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Letrozole step up versus fixed dose protocols in ovulation induction in patients with unexplained infertility

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Abstract

Unexplained infertility (UI) represents 25% of cases of infertility. The main treatment of UI is ovulation induction with or without intrauterine insemination (IUI). To compare between the step-up and conventional protocols in ovulation induction (OI) and subsequent pregnancy rate (PR) and live birth rate in women with UI. A prospective randomized controlled study 180 patients diagnosed with UI were enrolled in the study and randomized in 2 groups 1st group stimulated with letrozole step up protocol, the 2nd group with conventional fixed dose protocol for 1 to 3 cycles. There is statistically significant difference between the two groups regarding ovulation rate. As ovulation rate was higher in group A (Step up protocol) = 95.3% (221 of 232 cycles) than group B (Fixed dose) =89.1% (212 of 238 cycles), with (P = 0.013). Also, as well, there was statistically significant difference between the two groups regarding Number of follicles ≥18 mm with (P ≤0.001). There was no evidence of statistically significant difference between the two groups regarding endometrial thickness, trilaminar endometrial pattern or the spiral artery PI (Pulsatile index). Spiral artery RI (Resistance Index) showed statistically significant difference between groups. Regarding Pregnancy rate there was no evidence of statistically significant difference between the two groups regarding cumulative pregnancy rate, miscarriage or complications. Letrozole step up protocol was associated with multi-follicular development and better endometrial receptivity in the form of lower endometrial spiral artery RI with no significant increase in complications as OHSS (ovarian hyperstimulation syndrome), multifetal pregnancy or dug complications.

Keywords: Unexplained infertility, Infertility, Letrozole, Step-up protocol, Induction of ovulation.

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1. Introduction

Despite having frequent, unprotected sexual encounters for at least twelve months, a woman with infertility is nevertheless unable to have a biological child. This medical disorder impacts the reproductive system. But currently, the American Society for Reproductive Medicine recommends that women over the age of 35 have a fertility test if they still can't conceive after six months of trying [1]. Testing for tubal patency, ovulation, and semen analysis is commonplace in standard fertility evaluations. The disheartening diagnosis of "unexplained infertility" is handed down to around 10% of infertile couples when these studies fail to uncover a particular reason [2]. When an identifiable anomaly cannot be found, the treatment for unexplained infertility is mostly dependent on clinical observation and practical expertise. Ovarian stimulation (OS), IUI, expectant management, OS plus IUI, and assisted reproductive technology (ART) are all viable alternatives for treating this illness [1]. In order to assess different therapy options, Wang et al., (2019) conducted many trials. There is insufficient data to support the claim that expectant management has a lower live birth rate compared to the other treatments, including OS, IUI, ovarian stimulation with intrauterine insemination (OS-IUI), and ART [3]. Based on its less invasive nature and lower price tag, Oduola et al., (2019) found that OS-therapy might be a good alternative to IVF as a shorter treatment option [4]. One way to stimulate ovulation is through the oral administration of letrozole, a third-generation aromatase inhibitor. Comparing it to clomiphene citrate, it offers certain advantages [5]. During cycles 3–7, the recommended dose of letrozole is 2.5 mg daily. According to Legro et al., (2014), the daily dose shouldn't go beyond 7.5 mg [6].

The security of letrozole was investigated in a recent retrospective cohort study by Tatsumi et al., (2017) [7]. The

risk of miscarriage was shown to be lower in women who used letrozole as a stimulant. No indication of an elevated risk of serious congenital abnormalities, unfavorable pregnancy outcomes, or severe neonatal outcomes was also found. Many studies have covered the Letrozole step-up procedure in detail. It seems that this technique may produce multi-follicular growth without the requirement for extra gonadotropins, according to Kaur's (2019) observations [8]. Compared to injectable treatments, there are fewer side effects and cheaper costs, and the rates of multi-follicular formation, average endometrial thickness, and clinical pregnancy rates are similar. Therefore, the Letrozole step-up regimen makes injectable medication unnecessary, according to Kaur et al., (2019) [8]. Additional research is necessary to determine whether or not letrozole is effective in inducing ovaries in people with unexplained infertility and, if so, how. For women experiencing infertility for no apparent reason, this research set out to compare the success rates of conventional and step-up protocols in triggering ovulation and achieving the following outcomes: pregnancy and live birth rates.

