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Effect of Continuous Versus Flexible Recombinant Luteinizing Hormone Supplementation in the Luteal Phase Long Protocol on Pregnancy Outcomes in Advanced-Age Women: A Retrospective Cohort Study

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Data availability statement: The datasets generated and/or analyzed during the current study are not publicly available due to patient privacy concerns but available from the corresponding author on reasonable request.

Abstract

Objective: To evaluate the impact of different timings of recombinant luteinizing hormone (rLH) supplementation during controlled ovarian stimulation (COS) with the luteal phase long protocol on pregnancy outcomes in women of advanced reproductive age.

Methods: This retrospective study analyzed 727 infertile women aged 35–39 years who underwent COS with the luteal phase long protocol at the Reproductive Medicine Center of Xiamen Women and Children's Hospital between January 2017 and July 2023. Patients were categorized into two groups according to the timing of rLH supplementation: continuous and flexible. Baseline characteristics, stimulation parameters, and pregnancy outcomes were compared between groups. Multivariate logistic regression was used to assess the independent effect of rLH supplementation timing on pregnancy outcomes.

Results: Of the 727 patients, 300 were in the continuous supplementation group, whereas 427 were in the flexible supplementation group. Baseline characteristics were generally balanced; however, the continuous supplementation group had significantly higher basal follicle-stimulating hormone (FSH) and lower LH and progesterone levels on the human chorionic gonadotropin (hCG) trigger day ($P < 0.05$). Across fresh embryo transfer cycles, the live birth rate was significantly higher in the continuous supplementation group than in the flexible supplementation group (50.5% vs. 40.4%, $P = 0.039$). After adjusting for key confounders, including age, infertility duration, anti-Müllerian hormone, antral follicle count, basal FSH, number of oocytes retrieved, and number of embryos transferred, multivariate logistic regression confirmed that continuous supplementation was an independent predictor of a higher live birth rate (adjusted odds ratio [aOR] = 1.59, 95% confidence interval [CI]: 1.10–2.36, $P = 0.015$), as well as improved implantation (aOR = 1.65, 95% CI: 1.10–2.48, $P = 0.016$) and clinical

pregnancy rates (aOR = 1.51, 95% CI: 1.03–2.23, *P* = 0.036). No significant differences between groups were observed concerning gonadotropin starting dose, duration of stimulation, endometrial thickness on the hCG day, number of retrieved oocytes, or number of high-quality embryos (all *P* > 0.05).

Conclusion: For women of advanced reproductive age undergoing the luteal phase long protocol, the strategy of continuous rLH supplementation from the start of stimulation is superior to a flexible, delayed approach, significantly improving live birth rates in fresh transfer cycles. These findings suggest that early and sustained LH support may optimize outcomes in this patient population, including those with signs of diminished ovarian reserve.

Keywords: luteinizing hormone; luteal phase long protocol; embryo transfer; pregnancy outcomes

Introduction

Female fertility progressively declines with advancing age, particularly after 35 years, when a substantial reduction in oocyte quality and ovarian reserve results in lower implantation and pregnancy rates [1,2]. Given the increasing trend toward delayed childbearing, achievement of improved *in vitro* fertilization (IVF) outcomes in advanced-age women has become a pressing clinical challenge in reproductive medicine [3].

The gonadotropin-releasing hormone agonist (GnRH-a) long protocol remains a widely applied regimen for controlled ovarian stimulation (COS) in assisted reproductive technology (ART) [4,5]. This protocol provides benefits concerning pituitary downregulation, follicular synchronization, and endometrial receptivity. However, strong suppression of endogenous gonadotropins—particularly luteinizing hormone (LH)—may impair follicular development and oocyte maturation in some patients, especially those with diminished ovarian reserve [6–8].

Previous studies have highlighted the role of exogenous LH supplementation in follicular development, steroidogenesis, and endometrial preparation [9–12]. Nevertheless, the optimal timing of LH supplementation during COS remains controversial. Some evidence suggests that delayed or flexible supplementation, guided by ovarian response or serum hormone levels, is sufficient [13,14]; other reports indicate that continuous supplementation from the beginning of stimulation may better mimic physiological conditions and improve outcomes [15–17].

