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Ovulation Induction in Unexplained Infertility: A Comparative Analysis of Letrozole, Clomiphene Citrate, and Gonadotropins

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ABSTRACT

To compare the effects of three different ovulation induction protocols (clomiphene citrate (CC), letrozole, and gonadotropin) on pregnancy outcomes in women diagnosed with unexplained infertility undergoing intrauterine insemination (IUI).

This retrospective observational cohort study was conducted at the Infertility Unit, Department of Obstetrics and Gynecology, Etlik City Hsopital. A total of 156 women aged 18–42 years with unexplained infertility who underwent IUI between January 2022 and December 2024 were included. Ovarian stimulation was performed using CC, letrozole, or gonadotropins. Clinical pregnancy was confirmed using serum β-hCG followed by transvaginal ultrasound. Independent predictors of pregnancy were identified through multivariate logistic regression, adjusting for age, FSH, AMH levels, and the induction protocol used.

Pregnancy was achieved in 21 women (13.5%). Those who conceived had significantly lower FSH levels than those who did not (median 5.4 vs. 6.0 IU/L, $p = 0.046$). Letrozole use was notably more common among women who became pregnant (76.2% vs. 44.4%, $p = 0.025$). In multivariate analysis, letrozole remained an independent predictor of clinical pregnancy (adjusted OR: 3.589, 95% CI: 1.229–10.480, $p = 0.019$), while higher FSH levels were associated with a decreased likelihood of pregnancy (adjusted OR: 0.744, 95% CI: 0.557–0.996, $p = 0.047$).

In women with unexplained infertility, ovulation induction with letrozole prior to IUI is associated with higher pregnancy rates compared to other protocols. Considering its cost-effectiveness, lower risk of multiple pregnancies, and favorable endometrial effects, letrozole should be considered as a first-line treatment option in appropriate patients.

Keywords: Unexplained infertility; Ovulation induction; Letrozole; Clomiphene citrate; Gonadotropins; Intrauterine insemination

Introduction

Infertility is often characterized as the failure to achieve conception after a minimum of 12 months of consistent, unprotected sexual intercourse (1). However, in certain cases with clear underlying causes, a formal waiting period may not be necessary before diagnosis. Unexplained infertility, also known as subfertility, is a diagnosis of exclusion for couples who are unable to conceive despite engaging in regular unprotected intercourse and who do not fulfill the diagnostic criteria for male factor infertility, ovulatory disorders, or anatomical abnormalities such as tubal occlusion, endometriosis, uterine cavity anomalies, or cervical/vaginal obstruction (2). This form of infertility accounts for approximately 15% of heterosexual couples seeking care at tertiary fertility centers (3).

Intrauterine insemination (IUI) is a common treatment for unexplained infertility and is used in many countries. This procedure can be done during the natural cycle or with ovarian stimulation. The goal of ovarian stimulation in IUI is to raise the number of dominant follicles per cycle, with the idea that this will lead to more pregnancies (4).

Medications used for ovulation stimulation primarily include clomiphene citrate (CC), letrozole, and gonadotropins. While CC and letrozole are administered orally, gonadotropins are delivered via subcutaneous injection. Due to their higher cost and the increased risk of ovarian hyperstimulation

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syndrome and multiple gestations, injectable gonadotropins are used less frequently in contemporary practice. Nevertheless, they remain an important therapeutic option in selected clinical scenarios, such as hypothalamic amenorrhea (5).

However, oral agents such as clomiphene citrate and letrozole are generally preferred because they are less expensive, better tolerated, and demonstrate high efficacy (6). Following a standard infertility evaluation, oral ovulation induction is widely accepted as the first-line therapeutic approach. Although clomiphene citrate has long been considered a conventional agent, its anti-estrogenic effects on endometrial receptivity and cervical mucus may limit conception rates, even in cycles where ovulation is successfully achieved (7,8).