2. Patients and methods

An experiment was conducted at the outpatient clinic of the Obstetrics and Gynecology department at Beni-Suef University hospital. The research received approval from the medical ethics council and involved a group of 180 Egyptian patients who had been diagnosed with unexplained infertility. The research was conducted from May 2020 to May 2022. The sample size was calculated using G*Power Version 3.1.9.2, a computer program developed in Kiel, Germany. A power analysis was performed using G-POWER to determine the appropriate sample size for a chi-square test. The study was conducted using an alpha error probability of 0.05, a power of 0.95, a medium effect size (w = 0.3), and 1 degree of freedom. Based on the previously stated assumptions, a sample size of 145 is necessary. To ensure statistical significance, a minimum total sample size of 180 patients (90 patients in each group) is necessary, taking into account a dropout rate of 25%.

2.1. Inclusion Criteria

- Age: 20 -35 years old.
- No conception for at least one year.
- Diagnosis of unexplained infertility is established.

2.2. Exclusion Criteria

- Age < 20 and > 35 years old.
- Ovarian factor of infertility as PCOs.
- Tubal factor of infertility.
- Male factor of infertility.
- Uterine pathology e.g. fibroid or ovarian cyst.
- Hyperprolactinemia, hypo or hyperthyroidism.
- Impaired hepatic or renal function.
- History of hypersensitivity to study drugs.

2.3. Methods

Patients attending at infertility clinic were subjected to: Full history: regarding demographic, gravidity, parity, menstrual history, past history, period and type of infertility, drug hypersensitivity. Full examination was done, generally, abdominal and local pelvic examination. Every Patient and her husband were investigated to exclude causes of infertility: Semen analysis to exclude Male Factor, Basal hormonal profile D2 FSH, LH, E2, and midluteal progesterone to exclude ovarian factor of infertility, Hysterosalpingography postmenstrual to exclude tubal factor of infertility, pelvic ultrasound to exclude uterine pathology, Serum prolactin, thyroid profile. Then patients who were diagnosed with unexplained infertility were enrolled in the study and randomly divided into two study groups:

- **Group 1 (Step Up dose protocol group):** ovulation induction by oral letrozole was given as step-up protocol starting by 2.5 mg on D2-D5 of menstruation then increasing doses 5mg, 7.5mg, 10mg on the 3 successive days of menstruation respectively.
- **Group 2 (Fixed dose protocol group):** ovulation induction by oral Letrozole was given as fixed dose 5 mg starting from D2-D5 of menstruation and for 5 days.

Follow up of each group on D10 menstruation by transvaginal ultrasound to assess follicular size, number of growing follicles and endometrial thickness and layering, if follicular size still beyond 18mm, then TVS was repeated every 2 days till it reaches 18 mm or more, then triggering of ovulation by HCG 5,000 IU was given. The patients were told to have timed sexual intercourse 36 h after trigger injection. Midluteal spiral artery Doppler was done and RI, PI were recorded Ultrasound examination was performed by the same operator using a 2D ultrasound system equipped with a 12 MHz transvaginal transducer. Patients were followed up for cumulative pregnancy rates and live birth rates. Each patient in each group was followed by the same protocol for 1-3 months.

2.4. Outcomes

- Primary outcomes were: Cumulative pregnancy rate, abortion rate, live birth rate.
- Secondary outcomes were: Follicular size, number of growing follicles, endometrial thickness, endometrial layering, endometrial spiral artery Doppler RI, PI, complications (OHSS, Multifetal pregnancy, Headache and Bleeding).

2.5. Statistical Analysis

Data was described statistically by calculating the mean ± standard deviation (± SD), counting the number of occurrences, and, when relevant, calculating relative frequencies (percentages). When deemed appropriate, the following statistical tests were employed: Fisher's Exact Test, Chi-square, Mann-Whitney U, and Kolmogorov-Smirnov. We choose the probability value (P value < 0.05) as the threshold for statistical significance. A version 22 of the Statistics Package for the Social Sciences (SPSS Inc.) was used to do the statistical analyses.