Given these inconsistencies and the lack of high-quality evidence specifically focusing on women of advanced reproductive age undergoing the luteal phase long protocol, further research is warranted. Notably, existing meta-analyses have demonstrated that rLH supplementation confers greater benefits in women aged ≥35 years than in younger populations [4,15], making the question of whether to add LH largely settled for this age group. The more clinically relevant

question, therefore, is not whether but when to initiate LH supplementation during COS. This retrospective cohort study aimed to evaluate the impact of continuous versus flexible recombinant LH (rLH) supplementation on pregnancy outcomes in women aged 35–39 years undergoing the luteal phase long protocol [18]. We hypothesized that, by establishing a more stable physiological endocrine milieu from the early follicular phase, continuous rLH supplementation would be associated with improved live birth rates relative to a flexible, response-guided approach.

Materials and methods

Study design and participants

This retrospective cohort study was conducted at the Reproductive Medicine Center of Xiamen Women and Children's Hospital between January 2017 and July 2023. In total, 727 infertile women aged 35–39 years who underwent IVF/intracytoplasmic sperm injection (ICSI) treatment using the luteal phase long protocol were included. Exclusion criteria were as follows: (1) uterine abnormalities or intrauterine adhesions; (2) untreated hydrosalpinx; (3) chromosomal abnormalities in either partner; (4) stage III–IV endometriosis; (5) cycles without embryo transfer; and (6) incomplete clinical data [3,6].

Controlled ovarian stimulation protocol

All patients underwent pituitary downregulation using a GnRH-a (1.0–1.3 mg triptorelin acetate) administered in the mid-luteal phase of the preceding cycle. COS was initiated after confirmation of pituitary suppression, defined as serum estradiol < 50 pg/mL, LH < 5 mIU/mL, endometrial thickness < 5 mm, and absence of ovarian cysts. Recombinant follicle-stimulating hormone (rFSH; Gonal-F®, Merck Serono, Switzerland) was administered at individualized doses of 150–300 IU/day and adjusted according to ovarian response. rLH (Luveris®, 75 IU/day, Merck

Serono, Switzerland) was administered according to the attending physician's clinical judgment and preference. Patients were retrospectively divided into two groups based on the rLH supplementation strategy they received:

- **Continuous supplementation group:** 75IU/day rLH was added from the first day of gonadotropin stimulation and continued until the trigger day.
- **Flexible supplementation group:** 75IU/day rLH was initiated based on ovarian response or serum hormone levels (LH and estradiol), typically when LH < 1.2 mIU/mL or when serum estradiol was considered suboptimal [9,10,13].

In the flexible supplementation group, the decision to initiate rLH was governed by a unified departmental Standard Operating Procedure to minimize inter-operator variability. rLH administration was triggered strictly by quantitative physiological parameters rather than subjective judgment. Specifically, supplementation commenced if one or more of the following criteria were met during monitoring: (i) serum LH levels < 1.2 mIU/mL; (ii) suboptimal estradiol response, defined as serum estradiol < 150 pg/mL per follicle ≥ 14 mm; or (iii) impaired follicular dynamics, defined as a growth rate < 1.5 mm/day observed on two consecutive visits. Ovarian response was comprehensively assessed based on a composite of morphological and kinetic parameters, including: (i) the rate of follicular growth, with a threshold of ≥ 1.5 mm/day considered adequate; (ii) the number and diameter of dominant follicles; and (iii) concordance between serum estradiol levels and the developing follicle count. Final oocyte maturation was triggered with 6,000–10,000 IU of human chorionic gonadotropin (hCG) when at least two follicles reached ≥18 mm in diameter. Oocyte retrieval was performed 34–36 hours later under transvaginal ultrasound guidance [5,7].

Embryo culture and transfer

Oocytes were fertilized via IVF or ICSI according to semen quality. Embryos were cultured and assessed on day 3 (cleavage stage) and day 5 (blastocyst stage). Embryo quality was graded according to standard morphological criteria. One or two embryos were transferred under ultrasound guidance in fresh cycles; luteal support was provided with vaginal progesterone and/or oral dydrogesterone until 10 weeks of gestation if pregnancy was achieved [12,15].

