to be personally accountable for their own contributions.

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None.

Abstract

Background

Programmed frozen embryo transfer (FET) allows scheduling flexibility but lacks a corpus luteum, whereas natural-cycle and letrozole-stimulated FET preserve ovulation but require ovulation tracking or triggering. Natural proliferative phase FET (NPP-FET) combines scheduling flexibility with spontaneous ovulation but is limited to ovulatory women. We developed letrozole-induced proliferative phase FET (LPP-FET) for women with polycystic ovary syndrome (PCOS) or oligo-anovulation. We evaluated whether LPP-FET preserves spontaneous ovulation despite preovulatory progesterone (P) initiation without human chorionic gonadotropin (hCG) triggering and whether variation in P initiation timing within the protocol-defined window is independently associated with live birth.

Methods

This retrospective study included 233 intended LPP-FET cycles in women with PCOS or oligo-anovulation scheduled for their first single euploid FET between August 2022 and November 2025. Letrozole (5 mg/day for 5 days) induced follicular development under ultrasonographic and hormonal monitoring. Dydrogesterone was flexibly initiated before ovulation when the dominant follicle was ≥15 mm, endometrial thickness ≥7 mm, estradiol >130 pg/mL, and progesterone <1.5 ng/mL. Single euploid FET was performed on P administration day 6. Spontaneous ovulation was confirmed by ultrasound-documented ovulation (UDO) and FET-day serum progesterone >3.0 ng/mL. Multivariable logistic regression (MLR) evaluated associations between P initiation timing–related variables and live birth. Primary and secondary outcomes were spontaneous ovulation rate and live birth (LBR), ongoing pregnancy (OPR), and clinical pregnancy (CPR) rates, respectively.

Results

Of 233 intended cycles, 187 were letrozole-responsive, and P was initiated in 178. Spontaneous ovulation was confirmed in 176/178 P-initiated cycles (98.9%), corresponding to 176/187 letrozole-responsive cycles (94.1%) and 176/233 intended cycles (75.5%). Subsequent analyses included these 176 ovulation-confirmed cycles. The interval from first meeting P initiation criteria (eligibility) to UDO was 2–7 days (median 4, IQR 3–5), suggesting potential flexibility in P initiation. LBR, OPR, and CPR were 59.7%, 60.2%, and 67.6%, respectively. In MLR, follicular diameter and estradiol at P initiation and the eligibility-to-P initiation and P initiation-to-UDO intervals were not significantly associated with live birth, whereas blastocyst morphology and biopsy day were.

Conclusions

LPP-FET preserved spontaneous ovulation in most letrozole-responsive women with

PCOS or oligo-anovulation. No independent association between P initiation timing–related variables and live birth was detected within their observed ranges. LPP-FET may offer a corpus luteum–preserving option, with transfer timed from P initiation rather than ovulation detection or hCG triggering, pending comparative studies.

Keywords: Frozen embryo transfer; Letrozole; Polycystic ovary syndrome; Dydrogesterone; Spontaneous ovulation; Natural proliferative phase

Introduction

A freeze-all approach is frequently adopted in women with polycystic ovary syndrome (PCOS) undergoing in vitro fertilization (IVF) to mitigate the risk of ovarian hyperstimulation syndrome [1, 2]. Endometrial preparation for frozen embryo transfer (FET) in patients with PCOS may be achieved using a programmed cycle (PC) or mild ovarian stimulation with letrozole (LOS), with or without low-dose gonadotropins. In LOS-FET cycles, ovulation timing is commonly controlled using human chorionic gonadotropin (hCG) triggering (modified LOS-FET) or allowed to occur spontaneously (true LOS-FET) [3, 4]. A natural cycle (NC), either true or modified, may be considered for ovulatory women with PCOS who have regular menstrual cycles.

Although a PC allows for predictable scheduling, the absence of a corpus luteum (CL) has been associated with higher risks of hypertensive disorders of pregnancy and adverse obstetric outcomes compared with CL-preserving approaches, including NC in ovulatory women and LOS in women with PCOS or oligo-anovulation [5–7]. This association may reflect the absence of CL-derived vasoactive signals involved in normal maternal vascular adaptation [8] and provides a strong rationale for favoring CL-preserving FET strategies when feasible [5–7]. In women with PCOS, LOS-FET has been associated with higher live birth and lower miscarriage rates compared with PC [7, 9].

