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Effect of three different doses of letrozole in ovulation induction and its effect on gonadotropins dose in unexplained infertility

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Abstract
Background/purpose: To evaluate the effect of three different doses of letrozole in ovulation induction and its effect on gonadotropins dose in unexplained infertility.
Material and method: It was a prospective study conducted in the department of Obstetrics and Gynecology, Muzaffarnagar Medical College, Muzaffarnagar among 180 patients of infertility.
Selected infertility cases were divided in three groups i.e. 1) Group 1(60 cases) infertile women treat with letrozole 2.5 mg, 2) Group 2 (60 cases) infertile women treat with letrozole 5 mg and
3) Group 3 (60 cases) infertile women treat with letrozole 7.5 mg. The demographic details of the subjects were recorded. All women were evaluated on the 2nd day of cycle with transvaginal ultrasound for measuring endometrial thickness and an antral follicle count as well as follicular vascularity. Base line that is on 3rd day of cycle serum FSH, LH levels were also measured in initial assessment. Group I, II and III received letrozole (Letrofem®; Iran hormone, Tehran, Iran) from day 3 of the cycle, 2.5 mg, 5mg, 7.5 mg orally per day for five days.
Results: Maximum follicle and ET size was increased in subjects having letrozole 7.5 mg dose.
Follicular number was 8 was reported among 70%, 68.33% and 61.67% of the subjects with 2.5mg, 5mg and 7.5mg letrozole dose respectively. Gonadotropin dose 1 and 2 was required by 45% and 35% of the subject's 7.5mg letrozole dose respectively.
Conclusion: We have shown that letrozole, used in doses greater than those commonly employed, can produce enhanced follicular growth without detrimental effects upon the endometrium.

Keywords: proximal tibia fracture, MIPPO, knee stiffness, wound dehiscence

Introduction
Infertility is commonly defined as the failure of conception after at least twelve months of unprotected intercourse. Infertility is a common problem in gynaecological practice affecting about 21% of couples in the reproductive age group [1].Unexplained infertility comprises 10–20% among all infertility patients. Treatment of unexplained infertility includes superovulation and intrauterine insemination (IUI). The initial management of unexplained infertility is taken care of by oral drugs [2].
There are two main medications used for ovarian stimulation; an oral antiestrogen, clomiphene citrate (CC) and injectable gonadotrophins [3]. CC has a long half- life and accumulates in the body [4]. In anovulatory women, the use of CC is widely accepted as the first line therapy because of its low cost and easy administration [5, 6]. Its use is associated with a high ovulation rate of 60%-80% but with a lower pregnancy rate of about 50% and some adverse side effects. This may be due to a detrimental effect on the endometrium (an estrogen responsive site) and on the quality of cervical mucus [4].
Recently letrozole a third-generation aromatase inhibitor, has been successfully used for ovulation induction. Letrozole inhibits the aromatase enzyme by competitively binding to the heme of the cytochrome p450 subunit of the enzyme, resulting in a blockade of androgens conversion into estrogens with subsequent increase in intraovarian androgens. The original choice of dosing with letrozole was extrapolated from several studies performed on postmenopausal women being treated for breast cancer [7, 8]. Data derived from these patients suggested substantial inhibition of estradiol formation with doses of 2.5–5 mg daily. However, the application of these data to short-term use of the drug in reproductive age women is highly questionable. Nevertheless, clinical investigation of the drug in infertile women has been

generally limited to 5 days of treatment at doses of 2.5–7.5mg daily. The dose has varied from 2.5 being lowest to 7.5 mg being highest, and a single study has been also conducted on a single dose if 20 mg.

For several years, we have, in women felt to be sub-optimally responding to established doses of letrozole. Hence the aim of the present prospective randomized clinical trial was to compare the efficacy of three different doses of letrozole (2.5 mg, 5 mg and 7.5 mg) in women undergoing ovulation induction and timed intercourse for treatment of unexplained infertility.

Material and method

It was a prospective study conducted in the department of Obstetrics and Gynecology, Muzaffarnagar Medical College, Muzaffarnagar in patients of infertility. A total of 180 infertile women were enrolled for study. The time period of the study was 2017-2019. The study protocol for all procedures was approved by the Institutional Review Board for Ethical Clearance of Muzaffarnagar Medical College, Muzaffarnagar and was performed in accordance with the Code of Ethics of the World Medical Association according to the Declaration of Helsinki of 1975, as revised in 2000. All patients were asked to sign a written consent form prior to commencement of the study. The patients were selected according to the following inclusion and exclusion criteria:

Inclusion criteria

1) Age less than 40 years
2) They had had at least 18 months of infertility
3) Patient Fallopian tubes had been proven by hysteron-salpingography or laparoscopy
4) They had ovulatory midluteal progesterone concentrations
5) The husband's semen analysis has been normal.
6) No recent treatment for OI (with in 6 months)

