Original Article

A Randomized Trial of Stair-Step Letrozole versus Traditional Letrozole for Subfertile Women with Polycystic Ovary Syndrome

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Abstract

Background: Traditional letrozole or clomiphene treatment is started on cycle days 3 to 5, following a spontaneous menses or progestin-induced bleeding. If ovulation does not occur, the dose is increased following a progestin-induced bleeding. An alternative stair step protocol has been described, and if ovulation does not occur in this protocol, a higher dose is used without inducing withdrawal bleeding. There is no randomized trial comparing the traditional and stair-step letrozole protocols yet. **Aim:** To compare the efficacy of traditional and stair-step protocols for ovulation induction using letrozole in women with polycystic ovary syndrome (PCOS). **Methods:** A total of 200 eligible women were given 5 mg/day letrozole, and those 80 who did not respond were randomized in a 1:1 ratio to stair-step or traditional letrozole therapy for up to 10 mg/day. For the traditional protocol, higher doses of letrozole were used in each new cycle if no ovulation occurred. For the stair-step protocol, higher doses of letrozole were given 7 days after the last dose if no dominant follicles were seen on ultrasonography. The PCOS was defined according to modified Rotterdam criteria. Participants were 18 to 35 years of age, had a body mass index of <40 kg/m^2, had at least one patent fallopian tube, had a normal uterine cavity, and had normal spermiogram results. **Results:** The median follicle development time was significantly longer in the traditional protocol group than in the stair-step protocol group (41 days, 95% CI (40–42) vs 25 days, 95% CI (25–26); log rank 64; $P < 0.001$). Between traditional and stair step groups, the cumulative ovulation ratio (37/40 [%93] vs 36/40 [%90]; $P = 1$), endometrial thickness (9 [5–13] vs. 9 [5–11]; $P = 0.005$), mature follicule development (36/40 [%90] vs 36/40 [%90]; $P = 0.549$), pregnancy rates (3/40 [%8] vs 2/40 [%5]; $P = 1$), and antiestrogenic side effects were similar. **Conclusions:** The stair-step protocol shortens the treatment time without causing any detrimental effects on ovulation outcome.

Keywords: *Aromatase inhibitors, letrozole, ovulation induction, polycystic ovary syndrome, pregnancy rate*

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Introduction

Letrozole is suggested as first-line therapy over clomiphene citrate for oligo-ovulatory women with polycystic ovary syndrome (PCOS) undergoing ovulation induction. It results in higher live birth rates compared with clomiphene therapy in this group of women.[1]

Traditional letrozole or clomiphene treatment is started on cycle days 3 to 5, following a spontaneous menses or progestin-induced bleeding. If the cycle is ovulatory, but pregnancy has not occurred, the same dose is used in the next cycle. If ovulation does not occur, the dose increased following a progestin-induced bleeding. An alternative

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stair step protocol has been described for clomiphene.[2] In this protocol, if ovulation does not occur, a higher clomiphene is used without inducing withdrawal bleeding. The advantage of the stair-step protocol is the lack of a waiting period until the next menstruation. In a few retrospective and randomized controlled trials, it has been shown that the clomiphene stair-step protocol reduces the ovulation induction time with similar ovulation and pregnancy rates in patients with PCOS compared to the traditional protocol.[2-5] Recently, in a few retrospective studies, it was reported that both the stair-step letrozole and clomiphene protocols showed similar efficacy in ovulation induction.[6,7] However, there is no randomized trial comparing the traditional and stair-step letrozole protocols yet.

We designed a randomized trial to evaluate the cumulative ovulation rates of traditional and stair-step protocols of letrozole usage in ovulation induction.

Materials and Methods

This prospective randomized controlled study was conducted from February 2020 to June 2021 at the Infertility and Assisted Reproductive Techniques Center of Atatürk University Department of Obstetrics and Gynecology. The study was approved by the Clinical Research Ethics Committee of Ataturk University (date: 27.02.2020 and B.30.2.ATA.0.01.00/113). Patients were included in the study after approval by the Clinical Research Ethics Committee.