The main limitations of true-NC and true LOS-FET are the need for frequent monitoring and limited scheduling flexibility due to dependence on accurate ovulation timing [10]. Modified-NC and modified LOS-FET protocols partially address these limitations by using hCG triggering to improve scheduling flexibility; however, these approaches remain dependent on pharmacologic control of ovulation timing. Moreover, substantial variability exists across protocols regarding the criteria used for hCG administration and the subsequent timing of embryo transfer, and consensus regarding the optimal timing framework remains evolving [3, 4, 10, 11]. Although recent protocol modifications in ovulatory women undergoing modified-NC may further enhance flexibility [12–14], these findings require confirmation in future

randomized controlled trials. Comparable protocol modifications have been less well studied in women with PCOS or oligo-anovulation.

To address these limitations, the natural proliferative phase FET (NPP-FET) protocol has been proposed for ovulatory women [15], in which exogenous progesterone (P) is flexibly initiated in the mid- to late follicular phase—prior to spontaneous ovulation and without hCG triggering—with FET timing predefined based on the duration of P exposure. A limited number of studies employing NPP-FET–related protocols have reported reassuring reproductive outcomes across heterogeneous P initiation criteria [15–19], while more recent studies assessing ovulation have demonstrated preservation of spontaneous ovulation in the great majority of cycles [18, 19].

As NPP-FET is primarily applicable to women with predictable spontaneous follicular development, we developed the letrozole-induced proliferative phase FET (LPP-FET) protocol for women with PCOS or oligo-anovulation. The LPP-FET protocol is, in essence, a modification of the NPP protocol designed for this population. In this protocol, follicular development is induced with letrozole, and exogenous P is initiated in the mid- to late-follicular phase once predefined ultrasonographic and hormonal criteria are met, with the aim of allowing flexible FET timing while preserving spontaneous ovulation and CL formation. Unlike modified LOS-FET, scheduling flexibility in LPP-FET is achieved by anchoring FET timing to flexible P initiation without hCG triggering.

This study had two objectives in women with PCOS or oligo-anovulation undergoing LPP-FET: (1) to determine whether spontaneous ovulation occurs after letrozole-induced follicular development despite follicular-phase P initiation without hCG triggering, and (2) to explore whether variation in P initiation timing within the protocol-defined window is independently associated with live birth. By addressing these questions, we sought to assess the potential role of LPP-FET as an additional ovulation-preserving FET option for this population.

MATERIALS AND METHODS

Design, study population and main outcome measures

This retrospective observational study was approved by the Institutional Review Board of Tri-Service General Hospital, National Defense Medical University, Taipei, Taiwan (B202505121). All patients had consented to the anonymous use of their medical data for research and publication at the start of treatment, and the requirement for additional informed consent was waived because of the retrospective design.

Consecutive women with PCOS or oligo-anovulation scheduled for their first single euploid FET using LPP-FET at Taipei IVF, Center for Reproduction and Genetics, Taipei, Taiwan, between August 2022 and November 2025 were included. Each woman contributed one intended LPP-FET cycle.

Inclusion criteria were women aged <45 years with PCOS or oligo-anovulation who underwent pre-implantation genetic testing for aneuploidy (PGT-A) and were scheduled for LPP-FET. PCOS was diagnosed according to the Rotterdam criteria [20]. Baseline assessment included pelvic ultrasonography, uterine cavity evaluation, and standard endocrine testing. Exclusion criteria included oocyte donation, non-obstructive azoospermia, significant uterine pathology, untreated hydrosalpinx, or uncontrolled endocrine or systemic medical conditions.

Letrozole responsiveness was defined as development of a dominant follicle ≥14 mm within 15 days after the final letrozole dose. In letrozole-nonresponsive cycles, neither stair-step letrozole dosing nor gonadotropin supplementation was permitted; these cycles were converted to PC and excluded from the analysis.

Among 233 intended LPP-FET cycles, 46 were letrozole-nonresponsive, leaving 187 letrozole-responsive LPP-FET cycles. Of these, seven cycles were discontinued for personal reasons before P initiation, and spontaneous ovulation occurred before scheduled P initiation in two cycles; none of these nine cycles initiated P. The remaining 178 cycles initiated P, constituting the P-initiated cohort for the primary outcome analysis. Of the 178 P-initiated cycles, ovulation was not confirmed in two, and transfer was cancelled in both, leaving 176 cycles that comprised the ovulation-confirmed cohort for secondary outcome analyses. In the ovulation-confirmed cohort (n = 176), 127 women had PCOS and 49 had oligo-anovulation without PCOS. The study flow and derivation of the analysis cohorts are shown in Fig. 1.