3. Results

Table 1 shows that there was no statistically significant difference between the 2 groups regarding baseline characteristics. In Table 2 the basal hormonal profile detected no evidence of statistically significant difference between the two groups. In Table 3 there is statistically significant

difference between the two groups regarding ovulation rate and number of follicles ≥18 mm on day of HCG with (P ≤0.001). In table 4 there was no evidence of statistically significant difference between the two groups regarding neither the endometrial thickness nor the spiral artery PI. Although, spiral artery RI showed statistically significant difference between the two groups. In table 5 regarding Pregnancy on 1st, 2nd, 3rd month cumulative pregnancy rate, and occurrence of complications showed no statistically significant differences between groups. Table 6 shows highly significant correlation between pregnancy rate and the measured parameters (age, BMI, Basal FSH, AMH and number of follicles) with negative relation as regard age and BMI while positive relation regarding FSH and AMH and number of follicles. In figure 1, the median cumulative probability of pregnancy (Kaplan–Meier life table analysis) following Step up protocol was achieved at 2 months, and also was same for fixed protocol group. Overall comparison between groups shows no significant difference with P- value =0.74 (Log Rank (Mantel-Cox)).

4. Discussion

UI represent 25% of cases of infertility, UI should be diagnosed after exclusion of all possible causes by proper History, Examination and performing main investigations to exclude male, ovulatory, tubal and uterine factors of infertility. Main treatment of UI is ovulation induction with or without IUI, multi-follicular ovulation is better than mono ovulation, so induction with gonadotropins is better than oral drugs, but still have a financial burden on the patient. So letrozole step up protocol is discussed now to see its effect to achieve multi-follicular development and pregnancy rate among cases of UI [1-2]. 180 patients diagnosed with UI were enrolled in the study and randomized in 2 groups , group 1 : ovulation induction by oral letrozole was given as step up protocol starting by 2.5 mg on D2 of menstruation then increasing doses 5mg, 7.5mg, 10mg on the D3, D4, D5 successive days of menstruation respectively. , group 2: ovulation induction by oral Letrozole was given as fixed dose 5 mg starting from D2-D6 of menstruation and for 5 days CPR, LBR , Abortion Rate , Number of follicles, Endometrial layering, Endometrial thickness , Spiral artery doppler PI, RI , OHSS , Multifetal pregnancy and drug complications were recorded and statistically analyzed. There is statistically significant difference between the two groups regarding ovulation rate. As ovulation rate was higher in Group A (Step up protocol) = 95.3% (221 of 232 cycles) than group B (Fixed dose) =89.1% (212 of 238 cycles), with (P = 0.013). Also, as well, there was statistically significant difference between the two groups regarding Number of follicles ≥18 mm on day of HCG; the mean in Group A (Step up protocol) was 2 ± 0.83 and 1.12 ± 0.33 in group B (Fixed dose protocol), with (P ≤0.001). There was no evidence of statistically significant difference between the two groups regarding the Endometrial thickness, trilaminar endometrial pattern or the spiral artery PI. Although, spiral artery RI showed statistically significant difference between the two groups; the mean in Group A (Step up protocol) was 0.56 ± 0.08 and 0.61 ± 0.08 in group B (Fixed dose), with (P = 0.001). Regarding Pregnancy rate: finally, there was no evidence of statistically significant difference between the two groups regarding cumulative pregnancy rate, which was higher in Group A (36.7%) than Group B (30%). Seven out of 33 women (21.2%) had miscarriage in group A (Step-up) and 3 out of 27 (11.1%) women of pregnant women group B experienced miscarriage, with P-value=0.296. which statistically; of no significant difference. There was no evidence of significant difference statistically regarding complications. Step up protocol was tested before in many studies:

Kaur et al., (2019) compared the letrozole step-up regimen to letrozole plus gonadotropins for controlled ovarian stimulation and intrauterine insemination in 2019 [8]. 60 individuals and couples with UI attended a tertiary infertility clinic in North India for the research. These individuals were randomly split into two groups. Group A began COS on day 2 or 3 of the menstrual cycle with a step-up letrozole regimen. Starting at 2.5 mg, the dosage was increased by 2.5 mg daily for 3 days (2.5, 5, 7.5, and 10 mg). Group B's COS used letrozole and hMG. Starting on the second or third day of menstruation, 2.5 mg of letrozole pills were taken twice a day for 5 days. Starting on day 7, hMG 150 IU was injected intramuscularly every other day and modified depending on response. HCG was given once the dominant follicle reached 17 mm, followed by IUI 36 hours later. The results of the operation are available. The mean number of 16 mm or bigger follicles was comparable in both groups: 1.74 (± 0.83) in group A and 1.94 (± 0.68) in group B (p = 0.178). Ovulation rates were 90.9% (40 out of 44) in group A and 100% (55 out of 55) in group B (p = 0.022). No substantial change was seen in patient clinical pregnancy rates. The rate was 10.7% (3/28) in group A and 16.67% (5/30) in group B (p = 0.707). The average pharmaceutical cost in group A was Rs. 345.00 (00), significantly cheaper than group B's Rs. 2148.64 (515.67) [p < 0.0001]. Group B experienced one hyperstimulation and many pregnancies, whereas Group A had none. This showed that the letrozole step-up regimen can achieve multi-follicular growth without gonadotropins. The two groups had equal multi-follicular growth, mean endometrial thickness, and clinical pregnancy, with fewer side effects and cheaper costs. Therefore, injectable treatment may be discontinued**.** Mitwally et al. (2008) studied a step-up technique for multiple ovarian follicle growth [9]. Ovarian stimulation and intrauterine insemination were performed 28 times on 22 infertile couples. In the trial group of nine patients and 11 cycles, letrozole was stepped up. On menstrual cycle days 2, 3, 4, and 5, take one, two, three, and four 2.5 mg letrozole pills daily. On menstrual cycle day 3, 13 patients (17 cycles) received 100 mg/day CC for 5 days in the control group. After the dominant follicle reached a diameter of 20 mm, hCG induced ovulation, and insemination followed 24 hours later. The count of prominent follicles (R15 mm) the day after hCG treatment was the key parameter. At 6–7 weeks, ultrasonography showed a positive fetal heart, confirming clinical pregnancy. Results: The step-up letrozole medication produced many follicles in the ovaries throughout 11 cycles, averaging 2.2 ± 1.5 mature follicles per cycle, equivalent to CC.

Letrozole step-up therapy had a higher clinical pregnancy rate per treatment cycle than CC (27.3% vs. 11.8%). The step-up procedure involves taking one 2.5 mg tablet on menstrual cycle day 2, two on day 3, three on day 4, and four on day 5. The step-up letrozole regimen produced numerous ovarian follicles, the research found. Letrozole step-up therapy had a higher clinical pregnancy rate per cycle than CC (27.3% vs. 11.8%). Multi-follicular development