Eligibility criteria

The inclusion criteria were as follows: an age of 18–35, a body mass index (BMI) lower than 40, diagnosis with PCOS according to the revised Rotterdam criteria (with at least two of the following criteria: oligo-anovulation, clinical and/or biochemical signs of hyperandrogenism, and presence of a polycystic ovary image on ultrasonography), normal hysterosalpingogram, and the partner has normal spermiogram results (normal number and motility of sperms according to the World Health Organization 1999 criteria and normal morphology according to the Kruger strict criteria). To exclude the male factor, the patients' partners were required to have normal results from two spermiogram tests performed at least 15 days apart. The exclusion criteria were infertility due to causes other than anovulatory PCOS, having undergone ovarian or adnexal surgery, having any chronic or endocrine disease, having an infertile partner, and having tubal pathology.

Study design

All women who met the study eligibility criteria were invited to participate in the study, and those who gave written informed consent were recruited consecutively. First, all women received 5 mg/day letrozole divided into two doses. Then, those who did not have follicle development after induction with letrozole 5 mg/day were randomized into one of the groups below.

Traditional treatment protocol group: The next menstrual cycle was waited for dose increase. With each new cycle, the dose of letrozole was increased by 2.5 mg/day, up to a maximum of 10 mg/day.

Stair-step treatment protocol group: An increased dose was started immediately after nonresponse to the previous dose was found via ultrasound, without waiting for the next menstrual cycle for dose increase. Letrozole was increased to 2.5 mg/day, up to 10 mg/day, until mature follicle development occurred.

Randomization

Randomization was performed by a doctor who did not participate in the treatment of the patients. A randomization list was created by means of a computer program in blocks of 20 subjects. A letter identifying the treatment group allocation was prepared and placed in opaque envelopes. The envelopes were sealed and numbered according to the randomization list. During the randomization phase, each patient was assigned the next study number from the randomization list, and the group was determined by opening the envelope with this number.

Outcome measure

The primary outcome variable of the study was induction to follicle development time. The time was calculated from the first day of menstruation in the first cycle that induced with 5 mg letrozole to follicle development. Secondary outcome measures were as follows: Age at the beginning of ovulation induction, BMI, menstruation pattern, duration of infertility (years), FSH and LH values on the second day of the menstrual cycle, estradiol measurements, endometrial thickness, endometrial thickness on the day of follicle development, mature follicle count, mature follicle diameter (mm), pregnancy rate, and level of antiestrogenic effects due to the drug.

Sample size

We could not find a similar study in the literature to estimate the sample size. To calculate the sample size, a retrospective scan was performed on patients who meet study eligibility criteria and underwent ovulation induction with letrozole in the infertility clinic of the hospital. Thirty consecutive women in each group who underwent ovulation induction with traditional or stair-stepped letrozole and who met the study criteria were selected retrospectively. We compared two

groups by survival analysis according to the Kaplan–Meier method. The hazard ratio was calculated as 0.4. Accordingly, when this hazard ratio was accepted, it was calculated that there should be 38 patients in each group at 80% power for alpha 0.05. Considering the possible losses, 40 patients were included in each group.

Treatment protocol and patient follow-up

All patients were examined on the second or third day of their menstrual cycle via transvaginal ultrasound (Toshiba Applio 500, Toshiba Medical Systems Corporation, Japan), through hormone profiles, and those with a possible pregnancy status were excluded. The patients with follicles over 10 mm in size detected via USG on the second day of their menstrual cycle were not taken into that cycle treatment.

All 200 women were given 5 mg letrozole for 5 days, beginning third day of menses. A transvaginal ultrasound was performed 7 days after the last day of letrozole, and if a follicle >10 mm was detected, it was considered as a follicle development. Then all nonresponders randomized either traditional or stair-step protocol groups.

In the traditional protocol group, women were given letrozole 7.5 mg/day divided into three equal doses for 5 days after spontaneous menstruation or progesterone-induced withdrawal bleeding. Seven days after the last day of drug administration, the patients underwent ultrasound, and if a follicle development was not found, they were given letrozole 10 mg/day divided into four equal doses for 5 days after spontaneous menstruation or progesterone-induced withdrawal bleeding. The patients who did not respond to the maximum dose of 10 mg/day were regarded as letrozole-resistant.

In the stair-step protocol group, if a growing follicle was found on the seventh day after the last day of administration of letrozole 7.5 mg/day (divided into three equal doses), the dose was determined to be sufficient, and follicle follow-up was performed without increasing the dose. However, if the follicle size did not exceed the threshold value (>10 mm) or if there was no growing follicle on the 7th day, the dose of letrozole was immediately increased to 10 mg/day (divided into four equal doses) for 5 days. If there was still no response, the patients were considered letrozole-resistant. All letrozole-resistant women in both groups were offered another treatment option.