The primary outcome was the spontaneous ovulation rate in the P-initiated cohort. The secondary outcomes, evaluated in the ovulation-confirmed cohort, were live birth rate (LBR), ongoing pregnancy rate (OPR), clinical pregnancy rate (CPR), early pregnancy loss rate (EPLR), and implantation rate (IR). Clinical pregnancy was defined as visualization of an intrauterine gestational sac by transvaginal ultrasonography (TV-USG) at 6 weeks of gestation; ongoing pregnancy was defined as fetal cardiac activity beyond 12 weeks; live birth was defined as the delivery of a live-born infant at or beyond 22 weeks of gestation; and early pregnancy loss was defined as loss of a clinical pregnancy before 12 weeks. EPLR was calculated per clinical pregnancy. The IR was defined as the number of gestational sacs per

blastocyst transferred. Live birth outcomes were obtained through telephone follow-up. Follow-up was complete for all clinical pregnancies, with no pregnancy remaining ongoing at the time of analysis.

Controlled ovarian stimulation, preimplantation genetic testing for aneuploidy, blastocyst vitrification/warming

All patients underwent controlled ovarian stimulation using progestin-primed ovarian stimulation or GnRH antagonist protocols with agonist triggering [21, 22]. Following standard procedures for IVF, blastocyst morphology was assessed according to the Gardner criteria [23], with high-quality blastocysts defined as those with expansion stage 3–6 and inner cell mass and trophectoderm grades A or B. Blastocysts not meeting these criteria were classified as low quality [18]. Blastocysts were biopsied on day 5 or 6 for PGT-A and subsequently vitrified. Vitrification and warming were performed using the Cryotop method [24].

LPP-FET protocol

Transvaginal ultrasonography was performed on cycle days 3–5 of spontaneous or dydrogesterone-induced withdrawal bleeding. Letrozole, 5 mg/day for 5 days, was commenced on cycle days 3–5. A follow-up TV-USG was scheduled 4–5 days after the final letrozole dose. Cycles without a dominant follicle ≥14 mm within 15 days after the final letrozole dose were classified as letrozole-nonresponsive and converted to PC. Once a dominant follicle reached ≥14 mm, serum estradiol (E2), LH, and progesterone (P4) were measured. If serum E_2 was <100 pg/mL at that assessment, oral estradiol valerate (2 mg twice daily) was initiated to ensure adequate endometrial priming. The dose of estradiol valerate remained unchanged until pregnancy confirmation. Thereafter, serial monitoring continued until the predefined P initiation criteria were met.

Exogenous P was administered only when predefined eligibility criteria were met, including endometrial thickness ≥7 mm, dominant follicle ≥15 mm, serum E2 >130 pg/mL, and serum P4 <1.5 ng/mL. Once these criteria were first met, P could be initiated flexibly before spontaneous ovulation, provided that the eligibility criteria remained satisfied. The eligibility-to-P initiation interval was defined as the interval from first fulfillment of P initiation criteria to actual P initiation, reflecting the flexibility used in routine clinical practice. The interval from first fulfillment of P initiation criteria to ultrasound-documented ovulation (UDO) was defined as the eligibility-to-UDO interval and was assessed to characterize the potential pre-ovulatory window for P initiation. UDO was defined as the complete disappearance of

the follicle, a reduction in follicular size with wall thickening, or replacement by a spongy-appearing structure [25].

On the scheduled day of P initiation, TV-USG and serum hormone measurements were repeated, and dydrogesterone was initiated only if all eligibility criteria remained satisfied and the dominant follicle had not ovulated. Cycles with ovulation before scheduled P initiation were cancelled. Single euploid blastocyst transfer was scheduled on the sixth day of P administration, with day 1 defined as the first day of P initiation. No hCG trigger was administered.

Dydrogesterone (10 mg four times daily; Duphaston®, Abbott Biologicals, The Netherlands) was used for exogenous P administration. After dydrogesterone initiation, daily TV-USG was continued until UDO, without additional serum hormone measurements during this interval. On the scheduled day of FET, endometrial thickness was measured and ultrasonographic evidence of ovulation was reassessed before transfer. Serum P4 was also measured that day, and the result was available before the decision to proceed with transfer. For the primary outcome, spontaneous ovulation was considered confirmed when both UDO and a serum P4 level >3.0 ng/mL [26] were present. Transfer was cancelled if ovulation was not confirmed. A schematic representation of the LPP-FET protocol and the temporal relationships among eligibility, P initiation, and spontaneous ovulation is shown in Fig. 2. A pregnancy test was performed 11 days after FET. In cycles resulting in pregnancy, dydrogesterone was continued until 10 weeks of gestation. Upon pregnancy confirmation, estradiol valerate, if used, was reduced to 2 mg daily for two weeks, then discontinued.

Hormone assay

Serum LH and E2 were measured using the IMMULITE 2000 system (Siemens Medical Solutions, Malvern, PA, USA), and P4 using the Cobas e801 analyzer (Roche Diagnostics GmbH). The lower limits of detection were 20 pg/mL (E2), 0.05 ng/mL (P4), and 0.3 IU/L (LH). Intra- and inter-assay coefficients of variation were 4.3–9.9% and 6.7–16% for E2, 1.1–4.2% and 1.7–6.2% for P4, and 3.6–5.4% and 4.3–6.4% for LH.