was connected to the technique. The prolongation of estrogen inhibition may create this effect. In the early days after using an aromatase inhibitor, endogenous gonadotropins rise, causing granulosa cell development. Aromatase production and estrogen release increase. Aromatase inhibition must be enhanced to counterbalance the inhibitory impact of estrogen on endogenous FSH synthesis. Stepping up the aromatase inhibitor dose may accomplish this. The step-up letrozole regimen prolongs FSH levels, forming numerous follicles. This unique method may help with ovarian hyperstimulation in assisted reproduction. A prospective randomized controlled study by Galal (2015) compared Letrozole Step Up Plus IUI to Gonadotropin Plus IUI in unexplained infertility patients [10]. The research involved 100 IUI-undergoing, unexplained infertile couples. A computer number system randomly allocated couples to two groups of 50 women each. Study findings showed... Step-up letrozole led to reduce multi-follicular ovarian growth (average 1.5±0.7 follicles) compared to HMG (3.1±1.0 follicles). The clinical pregnancy rate was 16% in the letrozole group and 18% in the HMG group, showing no significant difference. The non-significant difference in endometrial thickness supports adequate oocyte quality and receptivity. Letrozole cycles cost much less than HMG. Thus, the letrozole step-up procedure may be a legitimate and novel induction protocol. This procedure has a comparable pregnancy rate as HMG ovulation induction but cheaper costs and better patient compliance. Vidya et al., (2020) studied 50 polycystic ovary syndrome-diagnosed infertile patients. OI and timed intercourse were used for 172 treatment cycles in these women [11]. The study comprised 25 women (80 menstrual cycles) who received one, two, three, or four 2.5 mg letrozole pills daily in a step-up method. The control group included 25 women (92 cycles) who received 5 mg of letrozole daily on days 2, 3, 4, 5, and 6. The step-up letrozole group had more mature follicles than the conventional group, and 88.7% (71 out of 80 cycles) of participants successfully induced ovulation, compared to 71.7% (66 out of 92 cycles). The difference was statistically significant ($p = 0.01$). The clinical pregnancy rate was 22.5% in the step-up letrozole group and 14.1% in the standard group. Though larger, the difference was not statistically significant. The step-up letrozole group had three multiple pregnancies, whereas the regular group had one twin pregnancy. OHSS was absent in both groups. In a prospective randomized controlled experiment, Elkhateeb and Mahran (2016) splitted 200 patients into two groups [12]. Letrozole was given to 100 individuals at 2.5 mg on cycle day 3. Starting at 2.5 mg daily, the dosage was raised to 10 mg on cycle day 6. The control group of 100 patients received 100 mg CC daily for 5 days, commencing on cycle day 3. Patients were observed for three treatment cycles. Clinical pregnancy rate was the key success metric, along with mature follicle count, endometrial thickness, blood progesterone levels, and dominant follicle development time. The two groups had similar numbers and times to grow fully mature follicles. The letrozole group had significantly higher endometrial thickness on HCG administration day compared to the CC group (10.1±0.22 mm vs. 8.2±0.69 mm, $p = 0.01$). Serum progesterone levels were considerably higher in the letrozole group compared to the CC group (19.3±3.1 vs. 15.3±2.2, $p<0.01$). Letrozole caused ovulation in 165 of 242 cycles (68.2%) and CC in 169 of 249 cycles (67.9%), with no statistically significant difference. The letrozole group had a significantly higher clinical pregnancy rate (14.8% versus 10.4%, $p<0.01$) than the CC group. Thus, increasing letrozole dosage increases the clinical pregnancy rate compared to CC. In PCOS women, it may be the initial treatment to stimulate ovulation. Our study includes 180 unexplained infertile people. Kaur et al., (2019) included 60 urinary incontinence patients [8]. Gala et al., (2015) evaluated 100 incontinent couples [10]. Mitwally et al., (2008) studied 22 infertile couples [9]. Vidya et al., (2020) examined 50 PCOS couples, whereas Elkhateeb and Mahran (2016) examined 100 [11-12]. Methodology: Our research group was randomly assigned to Group A and Group B. Group A included 90 participants. Women received oral letrozole in a step-up regimen, starting at 2.5 mg on the second day of menstruation and rising to 5 mg, 7.5 mg, and 10 mg on the third, fourth, and fifth days. Group B had 90 women who received conventional therapy. Starting on the second day of the menstrual cycle, the recommended amount of letrozole is 5 mg, given in two doses, for five days. In Kaur et al., (2019), participants were randomly allocated to two groups [8]. Group A had step-up letrozole- COS. The therapy started with 2.5 mg on day 2 or 3 of the menstrual cycle and increased by 2.5 mg each day for three days. Group B received letrozole and hMG for COS. Starting on the second or third day of menstruation, 2.5 mg of letrozole pills were taken twice a day for five days. Starting on day 7, 150 IU of hMG was injected intramuscularly every other day and adjusted depending on response. After the dominant follicle reached 17 millimeters, HCG was given, and IUI was done 36 hours later. Galal (2015) randomly split the group into two [10]. Group B will not get Letrozole step-up therapy with IUI, but Group A will. A patient is receiving gonadotropins and IUI. Vidya et al., (2020) randomly allocated 25 PCOS patients to the Step-up Protocol for Ovulation Induction or the Conventional Dosage of Letrozole [11]. Mitwally et al., (2008) gave nine patients (11 cycles) letrozole in a step-up strategy. On menstrual cycle days 2, 3, 4, and 5, take one, two, three, and four 2.5 mg letrozole pills daily [9]. Elkhateeb and Mahran (2016) separated 200 patients into two groups. The trial group included 100 individuals who received letrozole in escalating dosages [12]. The medication began at 2.5 mg on cycle day 3 and escalated by 2.5 mg daily to 10 mg on cycle day 6. The control group of 100 patients got 100 mg of CC daily for 5 days, commencing on cycle day 3. Our study confirmed the results of prior Letrozole step-up studies in UI or PCOS patients regarding follicle count during the hCG trigger. Mitwally et al., (2008) found a mean of 2.2 with a standard deviation of 1.5, ranging from 1 to 6 [9]. Galal (2015) reported a mean of 1.5 and a standard deviation of 0.7 [10]. Kaur et al., (2019) reported a 1.74 mean and 0.83 standard deviation [8].