In both groups, hCG was used as an ovulation trigger for synchronization in patients with a follicle diameter of 18–22 mm. The patients in the two groups were given 6500 IU of rhCG to trigger ovulation, and timed sexual intercourse was recommended for follow-up. The patients were also asked about the antiestrogenic effects side effects of letrozole. If there was no menstrual bleeding 15 days after hCG administration, an hCG in the blood was measured and pregnancy was diagnosed if its level was above 10 mIU/mL.

Statistical analysis

The statistical analysis was performed according to the intention-to-treat principle. The Kolmogorov–Smirnov test was used to test the normal distribution. Continuous variables were compared using the Mann–Whitney U test, and categorical variables were compared using the Pearson Chi-square test, Fisher's exact test, or Kolmogorov–Smirnov test. All the tests were performed in two directions, and statistical significance was set at $P < 0.05$. The IBM SPSS Statistics 20 (IBM Corp.) program was used for all the statistical analyses.

Results

A total of 244 infertile women were screened during the study period, and 200 of them who met the study criteria

Table 1: Demographic characteristics of a total of 200 women per cycle. Data are given as median (minimum–maximum), number (percentage) where is appropriate

	Variable
Age	25 (18-40)
Body mass index	27 (19-38)
Duration of infertility (years)	2 (1-12)
FSH	7 (2-21)
LH	6,86 (1-27)
Estradiol	32 (9-207)
Endometrium[1] (mm)	5 (3-9)
Menstruation	
Oligomenorrhea	% 77 (154)
Amenorrhea	% 8 (16)
Hypermenorrhea	% 2 (4)
Regular	% 13 (26)

[1]Thickness of the endometrium on the second day of the cycle

Table 2: Demographic data and induction results per cycle for letrozole 5 mg. Data are given as a median (minimum–maximum)

	Follicle Development		*P*
	-	+	
Age	25 (19-38)	18 (26-40)	0,27
BMI	26 (19-38)	21 (29-37)	0,00
Duration of infertility	2 (1-12)	2 (1-7)	0,23
FSH	7,16 (2-12,9)	7 (2-21)	0,224
LH	6,6 (1,64-25,6)	7 (1-27)	0,127
E2	9,02 (31-207)	33 (14-71)	0,282
Endometrium[1] (mm)	5 (1-8)	5 (1-9)	0,034