Statistical analysis

Statistical analyses were performed using SPSS version 21.0 (IBM Corp., Armonk, NY, USA). Normality was assessed, and continuous variables were presented as medians (interquartile range [IQR]) and compared using the Mann–

Whitney U test. Categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate. The 95% confidence interval for the primary outcome was calculated using the exact binomial (Clopper–Pearson) method. No data were missing for variables included in the secondary outcome analyses of the ovulation-confirmed cohort; therefore, no imputation was performed. No a priori sample-size calculation was performed.

Univariable logistic regression (ULR) was used to estimate crude associations with live birth. To evaluate whether variation in P initiation timing within the protocol-defined window was independently associated with live birth, four P initiation timing-related variables—follicular diameter and serum E2 on the day of P initiation, the eligibility-to-P initiation interval, and the interval from P initiation to UDO—were included in the multivariable logistic regression (MLR) model based on the study hypothesis and clinical relevance, together with key demographic and embryo-related prognostic factors (age at oocyte retrieval, embryo morphology, and biopsy day). Because the intervals from P initiation to UDO and from UDO to FET are structurally linked, only the interval from P initiation to UDO was included to avoid collinearity. All seven variables were entered simultaneously into the MLR model using forced entry, without data-driven variable selection. Results are presented as crude odds ratios (ORs) for ULR and adjusted odds ratios (aORs) for MLR, with 95% confidence intervals (CIs).

To assess the robustness of the primary multivariable analysis, sensitivity analyses were performed by additionally adjusting, in separate models, for follicular-phase duration (from cycle day 1 through the day of P initiation), serum P4 concentration on the day of FET, and estrogen supplementation status. Each variable was added separately to limit model complexity and to determine whether additional adjustment for these factors materially altered the associations observed in the primary analysis. A two-sided P value <0.05 was considered statistically significant.

RESULTS

Spontaneous ovulation and scheduling flexibility

In the P-initiated cohort (n = 178), spontaneous ovulation was confirmed in 176/178 cycles (98.9%; 95% CI, 96.0–99.9%) based on UDO and FET-day serum P4 >3.0 ng/mL. Using letrozole-responsive and all intended cycles as denominators, the corresponding rates were 94.1% (176/187; 95% CI, 89.7–97.0%) and 75.5% (176/233; 95% CI, 69.5–80.9%), respectively. Among the ovulation-confirmed cohort (n = 176), complete follicular disappearance was the most common ultrasonographic

finding, observed in 140/176 cycles (79.5%), whereas the remaining cycles demonstrated either a reduction in follicular size with wall thickening or replacement by a spongy-appearing structure. In the two cycles without confirmed ovulation, P was initiated at follicular diameters of 16.5 and 17.2 mm. Serial TV-USG showed no evidence of ovulation; on the planned FET day, the follicles remained unruptured and measured 32.3 and 32.6 mm, with serum P4 concentrations of 0.52 and 0.48 ng/mL, respectively. FET was cancelled in both cycles.

To characterize the potential pre-ovulatory window for flexible P initiation, the eligibility-to-UDO interval was assessed in the ovulation-confirmed cohort (n = 176). The interval ranged from 2 to 7 days (median 4, IQR 3–5); it was 2 days in 21.0% of cycles and 3–7 days in the remaining 79.0%. The eligibility-to-P initiation interval ranged from 0 to 5 days (median 1, IQR 0–1); it was 0 days in 49.4% of cycles, 1 day in 29.5%, and 2–5 days in 21.0%. The median number of visits from baseline assessment to P initiation, including the baseline and P initiation visits, was 3 (IQR 2–3); the median number after P initiation through the visit documenting UDO was also 3 (IQR 2–3).

Pregnancy outcomes and baseline characteristics

In the ovulation-confirmed cohort, the LBR was 59.7% (105/176), the OPR was 60.2% (106/176), and the CPR and IR were both 67.6% (119/176). The EPLR was 10.9% (13/119). When analyzed in the P-initiated cohort (n = 178), with the two cycles without confirmed ovulation considered as reproductive failures, the LBR, OPR, and CPR were 59.0% (105/178), 59.6% (106/178), and 66.9% (119/178), respectively.

As shown in Table 1, no significant differences were observed in demographic, clinical, or ultrasonographic characteristics between the live birth and non-live birth groups. However, cycles resulting in live birth had higher proportions of high-quality blastocysts (87.6% vs. 49.3%, $P < 0.001$) and blastocysts biopsied on day 5 (81.9% vs. 56.3%, $P < 0.001$).