Outcome measures

The primary outcome was the live birth rate per fresh transfer cycle. Secondary outcomes included implantation rate, clinical pregnancy rate, number of oocytes retrieved, number of high-quality embryos, and early miscarriage rate. Implantation was defined as the number of gestational sacs observed divided by the number of embryos transferred. Clinical pregnancy was defined as the presence of a gestational sac on transvaginal ultrasound at 5–7 weeks of gestation. Live birth was defined as the delivery of a viable infant beyond 28 weeks of gestation [1,2,16].

Statistical analysis

Data analysis was performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables were presented as mean ± standard deviation (SD) and compared using Student's t-test. Categorical variables were presented as frequencies or percentages and compared using the chi-square test. Multivariate logistic regression analysis was conducted to evaluate the independent effect of rLH supplementation timing on pregnancy outcomes, with adjustment for potential confounders including age, infertility duration, anti-Müllerian hormone (AMH), antral follicle count (AFC), basal FSH, number of oocytes retrieved, and number of embryos transferred. Two-sided *P*-values < 0.05 were considered statistically significant.

Results

Baseline characteristics

In total, 727 patients met the inclusion criteria: 300 in the continuous supplementation group and 427 in the flexible supplementation group. In the flexible supplementation group, 85.48% (*n* = 365/427) of patients ultimately received rLH supplementation at D5-D7 of the ovarian stimulation process, with a mean exposure duration of 3.7 ± 2.0 days. Baseline characteristics were generally comparable between groups with respect to age, BMI, infertility duration, infertility type, AFC, and AMH levels (Table 1). However, basal FSH levels were significantly higher in the continuous supplementation group than in the flexible supplementation group (*P* = 0.012). Basal LH levels were similar between groups (*P* = 0.599).

Ovarian stimulation outcomes

Ovarian stimulation and laboratory outcomes are summarized in Table 2. The total gonadotropin dose was significantly higher in the continuous supplementation group. No significant differences between groups were observed in the starting dose of gonadotropins, duration of stimulation, or number of oocytes retrieved (all $P > 0.05$). Serum estradiol, LH, and progesterone levels on the hCG day were significantly lower in the continuous supplementation group. The numbers of MII oocytes and available embryos were significantly lower in the continuous supplementation group (*P* = 0.010 and *P* = 0.026, respectively). However, the number of good-quality embryos did not differ significantly between groups.

Pregnancy outcomes in fresh transfer cycles

Pregnancy outcomes in fresh transfer cycles are presented in Table 3. The mean number of embryos transferred was comparable between groups (*P* = 0.951). Endometrial thickness on the day of transfer and the proportion of blastocyst transfers were also similar. The distribution of

single versus double embryo transfers was comparable (continuous: 65.6% vs. 34.4%; flexible: 66.3% vs. 33.7%; $P = 0.873$). The implantation rate (69.8% vs. 60.7%), clinical pregnancy rate (59.9% vs. 51.5%), and live birth rate (50.5% vs. 40.4%) were all higher in the continuous supplementation group than in the flexible supplementation group, while only the difference in live birth rate was statistically significant ($P = 0.039$). The miscarriage rate did not differ significantly between groups.

Multivariate logistic regression analysis

Multivariate logistic regression results are shown in Table 4. After adjustment for potential confounders, the analysis confirmed that continuous rLH supplementation was independently associated with improved outcomes: implantation rate (adjusted odds ratio [aOR] = 1.65, 95% confidence interval [CI]: 1.10–2.48, $P = 0.016$), clinical pregnancy rate (aOR = 1.51, 95% CI: 1.03–2.23, $P = 0.036$), and live birth rate (aOR = 1.59, 95% CI: 1.10–2.36, $P = 0.015$). These findings indicate that continuous LH supplementation exerted a positive effect on reproductive outcomes, independent of baseline characteristics.