Elkhateeb and Mahran (2016) discovered 3.4 with a 0.5 standard deviation [12]. Last, Vidya et al., (2020) found a mean of 2.5 and a standard deviation of 0.9 [11]. Our trigger-time endometrial thickness findings were comparable to earlier investigations. Mitwally et al., (2008) reported a mean thickness of 9.8 ± 2.1, Galal (2015) 9.5 ± 0.7, Kaur et al., (2019) 8.18 (1.80), Elkhateeb and Mahran (2016) 10.1 ± 0.22, and Vidya et al., (2020) 9.1 ± 0.22 [8-12]. We detected a statistically significant difference in ovulation rates across groups. Group A (step-up protocol) had a statistically significant higher ovulation rate (95.3%) (221 out of 232 cycles) than Group B (fixed dose) (89.1%) (212 out of 238

cycles). Kaur et al., (2019) reported that group A had an ovulation rate of 90.9% (40 out of 44) and group B had an ovulation rate of 100% (55 out of 55) [8]. A p-value of 0.022 indicated a significant difference between groups. Vidya et al., (2020) observed that the step-up letrozole group had 88.7% ovulation (71 out of 80 cycles) compared to 71.7% (66 out of 92) in the standard group [11]. The difference was statistically significant (p = 0.01). Elkhateeb and Mahran (2016) produced ovulation in 165 of 242 cycles (68.2%) with letrozole and 169 of 249 cycles (67.9%) with CC. The difference in ovulation rates between groups was not statistically significant [12].
We discovered 36.7% CPR for the step-up therapy and 30% for the standard procedure. The difference between regimens was not statistically significant (P = 0.343). According to Vidya et al., (2020), our step-up and conventional techniques had 22.5% and 14% success rates, respectively [11]. The difference in success rates between the two methods was not statistically significant (p = 0.15).
As for problems, Vidya et al., (2020) and Kaur et al., (2019) found no statistical significance in OHSS, multifetal pregnancy, headache, or bleeding [8,11].

5. Strengths of the study

1. Sample size was accurately calculated.
2. Each patient was assessed in a period up to 3 cycles.
3. Using specific protocol in patient selection and in induction protocols.
4. Endometrial receptivity was assessed by Spiral artery Doppler that was not assessed in the studies done before on the step-up protocol.
5. Patients were traced to calculate the Live birth rate.

6. Limitations of the study

- Further comparison of letrozole with Gonadotropins would be helpful to assess if letrozole step up multi-follicular ovulation would be helpful in decreasing the financial burden in patients with UI undergoing ovulation induction.
- Patient's dropouts after 1 or 2 failed cycles.
- The induction was not combined with IUI which is recommended in NICE guidelines about patients with UI [13].

7. Clinical implications of the study

Cost is still a challenge in treatment of patients with infertility specially those using Gonadotropins, So Letrozole step up could be used as a substitute to gonadotropins to achieve multi-follicular ovulation. OHSS and other complications are low compared to Gonadotropins, So, it is safer.