Follicular-phase P initiation and live birth

Table 2 summarizes the clinical parameters reflecting variation in follicular-phase P initiation. None differed significantly between cycles with and without live birth.

Estrogen supplementation

Estrogen supplementation was administered in 29.0% (51/176) of cycles. Median serum E2 levels on the day of P initiation were 246 and 217 pg/mL in supplemented and non-supplemented cycles, respectively (P = 0.068). No significant differences were observed in the other clinical or cycle characteristics or in pregnancy outcomes between the two groups (Table 3).

Regression and sensitivity analyses

In univariable analyses, none of the four P initiation timing-related variables—follicular diameter and serum E2 on the day of P initiation, the eligibility-to-P initiation interval, and the interval from P initiation to UDO—was significantly associated with live birth, whereas high-quality blastocyst morphology and day-5 biopsy were significantly associated with live birth. In the primary multivariable model, no statistically significant independent association with live birth was detected for any of these four variables. In contrast, high-quality blastocyst morphology (aOR 6.62, 95% CI 2.90–15.08; P < 0.001) and day-5 biopsy (aOR 2.99, 95% CI 1.40–6.42; P = 0.005) remained independently associated with live birth (Table 4). These results remained materially unchanged in sensitivity analyses additionally adjusting, in separate models, for follicular-phase duration, serum P4 on the day of FET, or estrogen supplementation (Supplementary Table S1).

DISCUSSION

To our knowledge, this is the first study to evaluate a proliferative-phase FET approach—pre-ovulatory P initiation without ovulation triggering—in women with PCOS or oligo-anovulation following letrozole-induced follicular development. This study supports the clinical feasibility of LPP-FET as an additional ovulation-preserving FET option for letrozole-responsive women, with potential flexibility in follicular-phase P initiation. Spontaneous ovulation was confirmed in 98.9% of P-initiated cycles (176/178). The eligibility-to-UDO interval ranged from 2 to 7 days (median 4, IQR 3–5). No independent association between P initiation timing–related variables and live birth was detected within their observed ranges, whereas blastocyst morphology and biopsy day showed independent associations.

Previous NPP-FET studies [15–19] were restricted to ovulatory women. Ovulatory NPP-FET cohorts, including our previous study [18], cannot answer whether a letrozole-induced follicle still ruptures spontaneously after early exposure to exogenous progestogen in women with PCOS or oligo-anovulation. A previous study reported a mean largest follicular diameter before ovulation of 22.18 ± 3.72 mm

in untriggered letrozole cycles [27]. In our cohort, the median follicular diameter one day before UDO was 21.1 mm (IQR 19.6–23.5 mm). Together, the high spontaneous ovulation rate and broadly comparable preovulatory follicular size support the physiological plausibility of the LPP-FET protocol.

The clinical use of the potential pre-ovulatory window for P initiation, suggested by the observed eligibility-to-UDO interval (Fig. 2), may depend on center-specific scheduling practices. In the 176 ovulation-confirmed cycles, the eligibility-to-P initiation interval was 0–5 days under our center's scheduling practices. P was initiated on the day the eligibility criteria were first met or on the following day in 139 cycles (79.0%); therefore, findings for the 37 cycles with delays of 2–5 days should be interpreted with caution. In addition, two letrozole-responsive cycles were cancelled because ovulation occurred before scheduled P initiation and were therefore not included in the P-initiated cohort (Fig. 1).

In multivariable analysis, none of the four P initiation timing–related variables showed a statistically significant independent association with live birth after adjustment for age at oocyte retrieval, blastocyst morphology, and biopsy day (Table 4). These findings remained materially unchanged in sensitivity analyses (Supplementary Table S1). In contrast, high-quality blastocyst morphology and day-5 biopsy remained independently associated with live birth. Both factors were also independently associated with higher clinical pregnancy rates in a cohort of 1,037 single euploid transfers [28]. These findings suggest potential flexibility in P initiation timing within the observed ranges, although comparable live birth outcomes across different initiation times remain to be established.

Dydrogesterone was used because it does not cross-react with conventional P4 immunoassays, allowing measurement of endogenous luteal P4 [29]. The dose of 40 mg/day was consistent with common clinical practice (30–40 mg/day) [30]. FET-day serum P4 did not differ significantly between cycles with and without live birth (Table 1), and additional adjustment for FET-day P4 did not materially change the main findings (Supplementary Table S1). A similar lack of significant association between FET-day serum P4 and live birth was reported in a dydrogesterone-based NPP-FET–related protocol [19]. Whether a lower dydrogesterone dose would suffice after spontaneous ovulation warrants further investigation. In LPP-FET, exogenous P is initiated before spontaneous ovulation; therefore, the duration of endogenous luteal P4 exposure before FET varies according to the timing of ovulation. Whether these variations influence endometrial receptivity or embryo–endometrial synchrony remains to be determined. FET-day P4 alone also cannot show whether other CL-

derived endocrine and vascular functions are equivalent to those in NC-FET or LOS-FET.