Letrozole offers several advantages owing to its lack of anti-estrogenic effects and its relatively short half-life. It has been associated with improved endometrial thickness and a lower likelihood of multiple gestations compared with other ovulation induction agents

(6,9,10). Notably, in patients with polycystic ovary syndrome (PCOS), letrozole has been shown to achieve higher live birth rates, further supporting its role as an effective first-line treatment option (11).

In this context, the aim of our study was to compare the effects of three commonly used ovulation induction protocols, clomiphene citrate, letrozole, and gonadotropins, on pregnancy outcomes in women with unexplained infertility. In this retrospective analysis, treatment cycles of patients diagnosed with unexplained infertility and managed in our clinic were reviewed, and the outcomes associated with each protocol were evaluated in terms of clinical pregnancy rates. By directly comparing these three frequently utilized treatment options, our study seeks to contribute to the existing literature and to provide evidence-based guidance for clinicians in selecting the most appropriate ovulation induction strategy tailored to the characteristics of women with unexplained infertility.

Materials and Methods

Study Design and Participants: This retrospective observational cohort study was conducted at the Infertility Unit of the Department of Obstetrics and Gynecology, Etlik City Hospital. Ethical approval was obtained from the Etlik City Hospital Clinical Research Ethics Committee (Approval No: AEŞH-BADEK1-2025-263, Date: 09/07/2025), and all procedures complied with the ethical standards of the Declaration of Helsinki and its later amendments.

Women aged between 18 and 42 years who underwent intrauterine insemination (IUI) for unexplained infertility between May 2024 and March 2025 were eligible. Unexplained infertility was defined as the inability to conceive after at least 12 months of regular unprotected intercourse despite normal ovulatory function, patent fallopian tubes confirmed by hysterosalpingography or laparoscopy, and normal semen parameters.

Participants were categorized into two groups based on pregnancy outcome following IUI:

- **Pregnancy group**: Serum β-hCG positivity followed by ultrasound evidence of a gestational sac.
- **Non-pregnancy group**: Negative β-hCG or biochemical pregnancy without clinical confirmation.

Inclusion criteria:

- Age 18–42 years
- Diagnosis of unexplained infertility
- At least one completed IUI cycle
- Complete medical and laboratory records available

Exclusion criteria:

- Endometriosis, tubal factor infertility, uterine anomalies, male factor infertility, or polycystic ovary syndrome (PCOS)
- History of in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) cycles
- Incomplete or missing clinical data

2.2 Ovulation Induction and IUI Protocol

Ovulation induction was performed using one of three regimens:

- **Letrozole**: 5 mg/day orally from cycle days 3–7
- **Clomiphene citrate (CC)**: 100 mg/day orally from cycle days 3–7
- **Gonadotropins**: Recombinant FSH at 50–75 IU/day subcutaneously starting on cycle day 3, adjusted according to follicular growth and estradiol levels

Follicular monitoring was initiated on day 9 using transvaginal ultrasonography (Voluson S10, GE Healthcare, USA) and repeated every 1–3 days until the lead follicle reached ≥18 mm with adequate endometrial thickness (≥7 mm).

hCG trigger: Recombinant hCG (250 µg choriogonadotropin alfa; Ovitrelle®, Merck

Serono) was administered to induce final oocyte maturation.

Cycle cancellation criteria:

- More than three follicles ≥16 mm
- Serum estradiol (E2) >1500 pg/mL
- Patient request or medical contraindication

Cycle cancellation criteria were applied primarily to minimize the risk of multiple gestation and ovarian hyperstimulation, in accordance with current clinical practice recommendations.

IUI was performed approximately 36 hours after the trigger. Semen samples were collected following 2–5 days of abstinence and prepared via density gradient centrifugation. A post-wash total motile sperm count (TMSC) ≥1 × 10^6 was required. A soft IUI catheter was used to deposit the sperm into the uterine cavity under sterile conditions.