Discussion

This retrospective cohort study demonstrated that continuous rLH supplementation throughout COS using the luteal phase long protocol significantly improved implantation, clinical pregnancy, and live birth outcomes among women aged 35–39 years compared with flexible supplementation. Notably, although the continuous supplementation group yielded significantly fewer MII oocytes and available embryos, the number of good-quality embryos was comparable between groups, and the live birth rate per transfer was even significantly higher. This finding supports a each retrieved oocyte, resulting in embryos with greater implantation potential. Importantly, the continuous supplementation group exhibited higher basal FSH levels—

indicative of reduced ovarian reserve—yet still achieved superior pregnancy outcomes, underscoring the potential benefits of early and sustained rLH support.

The physiological role of LH in folliculogenesis and steroidogenesis has been well documented [7–9]. Adequate LH is required for theca cell androgen production, which constitutes the substrate for granulosa cell aromatization to estradiol under the influence of FSH [8]. Excessive suppression of endogenous LH during GnRH-a protocols may disrupt this balance, compromising oocyte competence and endometrial receptivity [10,11]. Exogenous rLH supplementation might restore this physiological milieu, enhancing both oocyte and embryo quality [7,15].

Our findings align with previous indications that LH supplementation is particularly beneficial in advanced-age women, who already exhibit compromised ovarian reserve and endocrine function [13–15]. For example, Conforti et al. [15] reported that rLH co-treatment improved outcomes among women of advanced reproductive age; Lehert et al. [16] confirmed through meta-analysis that combined rFSH and rLH stimulation was associated with higher pregnancy rates relative to rFSH alone. More recently, Bielfeld et al. [17] demonstrated that combined rFSH and rLH therapy improved effectiveness in women aged 35–40 years, further supporting our results.

The observation of better outcomes with continuous supplementation compared with flexible supplementation may be explained by early restoration of LH support, which could prevent premature progesterone elevation, promote synchronized follicular development, and optimize endometrial receptivity [6,12,14]. In contrast, flexible supplementation may provide insufficient LH exposure during the critical early follicular phase, potentially limiting its effectiveness.

Strengths of this study include its relatively large sample size, its focus on women of advanced reproductive age, and its comparison of two clinically relevant supplementation strategies within

the widely used luteal phase long protocol. However, several limitations should be acknowledged. First, given the retrospective design, group allocation was based on attending physician preference rather than randomization, which may introduce selection bias and confounding by indication. Although we adjusted for multiple covariates in the logistic regression model, residual confounding cannot be entirely excluded. Second, the single-center framework may limit generalizability. Third, live birth outcomes were assessed only in fresh transfer cycles; cumulative outcomes, including frozen transfers, were not analyzed. Finally, the long study period introduces potential temporal bias, as advancements in laboratory techniques and clinical management over this interval could have influenced outcomes in a manner independent of the LH supplementation strategy. Although these advancements were likely applied to both groups in a similar manner, their confounding effect cannot be completely excluded. Future randomized controlled trials are warranted to confirm the present findings and to elucidate the underlying mechanisms by which continuous rLH supplementation enhances reproductive outcomes.

Conclusion

In this large retrospective cohort of women aged 35–39 years undergoing a luteal phase long protocol, continuous rLH supplementation was associated with significantly higher live birth rates per fresh embryo transfer relative to a flexible supplementation approach. Our findings suggest that, for this specific patient population, efforts to ensure adequate LH activity from the onset of stimulation may represent a critical factor in improving oocyte quality and pregnancy success. Although the present results are promising, they require confirmation through prospective randomized controlled trials that also assess cumulative live birth rates.

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Table 1. Baseline characteristics of patients in the two supplementation groups

Variable	Continuous (n = 300)	Flexible (n = 427)	P value
Age (years)	36.50 ± 1.35	36.36 ± 1.29	0.167
Duration of infertility (years)	3.8 ± 1.8	3.7 ± 1.7	0.143
BMI (kg/m²)	22.33 ± 2.54	22.05 ± 2.39	0.137
AFC	12.52 ± 8.49	12.6 ± 6.88	0.294
AMH (ng/mL)	3.58 ± 2.81	3.93 ± 3.09	0.124
Basal FSH (mIU/mL)	7.82 ± 1.95	7.40 ± 1.75	0.012
Basal LH (mIU/mL)	4.58 ± 2.21	4.58 ± 2.45	0.599
Infertility type % (n)			0.872
Primary infertility	38.0 (114)	38.9 (166)	
Secondary infertility	62.0 (186)	61.1 (261)	
Fertilization method % (n)			0.713
IVF	82.3 (247)	83.6 (357)	
ICSI	17.0 (51)	15.7 (67)	
Etiology of infertility % (n)			0.472
Tubal factors	61.3 (184)	56.0 (239)	
Endometriosis	17.0 (51)	21.1 (90)	
Ovarian dysfunction	4.3 (13)	4.4 (19)	
Other factors	17.3 (52)	18.5 (79)	