The E2 criterion for P initiation was lowered from >150 pg/mL in our NPP-FET protocol [18] to >130 pg/mL in LPP-FET, considering the lower E2 levels expected in letrozole-stimulated cycles. However, the optimal periovulatory E2 threshold in letrozole-based FET remains uncertain. In modified LOS-FET cycles, Zhang et al. [31] reported higher miscarriage and lower live birth rates with trigger-day E2 <91 pg/mL, whereas another study observed a nonsignificant trend toward lower live birth rates below 100 pg/mL [32]. Evidence from letrozole-IUI cycles also suggested 125–150 pg/mL as a potentially favorable periovulatory E2 range [33]. These findings provide indirect support for avoiding excessively low E2 levels but do not establish an optimal threshold for LPP-FET. In our protocol, estrogen was supplemented when E2 was <100 pg/mL at the first observation of a dominant follicle ≥14 mm. Supplementation was used in 29.0% of cycles, with no significant differences in pregnancy outcomes between supplemented and non-supplemented cycles (Table 3). Adjustment for estrogen supplementation did not materially alter the main findings (Supplementary Table S1). However, its therapeutic efficacy remains uncertain without an untreated low-E2 comparison group. Estrogen supplementation has also been reported in selected letrozole-based FET cycles, with protocol-specific indications [31, 34].

We selected a more conservative follicular diameter threshold of ≥15 mm for P initiation, compared with ≥14 mm in our NPP-FET protocol [18], to allow further follicular development before P initiation. Previously reported NPP-FET protocols used thresholds of ≥12 mm, ≥14 mm, or >16 mm [15–19]. A threshold of ≥15 mm has also been reported for hCG triggering in modified LOS-FET [11]. Two large retrospective studies of modified NC-FET reported hCG triggering across a broad range of follicular diameters, with no significant differences in pregnancy outcomes after adjustment [12, 13]. Taken together, these reports suggest potential scheduling flexibility in these protocols, but their findings cannot be directly extrapolated to LPP-FET. Whether initiating P at <15 mm could expand scheduling flexibility without compromising spontaneous ovulation or reproductive outcomes warrants further investigation.

A key distinction between modified LOS-FET and LPP-FET is how FET timing is scheduled. In modified LOS-FET, hCG is administered when prespecified follicular criteria are met to induce ovulation, and FET is scheduled relative to the trigger. However, the optimal follicular diameter for hCG triggering remains uncertain, with thresholds varying across protocols [3, 4, 11, 31, 32]. In a multicenter observational study of 170 modified LOS-FET cycles, crude OPR did not differ significantly across

trigger-day follicular diameter groups (15–17, 18–20, and 21–24 mm), although larger follicular diameter at triggering was independently associated with higher odds of ongoing pregnancy [11]. In LPP-FET, a follicular diameter of ≥15 mm serves as the threshold for exogenous P initiation rather than ovulation triggering. In our cohort, follicular growth continued after P initiation, with spontaneous ovulation documented a median of three days later and a median follicular diameter of 21.1 mm measured one day before UDO. Both approaches offer scheduling flexibility, but modified LOS-FET relies on timed ovulation triggering, whereas LPP-FET temporally separates P initiation from spontaneous ovulation, anchoring FET timing to P initiation while follicular development continues. This conceptual distinction does not establish superiority, as the present study was not designed for direct protocol comparison. Rather, LPP-FET may provide an additional ovulation-preserving option for FET scheduling in appropriately selected patients.

During the study, daily TV-USG was performed after P initiation until UDO to document spontaneous ovulation timing. The high confirmed spontaneous ovulation rate (176/178, 98.9%) subsequently informed our center's decision to discontinue daily post-P-initiation TV-USG in routine practice. However, FET-day assessment of endometrial thickness, ultrasonographic evidence of ovulation, and serum P4 has been retained.

The strengths of this study include a well-defined LPP-FET protocol and combined ultrasonographic and biochemical confirmation of spontaneous ovulation. Using dydrogesterone allowed assessment of endogenous P4. Restriction to first single euploid blastocyst transfers reduced embryo-related heterogeneity when assessing live birth. Variables in the primary multivariable model were selected based on the study hypothesis and clinical relevance rather than data-driven selection, with sensitivity analyses assessing the robustness of the findings.