Laboratory Assessments: Baseline hormonal evaluation was performed on cycle days 2–4 and included anti-Müllerian hormone (AMH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), prolactin (PRL), and thyroid-stimulating hormone (TSH). Serum β-hCG was measured 14 days after IUI, and clinical pregnancy was confirmed by transvaginal ultrasonography demonstrating a gestational sac and fetal heartbeat at 6–7 weeks.

Statistical Analysis: Statistical analysis was performed using SPSS Statistics version 29.0 (IBM Corp., Armonk, NY, USA).

Based on the effect size reported by Pourali et al., an a priori power analysis (two-tailed t-test, $\alpha = 0.05$, power = 70%) indicated that a minimum of 21 participants per group (total n = 42) was required to detect a significant difference.

The distribution of continuous variables was evaluated using the Kolmogorov–Smirnov test. Normally distributed variables were compared using the independent samples t-test, while non-normally distributed variables were analyzed using the Mann–Whitney U test. Categorical variables were assessed with the Chi-square test or Fisher's exact test when appropriate.

To identify independent predictors of pregnancy, variables with a p-value <0.10 in univariate analysis were entered into a multivariate logistic regression model using the enter method. The results were expressed as odds ratios (ORs) with 95% confidence intervals (CIs). A p-value <0.05 was considered statistically significant.

Results

A total of 156 women with unexplained infertility who underwent intrauterine insemination (IUI) were included in the study. Among them, 21 women (13.5%) achieved pregnancy, while 135 (86.5%) did not.

As shown in Table 1, there were no significant differences between pregnant and non-pregnant groups in terms of age, BMI, duration of infertility, gravida, or nulliparity (all p>0.050). Similarly, laboratory findings, including anti-Müllerian hormone (AMH), luteinizing hormone (LH), estradiol (E2), prolactin (PRL), and thyroid-stimulating hormone (TSH) levels, did not differ significantly between groups (p>0.050). However, follicle-stimulating hormone (FSH) levels were significantly lower in the pregnancy group compared to the non-pregnancy group (median 5.4 vs. 6.0 IU/L, p=0.046).

Table 2 summarizes the IUI stimulation protocols and medication doses. Use of letrozole was significantly higher in the pregnancy group compared to the non-pregnancy group (76.2% vs. 44.4%, p=0.025). There were no significant differences between the groups in terms of clomiphene citrate (CC) or gonadotropin use, treatment duration, or medication dosages (all p>0.050).

Univariate and multivariate logistic regression analyses are presented in Table 3. In univariate analysis, FSH was negatively associated with pregnancy (OR: 0.744, 95% CI: 0.557–0.996, p=0.047), and use of letrozole significantly increased pregnancy odds (OR: 4.000, 95% CI: 1.385–11.546, p=0.010).

In the multivariate model adjusted for age, FSH, AMH, and IUI protocol, letrozole remained an independent predictor of pregnancy (adjusted OR: 3.589, 95% CI: 1.229–10.480, p=0.019). No other variables showed significant associations in the adjusted model.

Discussion

In this study, the effects of three different ovulation induction protocols (clomiphene citrate, letrozole, and gonadotropin) on intrauterine insemination (IUI) outcomes were compared in women diagnosed with

Table 1: Baseline Characteristics of Women with Unexplained Infertility Undergoing Intrauterine Insemination: Comparison Between Pregnancy and Non-Pregnancy Groups

	Pregnancy (+) N:21	Pregnancy (-) N:135	P value
Demographics, Median (IQR) – n (%)			
Age (years)	26.0 (23.0, 31.0)	29.0 (26.0, 32.5)	0.096
BMI (kg/m²)	25.0 (22.0, 28.0)	24.0 (22.0, 28.0)	0.712
Duration of infertility (years)	2.5 (1.2, 4.0)	2.0 (1.5, 3.5)	0.898
Gravida	0.0 (0.0, 1.0)	0.0 (0.0, 1.0)	0.772
Nulliparity	18 (86.7)	126 (90.3)	0.676
Laboratory Findings, Median (IQR)			
AMH (ng/mL)	3.30 (1.60, 6.00)	2.70 (1.60, 4.50)	0.355
FSH (IU/L)	5.4 (5.0, 7.0)	6.0 (5.0, 7.0)	0.046
LH (IU/L)	5.0 (3.0, 9.0)	6.0 (5.0, 8.0)	0.297
Estradiol (pg/mL)	38.0 (27.8, 51.3)	38.0 (30.0, 53.0)	0.785
Prolactin (ng/mL)	17.5 (12.5, 25.8)	16.0 (13.0, 22.0)	0.655
TSH (μIU/mL)	1.70 (1.15, 2.05)	2.00 (1.70, 2.80)	0.100