Table 1: Baseline characteristics of both groups.

	Step Up dose protocol group Mean (SD) or Frequency (%)	Fixed dose protocol group Mean (SD) or Frequency (%)	P-value
Age **	25.68(3.35)	26.21 (3.54)	0.397
BMI **	26.07 (2.57)	25.97 (2.41)	0.624
Gravidity**	1.16(1.34) IQR=2	1.38 (1.39) IQR=3	0.304
Parity **	0.86(1.01) IQR=2	0.96(1.02) IQR=2	0.464
Period of infertility (year)**	2.21 (0.46)	2.26 (0.49)	0.477
Type of infertility ***	• Primary 45(50%) • Secondary 45 (50%)	• Primary 40(44.4%) • Secondary 50(55.6%)	0.455

* P value: Probability value: non-significant, ** Independent-samples Mann-Whitney U test, *** Chi square (χ^2) test.

Table 2: Comparison between group A (Step up protocol) and group B (Fixed dose protocol) according to investigations.

Hormonal Profile	**Step Up dose Mean (SD)**	**Fixed dose Mean (SD)**	**P-value**
Basal FSH **	7.09(1.69)	7.55(1.77)	0.122
Basal LH **	5.23(1.56)	5.63(2.05)	0.062
AMH**	1.57(0.68)	1.73(0.7)	0.113

* P value: Probability value: non-significant, **Independent-samples with Mann-Whitney U test.

Table 3: Comparison between group A (Step up protocol) and group B (Fixed dose protocol) according to ovulation.

	Step Up dose	Fixed dose	P-value
No. of cycles (No)	232	238	
Ovulation rate (%) ***	95.3% (n=221)	89.1%(n=212)	0.013
No. of follicles ≥18 mm on day of HCG Mean ±(SD) **	2 ± 0.83	1.12 ± 0.33	≤0.001

* P value: Probability value: non-significant, ** Independent-samples Mann-Whitney U test, *** Chi square (χ^2) test.

Table 4: Comparison between group A (Step up protocol) and group B (Fixed dose protocol) according to Endometrium.

	Step Up dose	Fixed dose	P-value
ET on day of hCG (mm) Mean ±(SD) **	9.9 ± 1.54	9.8 ± 1.52	0.677
Tri-laminar Endometrial Pattern (%) ***	84 (93.3%)	85 (94.4%)	0.756
Spiral artery PI **	1.1 (0.06)	1.1(0.08)	0.069
Spiral artery RI **	0.56(0.08)	0.61 (0.08)	0.001

* P value: 0.001: significant, ** Independent-samples Mann-Whitney U test, *** Chi square (χ^2) test.

Table 5: Comparison between group A (Step up protocol) and group B (Fixed dose protocol) regarding study outcomes.

	Step Up dose	**Fixed dose**	**P-value**
Pregnancy Rate (1^{st} month) Frequency (%)	13/90 (14.4%)	9/90 (10%)	0.363
Pregnancy Rate (2^{nd} month) Frequency (%)	12/77 (15.6%)	11/81 (13.6%)	0.721
Pregnancy Rate (3^{rd} month) Frequency (%)	8/65 (12.3%)	7/70 (10%)	0.67
Cumulative Pregnancy Rate	33/90 (36.7%)	27/90 (30%)	0.343
Miscarriage	7 (21.2%)	3 (11.1%)	0.296
Live birth	26 (78.8%)	24 (88.9%)	
OHSS (ovarian hyperstimulation syndrome)	2/90	0	0.202
Multi-fetal Pregnancy	(2.2%)	0	
Bleeding	3/90	1/90	
Headache	(3.3%)	(1.1%)	

* P value: Probability value: non-significant, Chi square (χ^2) test.

Table 6: Correlation between pregnancy achieved and different Parameters.