This study is limited by its retrospective design and the absence of a concurrent control group, which precludes direct comparison with other FET protocols. P initiation timing was not randomized, and residual confounding remains possible. Relatively wide confidence intervals for adjusted associations between P initiation timing–related variables and live birth indicate that clinically important associations cannot be excluded despite nonsignificant results. Serial hormone measurements were not performed between P initiation and UDO, limiting assessment of periovulatory LH and P4 dynamics. The predominantly lean cohort and assessment of reproductive outcomes primarily in ovulation-confirmed cycles may limit generalizability.

Conclusions

This study supports the clinical feasibility of LPP-FET and suggests potential scheduling flexibility in letrozole-responsive women with PCOS or oligo-anovulation. Spontaneous ovulation was confirmed in 98.9% of P-initiated cycles. No independent association between P initiation timing–related variables and live birth was detected within their observed ranges. LPP-FET may represent an additional CL-preserving FET option, with transfer timed from P initiation rather than ovulation detection or hCG triggering. Further studies are warranted to refine P initiation thresholds, optimize dydrogesterone dosing, evaluate reproductive and obstetric outcomes, and compare LPP-FET with other FET protocols.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the principles of the Declaration of Helsinki. This study was approved by the Institutional Review Board of Tri-Service General Hospital, National Defense Medical University, Taipei, Taiwan (IRB No. B202505121). All patients who visited our center had consented to the anonymous use of their medical data for scientific research and publication, and informed consent was signed at the beginning of treatment. Because the study retrospectively analyzed the data of patients who had completed treatment, further consent was not necessary.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no competing interests.

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Authors' contributions

CC Huang and TC Huang conceived and designed the study. FW Chang, CY Huang, and GJ Wu were responsible for data acquisition and analysis. JH Yang and JL Hwang analyzed and interpreted the data. MJ Chen performed the statistical analysis. TC Huang and JL Hwang drafted the article. CC Huang, H Yarali, and KH Tsui reviewed and revised the manuscript. All authors approved the submitted version of the manuscript and agree to be personally accountable for their own contributions.

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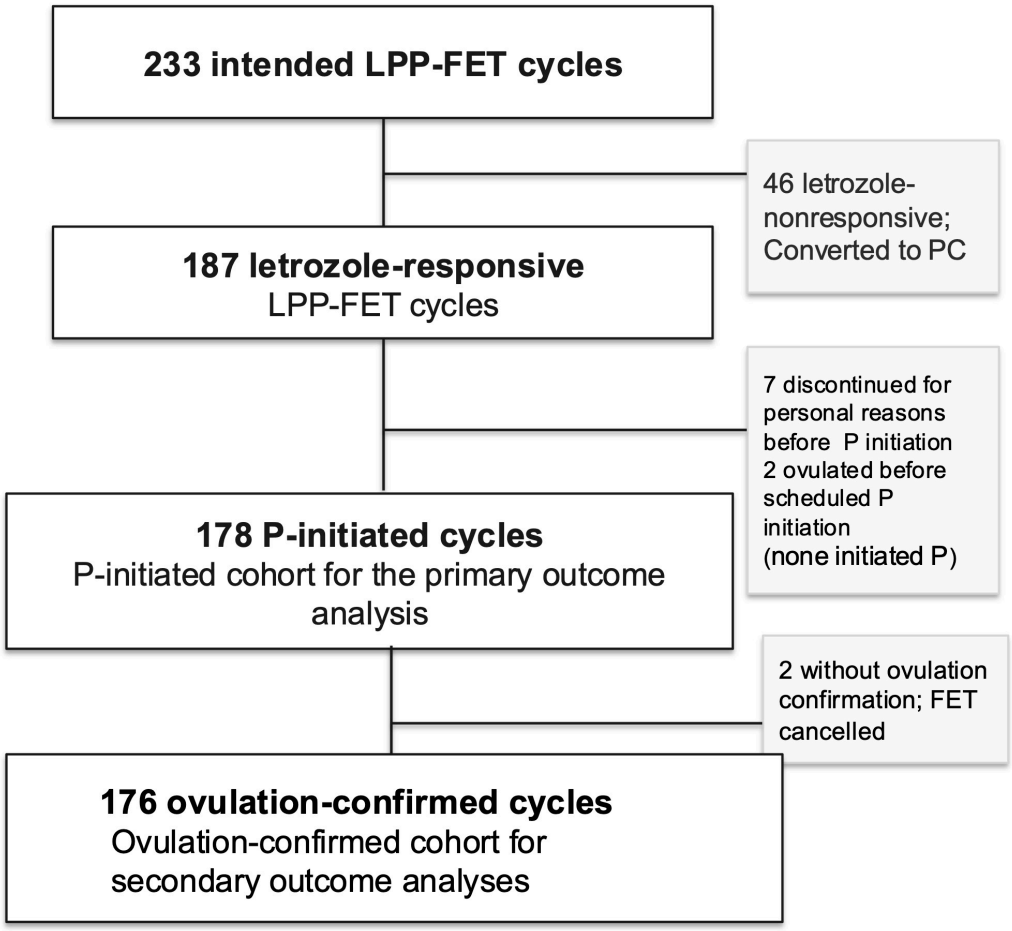
Figure legends

Fig. 1 Study flow and derivation of the analysis cohorts. LPP-FET, letrozole-induced proliferative phase frozen embryo transfer; PC, programmed cycle; P, progesterone.