Values are presented as median (interquartile range) unless otherwise specified. Comparisons between pregnancy and non-pregnancy groups were performed using the Mann–Whitney U test for continuous variables and the Chi-square test for categorical variables. A p-value of <0.050 was considered statistically significant.
BMI: Body-mass index; **FSH:** Follicle-stimulating hormone; **AMH:** Anti-Müllerian hormone; **LH:** Luteinizing hormone; **TSH:** Thyroid-stimulating hormone

Table 2: Comparison of Intrauterine Insemination (IUI) Stimulation Protocols, Treatment Duration, and Medication Doses Between Pregnant and Non-Pregnant Women

	Pregnancy (+) N:21	Pregnancy (-) N:135	P value
IUI stimulation protocol			0.025
CC	1/21 (4.8)	20/135 (14.8)	
Letrozole	16/21 (76.2)	60/135 (44.4)	
Gonadotropins	4/21 (19.0)	55/135 (40.7)	
Treatment Duration in days	10.5 (9.0, 12.0)	10.0 (9.0, 12.0)	0.387
CC dose in mg	100.0 (100.0, 100.0)	100.0 (100.0, 100.0)	0.571
Letrozole dose in mg	5.0 (5.0, 5.0)	5.0 (5.0, 5.0)	0.853
Gonadotropins dose in IU	50.0 (50.0, 50.0)	50.0 (50.0, 75.0)	0.383

Values are presented as median (interquartile range) unless otherwise specified. Comparisons between pregnancy and non-pregnancy groups were performed using the Mann–Whitney U test for continuous variables and the Chi-square test for categorical variables. A p-value of <0.050 was considered statistically significant.
IUI: Intrauterine insemination; **CC:** Clomiphene Citrate

unexplained infertility. Our findings revealed that letrozole use significantly increased pregnancy rates and that FSH levels were inversely related to the likelihood of pregnancy. The pregnancy rate obtained in our study was 13.5%, which is consistent with previous studies reporting IUI success rates ranging from 10% to 20% in unexplained infertility (3,4).

The efficacy of letrozole in cases of unexplained infertility is consistent with numerous randomized controlled trials and meta-analyses in the current literature. It has been reported that letrozole may positively affect endometrial thickness and increase uterine receptivity in patient groups other than those with PCOS, and that it may increase the chances of implantation because, unlike clomiphene citrate, it does not have antiestrogenic adverse effects on cervical mucus (6,9). These biological advantages support the finding in our study that letrozole is an independent predictor of pregnancy in multivariate analysis (aOR: 3.589; p=0.019).

Table 3: Univariate and Multivariate Logistic Regression Analyses of Factors Associated with Pregnancy in Women with Unexplained Infertility Undergoing Intrauterine Insemination

	Univariate LR		Multivariate LR	
	OR (95% CI)	P value	aOR (95% CI)	P value
Age (years)	0.915 (0.826 – 1.010)	0.090		
FSH	0.744 (0.557 – 0.996)	0.047	0.769 (0.572 – 1.030)	0.082
AMH	1.163 (0.947 – 1.429)	0.148		
IUI Protocol				
CC	0.288 (0.036 – 2.264)	0.236		
Letrazole	4.000 (1.385 – 11.546)	0.010	3.589 (1.229 – 10.480)	0.019
Gonadotropin	0.109 (0.109 – 1.072)	0.066		

Values are presented as odds ratio (OR) or adjusted odds ratio (aOR) with 95% confidence intervals (CI). A p value < 0.05 was considered statistically significant.