Parameters	Correlation Coefficient	p -value
Age	-0.175	0.004
BMI	-0.303	<0.001
Parity	-0.057	0.386
Duration of infertility	-0.073	0.299
Basal FSH	0.208	<0.001
AMH	0.353	<0.001
Endometrial Thickness	0.048	0.45
Number of follicles	0.152	0.041

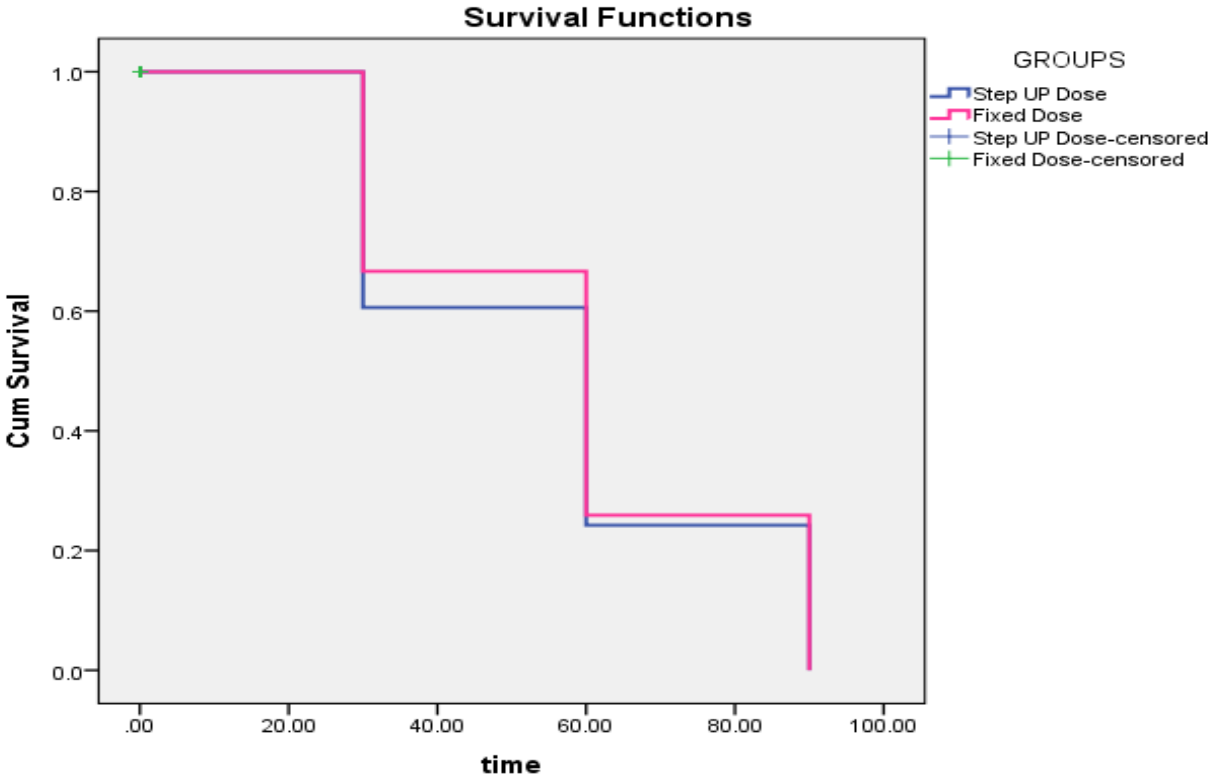

Figure 1: Kaplan–Meier survival curves showing probability of [pregnancy versus months of induction by step up protocol and fixed dose protocol groups.

8. Conclusions

Compared to the mono-follicular development of the conventional protocol, letrozole step up protocol was associated with multi-follicular development and better endometrial receptivity in the form of lower endometrial spiral artery RI with no significant increase in complications as OHSS, multifetal pregnancy or dug complications.

9. Recommendations

We recommend larger sample size. Meta-analysis and randomized controlled trials on letrozole step up protocol. Also, combination of letrozole step-up protocol with IUI and assessment of CPR in patients with UI is recommended. Further studies to compare letrozole with Gonadotropins would be helpful to assess if letrozole step up multi-follicular ovulation would be helpful in decreasing the financial burden in patients with UI undergoing ovulation induction.

Conflict of interest

Nil.

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