[1]Thickness of the endometrium on the second day of the cycle

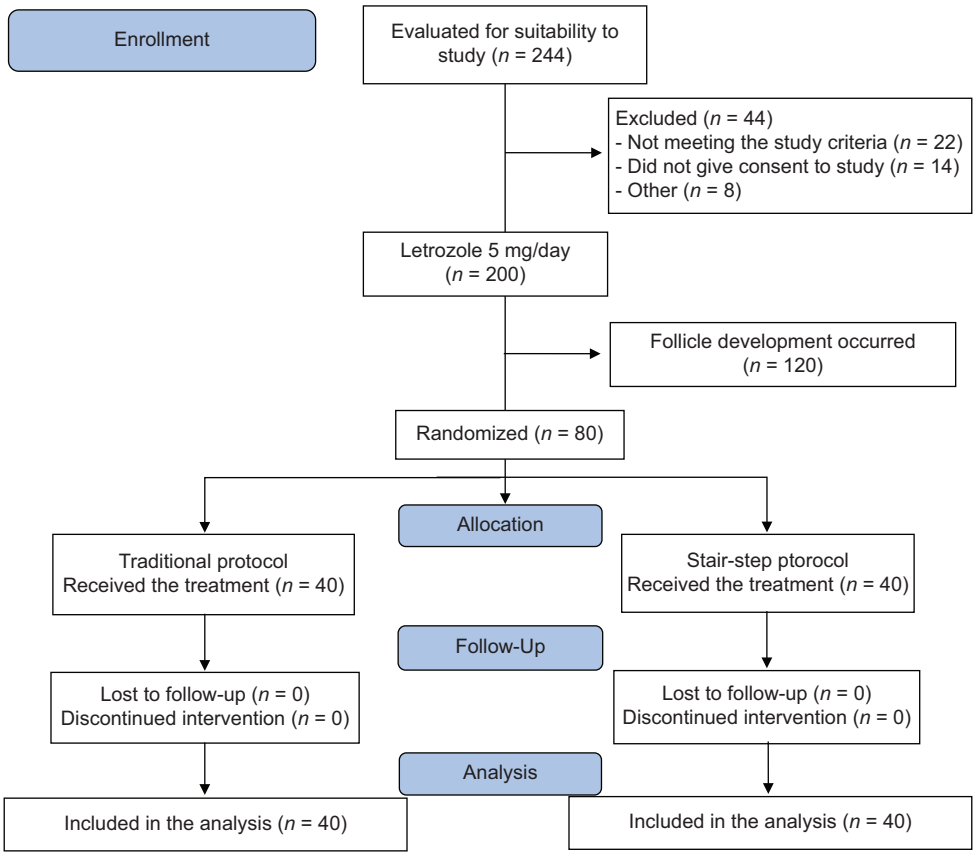

Figure 1: Consort 2010 flow diagram

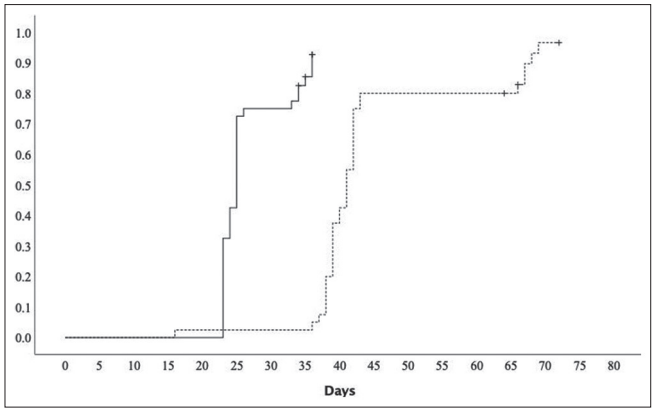

Figure 2: Percentage of patients without follicle development between groups. Straight line stair-step method, dotted line traditional protocol, + cases without follicle development (Log Rank 64, $P < 0.001$)

were given letrozole 5 mg/day [Figure 1]. The demographic characteristics of the 200 women are shown in Table 1.

A total of 120 (60%) women in our study responded to letrozole 5 mg/day [Table 2]. There were no significant differences in baseline variables among responders and nonresponders except BMI. The BMI was significantly higher in nonresponders. Seven (6%) women who received letrozole 5 mg/day became pregnant, and all pregnancies ended with term delivery.

The 80 (40%) women who did not respond to letrozole 5 mg/day were randomized to either the traditional or stair-step protocol group [Figure 1]. The baseline variables were similar among the study groups [Table 3]. The median follicle development time was statistically significantly longer in the traditional protocol group than in the stair-step protocol group (41 days, 95% CI (40–42); 25 days, 95% CI (25–26); log rank 64; $P < 0.001$) [Figure 2].

The induction results per treatment cycle are shown in Table 4. There were no significant differences in endometrial thickness, follicle development rate, number of mature follicles, largest mature follicle diameter, or pregnancy rate between the groups after the administration of letrozole 5 mg/day. The response rate to induction with 7.5 and 10 mg letrozole was similar in both groups. At the end of the study, despite receiving letrozole 10 mg/day, 3 (8%) patients in the traditional protocol group and 4 (10%) patients in the stair-step protocol group did not develop follicles and were thus considered letrozole-resistant.

Pregnancy occurred in five women (7.3%). There was no statistical difference in pregnancy rate between the groups. All pregnancies ended with term delivery.

Table 3: Demographic characteristics of randomized women per cycle. Data are given as median (minimum–maximum), number (percentage) where appropriate

	Traditional protocol (*n*=40)	Stair step protocol (*n*=40)	*P*
Age	20 (20-33)	26 (18-40)	0.281[1]
Body mass index	28 (21-37)	30 (22-37)	0.096[1]
Duration of infertility (years)	2 (1-7)	2 (1-6)	0.580[1]
FSH	7 (4-13)	7 (2-21)	0.616[1]
LH	7 (1-24,5)	7,5 (3-27)	0.783[1]
Estradiol	34 (19-71)	31,5 (14-71)	0.166[1]
Endometrium[2]	5 (3-9)	5 (3-9)	0.166[1]
Menstruation			0.4[3]
Oligomenorrhea	27 (27)	35 (35)	
Amenorrhea	7 (7)	4 (4)	
Hypermenorrhea	0 (0)	0 (0)	
Regular	6 (6)	1 (1)	