LR: Logistic regression; **OR:** Odds ratio; **aOR:** Adjusted odds ratio; **CI:** Confidance interval; **FSH:** Follicle-stimulating hormone; **AMH:** Anti-Müllerian hormone; **IUI:** Intrauterine insemination; **CC:** Clomiphene Citrate

Randomized controlled trials have shown that there is no significant difference between letrozole and clomiphene citrate in terms of clinical pregnancy, live birth, and miscarriage rates, but that a 2.5 mg dose of letrozole may increase clinical pregnancy rates compared to 100 mg of clomiphene (12). Meta-analyses conducted by Eskew et al. (13) and Liu et al. (12) also revealed that letrozole is as effective as clomiphene in unexplained infertility and may be superior in some subgroups. Qin et al. (14) reported that letrozole may increase the number of dominant follicles and reduce the miscarriage rate in IUI cycles.

Evidence regarding gonadotropin use presents a more complex situation. Zolton et al. (15) meta-analysis found that the additional births achieved with gonadotropins were associated with a similar increase in the risk of multiple pregnancies and that there was insufficient randomized data to support the use of gonadotropins in OS-IUI protocols for unexplained infertility. Wessel et al. (16) found that while low-quality evidence indicates a higher risk of multiple pregnancies, especially triplet pregnancies, moderate-quality evidence suggests gonadotropins may increase the chance of live birth when compared to both CC and letrozole. In protocols with stringent cancellation criteria, gonadotropins can achieve acceptable rates of multiple pregnancies without compromising efficacy, but treatment planning must take patient preferences and cost into account, according to Danhof et al. (17).

The CC group had the lowest cumulative live birth rates after four cycles of OS-IUI (29.4%), followed by letrozole (32.0%) and gonadotropins (34.5%) (18). This suggests that letrozole may be a good choice in terms of both effectiveness and cost-effectiveness. In a randomized controlled trial conducted by Legro et al. (11) involving women with PCOS, letrozole demonstrated superior live birth and ovulation rates in comparison to clomiphene.

The negative correlation between FSH levels and the likelihood of pregnancy can be explained by the known biological mechanism between ovarian reserve and oocyte quality. High FSH levels are an indicator of reduced ovarian reserve and suggest that the response to treatment may be poor (19).

The strengths of our study include its conduct at a single center using standardized protocols, its comprehensive dataset, and the direct comparison of three commonly used stimulation strategies. However, several limitations should be acknowledged. The retrospective design may be associated with selection bias, and the relatively limited sample size may have reduced statistical power. Furthermore, pregnancy rates rather than live birth rates were evaluated, which may partially limit the clinical significance of the findings. In addition, endometrial parameters such as endometrial thickness, although routinely assessed during cycle monitoring, were not included in the comparative analyses and therefore could not be evaluated as potential differentiators between stimulation protocols. Finally, heterogeneity in the existing literatüre,stemming from variations in medication regimens, patient populations, and stimulation criteria, should be considered when comparing our results with previous studies.

In conclusion, ovulation induction with letrozole appears to increase pregnancy rates in cases of unexplained infertility. Considering its cost-effectiveness, lower risk of multiple pregnancies, and patient comfort, letrozole is a strong first-line treatment option for suitable candidates. Nevertheless, personalized treatment plans should be developed for each patient, taking into account the

potential additional benefits of gonadotropins, the risk of multiple pregnancies, and the economic burden. Our findings must be validated by large-scale, prospective, randomized, controlled trials that also evaluate live birth rates.

In this retrospective case-control study evaluating three different ovulation induction protocols in unexplained infertility, letrozole use prior to IUI was associated with a significant increase in pregnancy rates, while higher baseline FSH levels were negatively correlated with pregnancy likelihood.

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