Fig. 2 Schematic representation of the LPP-FET protocol and the temporal relationships among eligibility, P initiation, and spontaneous ovulation. Confirmed spontaneous ovulation was defined as UDO plus FET-day serum P4 >3.0 ng/mL. The timeline is schematic and not drawn to scale; eligibility and P initiation could occur on the same day. DF, dominant follicle; EMT, endometrial thickness; E2, estradiol; EV, estradiol valerate; FET, frozen embryo transfer; LH, luteinizing hormone; P, progesterone; P4, serum progesterone; TV-USG, transvaginal ultrasonography; UDO, ultrasound-documented ovulation.

Figures

Figure 1. Study flow and derivation of the analysis cohorts

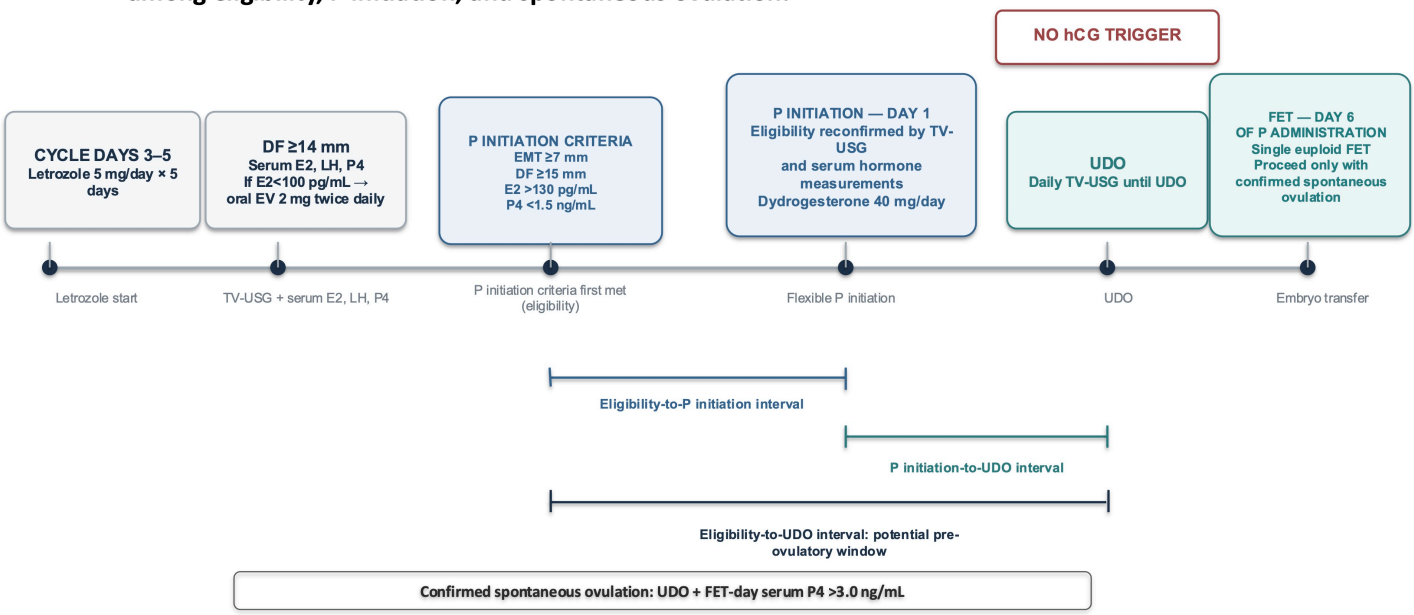


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Figure 2

Schematic representation of the LPP-FET protocol and the temporal relationships among eligibility, P initiation, and spontaneous ovulation. Confirmed spontaneous ovulation was defined as UDO plus FET-day serum P4 >3.0 ng/mL. The timeline is schematic and not drawn to scale; eligibility and P initiation could occur on the same day. DF, dominant follicle; EMT, endometrial thickness; E2, estradiol; EV, estradiol valerate; FET, frozen embryo transfer; LH, luteinizing hormone; P, progesterone; P4, serum progesterone; TV-USG, transvaginal ultrasonography; UDO, ultrasound-documented ovulation.

Supplementary Files

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- Tables14LPPFETFinalJOR20261005.docx
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