[1]Mann-Whitney-*U*, [2]Thickness of the endometrium on the second day of the cycle, [3]Kolmogorov-Smirnov

Table 4: Results of induction per cycle after randomization. Data are given as median (minimum–maximum), number (percentage), number/total number (percentage) where appropriate

	Traditional protocol	Stair-step protocol	*P*
Endometrium1	9 (5-13)	9 (5-11)	0.005[2]
Number of mature follicles	1 (0-3)	1 (0-2)	0.330[2]
Mature follicle diameter (mm)	19 (16-26)	20 (17-23)	0.110[2]
Number of mature follicles			0.549[3]
0	3 (7,5)	4 (10)	
1	20 (50)	21 (52.5)	
2	16 (40)	15 (37.5)	
3	1 (3)	0	
Response to letrozole			1[3]
7.5 mg	33 (83)	31 (77.5)	
10 mg	4 (10)	5 (12.5)	
Resistant	3 (8)	4 (10)	
Pregnancy	3 (8)	2 (5)	1[4]

[1]Endometrium thickness when folliculometry performed, [2]Mann-Whitney-*U* test, [3]Kolmogorov-Smirnov, [4]Fisher-Exact Test

Table 5: Letrozole-related side effects per cycle. Data are given as number (percentage)

Side effects	5 mg letrozole (*n*=200)	Randomized group		*P*
		Traditional (*n*=40)	Stair step (*n*=40)	
Flushing on the face	3 (4)	1 (3)	2 (5)	0.913[1]
Headache	15 (19)	6 (15)	6 (23)	
Bleeding	1 (1)	1 (3)	0	
Multiple	2 (3)	0	2 (5)	

[1]Kolmogorov-Smirnov

Side effects of letrozole are shown in Table 5. None of the patients developed ovarian hyperstimulation syndrome during induction. There was no significant difference in side effects between the groups.

Discussion

We found that the stair-step protocol has a shorter duration of treatment for ovulatory follicle development and a shorter time for determining letrozole resistance when compared to the traditional protocol. The two protocols had similar outcomes of ovulation and pregnancy.

We used 5 mg/day letrozole as the initial dose in the study. A starting dose of 2.5 mg is usually used empirically for induction of ovulation with letrozole. However, the optimal dose of letrozole for ovulation induction is not yet clear. It is reported that a daily dose of 5 mg/day produced more mature follicles.[8] Clinical experience also shows that the use of 2.5 mg/day as the initial letrozole dose has a low rate of ovulation induction. If we started with 2.5 mg/day letrozole, the difference in time to ovulation between groups would have been greater.

At our best knowledge, this is the first randomized trial comparing the stair-step letrozole protocol and traditional protocol. The study has a few strengths. One, it was a randomized study and both groups were similar in terms of demographic and clinical data. The other, there was no dropout in the study. Finally, the power of the study was sufficient to see the difference of a hazard ratio of 0.4 between the groups. The study has a potential weakness. A lifestyle intervention was considered the optimal first-line treatment for anovulation in women with PCOS and infertility.[9] As reported previously, we found that BMI had a significant impact on ovulation rates. BMI was the only difference between responder and nonresponder women to an initial dose of 5 mg letrozole. A lifestyle intervention was not required for study before enrolment but might be important on ovulation outcomes.

Induction results per cycles were similar between treatment groups. It is already known that letrozole results in monofollicular ovulation in most cases which reduce the risk of multiple pregnancies, has no direct antiestrogenic adverse effects on the endometrium, and is well tolerated due to an absence of estrogen receptor blockade and the relatively short half-lives of 2 days.[10,11] We found that the endometrial thickness and the number of mature follicles were similar in both stair-step and traditional treatment groups. Except for one woman in the traditional protocol who developed three follicles, the number of mature follicles was less than three in both treatment groups. Although we used a high letrozole dose up to 10 mg/day, the rates of

antiestrogenic side effects were similar in both treatment groups. Side effects also appeared similar between the 5 mg letrozole group and the treatment groups, although a direct statistical comparison has not been made. It seems there is no cumulative effect of letrozole in stair-step treatment due to the half life of the drug. In all these considerations of the secondary outcome variables of the study, it should be noted that the sample size of the study was not selected for these secondary outcome variables.