Note: Continuous data are presented as mean ± SD unless otherwise indicated. Categorical variables are presented as percentages (counts).

Table 2. Ovarian stimulation and laboratory outcomes

Variable	Continuous (*n* = 300)	Flexible (*n* = 427)	*P* value
Initial Gn dose (IU)	265.75 ± 48.57	262.32 ± 50.01	0.847
Total Gn dose (IU)	2795.17 ± 719.59	2639.90 ± 650.49	0.001
Duration of stimulation (days)	10.1 ± 1.54	10.08 ± 1.27	0.062
FSH levels on Gn starting day (mIU/mL)	3.80 ± 1.55	3.39 ± 1.53	< 0.001
LH levels on Gn starting day (mIU/mL)	0.52 ± 0.38	1.17 ± 0.74	< 0.001
Estradiol on hCG day (pg/mL)	2705.18 ± 1944.53	3112.98 ± 1925.82	< 0.001
LH levels on hCG day (mIU/mL)	1.38 ± 1.33	1.68 ± 1.20	< 0.001
Progesterone on hCG day (ng/mL)	0.84 ± 0.42	0.92 ± 0.45	0.006
No. of oocytes retrieved	10.2 ± 5.86	10.73 ± 5.27	0.098
No. of MII oocytes	9.0 ± 5.86	9.9 ± 5.29	0.010
No. of available embryos	5.41 ± 4.00	5.99 ± 4.00	0.026
No. of good quality embryos	2.15 ± 2.29	2.30 ± 2.24	0.215
Percentage of freeze-all strategy	36.0 (108/300)	36.8 (157/427)	0.832

Note: Continuous data are presented as mean ± SD unless otherwise indicated. Categorical variables are presented as percentages (counts).

Table 3. Pregnancy outcomes in fresh embryo transfer cycles

Variable	Continuous (*n* = 192)	Flexible (*n* = 270)	*P* value
No. of embryos transferred	1.52 ± 0.50	1.52 ± 0.50	0.951
Endometrial thickness on transfer day (mm)	11.06 ± 2.32	11.11 ± 2.16	0.222
Blastocyst transfer rate (%)	30.7	28.1	0.619
Single embryo transfer (%)	65.6	66.3	0.873
Implantation rate (%)	69.8	60.7	0.057
Clinical pregnancy rate (%)	59.9	51.5	0.090
Live birth rate (%)	50.5	40.4	0.039
Early miscarriage rate (%)	14.8 (17/115)	19.4 (27/139)	0.420

Note: Continuous data are presented as mean ± SD unless otherwise indicated. Categorical variables are presented as percentages (counts).

Table 4. Multivariate logistic regression analysis of pregnancy outcomes

Outcome	**OR (95% CI)**	***P* value**	**Adjusted OR (95% CI)**	***P* value**
Blastocyst implantation rate	1.13 (0.76–1.70)	0.55	1.73 (0.88–3.43)	0.119
Implantation rate	1.49 (1.01–2.21)	0.046	1.65 (1.10–2.48)	0.016
Clinical pregnancy rate	1.41 (0.97–2.05)	0.074	1.51 (1.03–2.23)	0.036
Early miscarriage rate	0.72 (0.37–1.40)	0.332	0.71 (0.36–1.40)	0.317
Live birth rate	1.51 (1.04–2.19)	0.031	1.59 (1.10–2.36)	0.015

Note: Adjusted for age, infertility duration, AMH, AFC, basal FSH, number of oocytes retrieved, and number of embryos transferred.