In the ovulation induction with letrozole in infertile patients with PCOS in our study, the time for mature follicle development in the patients who underwent the stair-step protocol was found to be shorter than that in the patients who underwent the traditional protocol. Thus, the stair-step protocol saved much time for the first-line treatment of the infertile patient group. Thus, when letrozole is used in ovulation induction therapy, the stair-step protocol can be applied to patients who do not respond to the initial dose of the drug instead of making them wait for their next menstrual cycle for dose increase, to avoid losing time.

In conclusion, ovulation induction with the stair-step letrozole protocol has a significantly shorter overall treatment period when compared to the traditional protocol without any adverse effect on the ovulation and the pregnancy rates and without any increase in adverse side effects in patients with PCOS.

Authors' contributions

SK and YK conceptualized and designed the study. RA, GC and PY were involved in data collection/acquisition and statistical analysis; All authors (Sevda Karakaya, Yakup Kumtepe, Ragıp Atakan Al, Gamze Nur Cimilli Senocak and Pinar. Topdagi Yilmaz) were involved in the writing and revising the manuscript for intellectual content. All authors read, and approved the final manuscript and agreed to be accountable for all aspects of the work.

Ethical approval

The study was approved by the Clinical Research Ethics Committee of Ataturk University (date: 27.02.2020 and B.30.2.ATA.0.01.00/113). Patients were included in the study after approval by Clinical Research Ethics Committee.

Informed consent

A written informed consent was obtained from each participant before enrollment into the study.

Helsinki Declaration

The study was conducted according to the principles of Helsinki Declaration.

Availability of research data

Authors are available and ready to supply the data upon any requests through the corresponding author.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Reeferences

1. Franik S, Eltrop SM, Kremer JA, Kiesel L, Farquhar C. Aromatase inhibitors (letrozole) for subfertile women with polycystic ovary syndrome. Cochrane Database Syst Rev 2018;5:CD010287.
2. Hurst BS, Hickman JM, Matthews ML, Usadi RS, Marshburn PB. Novel clomiphene "stair-step" protocol reduces time to ovulation in women with polycystic ovarian syndrome. Am J Obstet Gynecol 2009;200:510 e1-4.
3. Deveci CD, Demir B, Sengul O, Dilbaz B, Goktolga U. Clomiphene citrate 'stair-step' protocol vs. traditional protocol in patients with polycystic ovary syndrome: A randomized controlled trial. Arch Gynecol Obstet 2015;291:179-84.
4. Agrawal K, Gainder S, Dhaliwal LK, Suri V. Ovulation induction using clomiphene citrate using stair-step regimen versus traditional regimen in polycystic ovary syndrome women-A randomized control trial. J Hum Reprod Sci 2017;10:261-4.
5. Jones T, Ho JR, Gualtieri M, Bruno-Gaston J, Chung K, Paulson RJ, *et al.* Clomiphene stair-step protocol for women with polycystic ovary syndrome. Obstet Gynecol 2018;131:91-5.
6. Sakar MN, Oğlak SC. Comparison of the efficacy of letrozole stair-step protocol with clomiphene citrate stair-step protocol in the management of clomiphene citrate-resistant polycystic ovary syndrome patients. J Obstet Gynaecol Re 2021;47:3875-82.
7. Thomas S, Woo I, Ho J, Jones T, Paulson R, Chung K, *et al.* Ovulation rates in a stair-step protocol with Letrozole vs clomiphene citrate in patients with polycystic ovarian syndrome. Contracept Reprod Med 2019;4:20.
8. Biljan MM, Tan SL, Tulandi T. Prospective randomized trial comparing the effects of 2.5 and 5.0 mg of letrozole (LE) on follicular development, endometrial thickness and pregnancy rate in patients undergoing super-ovulation. Fertil Steril. 2002; 78:S55.
9. Al Wattar BH, Fisher M, Bevington L, Talaulikar V, Davies M, Conway G, *et al.* Clinical practice guidelines on the diagnosis and management of polycystic Ovary syndrome: A systematic Review and quality assessment study. J Clin Endocrinol Metab 2021;106:2436-46.
10. Legro RS, Brzyski RG, Diamond MP, Coutifaris C, Schlaff WD, Casson P, *et al.* Letrozole versus clomiphene for infertility in the polycystic ovary syndrome. N Engl J Med 2014;371:119-29.
11. Casper RF, Mitwally MF. Review: aromatase inhibitors for ovulation induction. J Clin Endocrinol Metab 2006;91:760-71.