Exclusion criteria

1) PCOS
2) Endometriosis
3) Male infertility
4) Chromosomal abnormalities in women

Selected infertility cases were divided in three groups as follows (60 cases in each group) and the subjects were enrolled according to the inclusion and exclusion criteria-

1) Group 1(60 cases) infertile women treat with letrozole 2.5 mg
2) Group 2 (60 cases) infertile women treat with letrozole 5 mg
3) Group 3 (60 cases) infertile women treat with letrozole 7.5 mg

Procedure (Figure 1)

The demographic details of the subjects were recorded. All women were evaluated on the 2nd day of cycle with transvaginal ultrasound (4.5-7 MHz vaginal probe, Sono line G-40, Siemens, Germany) for measuring endometrial thickness and performing an antral follicle count as well as follicular vascularity. Base line serum FSH, LH levels were also measured in initial assessment. Group I, II and III received letrozole (Letrofem®; Iran hormone, Tehran, Iran) from day 3 of the cycle, 2.5 mg, 5mg, 7.5 mg orally per day for five days.

In addition, all the patients received a daily intramuscular (IM) human menopausal gonadotropin (HMG, Pergonal, Serono, 65TSwitzerland65T) injection. The dosage was 75 IU starting on day 7 of menstrual cycle until hCG administration. Gonadotropins given on alternate day from day 7 after 5 days of letrozole till the follicular size reached to 18 to 20mm in a dose of 150 IU on the basis of size of follicle. Transvaginal ultrasonography was done in the days 2, 7, 9, 12 & 14 of the cycle and then every alternate day, until follicle size reached to 18 to 20 mm in size.

Alternate day TVS was performed to measure the total number of developing follicles, the size of follicles on both the sides and endometrial thickness (ET). The day when the size of at least one dominant follicle reached 20mm, ovulation was triggered by an intramuscular injection of 5,000 units of human chorionic gonadotropin (hCG). Ovulation was expected after 36 h of injection hCG, and it was confirmed by TVS.

Crenation of the follicle and appearance of fluid in the pouch of Douglas were considered to be the signs of rupture of follicle. IUI was performed after 36 h of trigger either by swim up technique or single density method depending on the sperm count and motility. Serum oestradiol levels were noted on day 10 and on the day of hCG administration. Serum progesterone level was done on day 21 to check ovulation and adequacy of luteal phase support. Both serum oestradiol and progesterone levels were recorded by competitive immunoassay using the direct chemiluminescent technology. For all the hormone levels, the kit used was ADIANA Siemens. Cycles with large follicles more than six in number were deferred from trigger to prevent ovarian hyperstimulation syndrome (OHSS). The primary outcomes were number and size of follicles, ET and secondary outcome was pregnancy rate.

Statistical analysis

Data so collected was tabulated in an excel sheet, under the guidance of statistician. The means and standard deviations of the measurements per group were used for statistical analysis (SPSS 22.00 for windows; SPSS inc, Chicago, USA). For each assessment point, data were statistically analyzed using factorial ANOVA. Difference between two groups was determined using student t-test as well as chi square test and the level of significance was set at $p < 0.05$.

Results

The mean age among group 1, 2 and 3 was 31.12±4.11, 30.85±3.78 and 31.98±3.90 years respectively. In the present study, most of the subjects among all the groups have menstrual flow between 3-5 days followed by 5-6 days. Mean duration of infertility (Years) was 3.90±1.42, 4.31±1.24 and 4.34±1.29 years among the subjects with 2.5mg, 5mg and 7.5mg letrozole dose respectively (table 1).

Mean FS/LSH on day 3 was 0.94±0.08, 0.94±0.12 and 0.90±0.11 among the subjects with 2.5mg, 5mg and 7.5mg letrozole dose respectively (table 2).

Table 3 shows the comparison of follicle size and ET at different intervals among the study groups. Mean follicle size was 2.1, 2.4 and 2.6 at day 3 among the subjects with 2.5mg, 5mg and 7.5mg letrozole dose respectively with statistically insignificant difference. Mean follicle size increased to 4.4, 5.9 and 6.8 among the subjects with 2.5mg, 5mg and 7.5mg letrozole dose respectively. Maximum follicle size and ET was increased in subjects having letrozole 7.5mg dose. When mean follicle size and ET was compared statistically among the three groups at day 7, it was found to be statistically significant as $p<0.05$.

Gonadotropin dose 1 and 2 was required by 45% and 35% of the subject's 7.5mg letrozole dose respectively. Gonadotropin dose 3 was needed by 8.3%, 48.3% and 20% of the subjects with 2.5mg, 5mg and 7.5mg letrozole dose respectively. When gonadotropin dose was compared statistically among the three groups using chi square test, it was found to be statistically insignificant as $p>0.05$ (table 5).

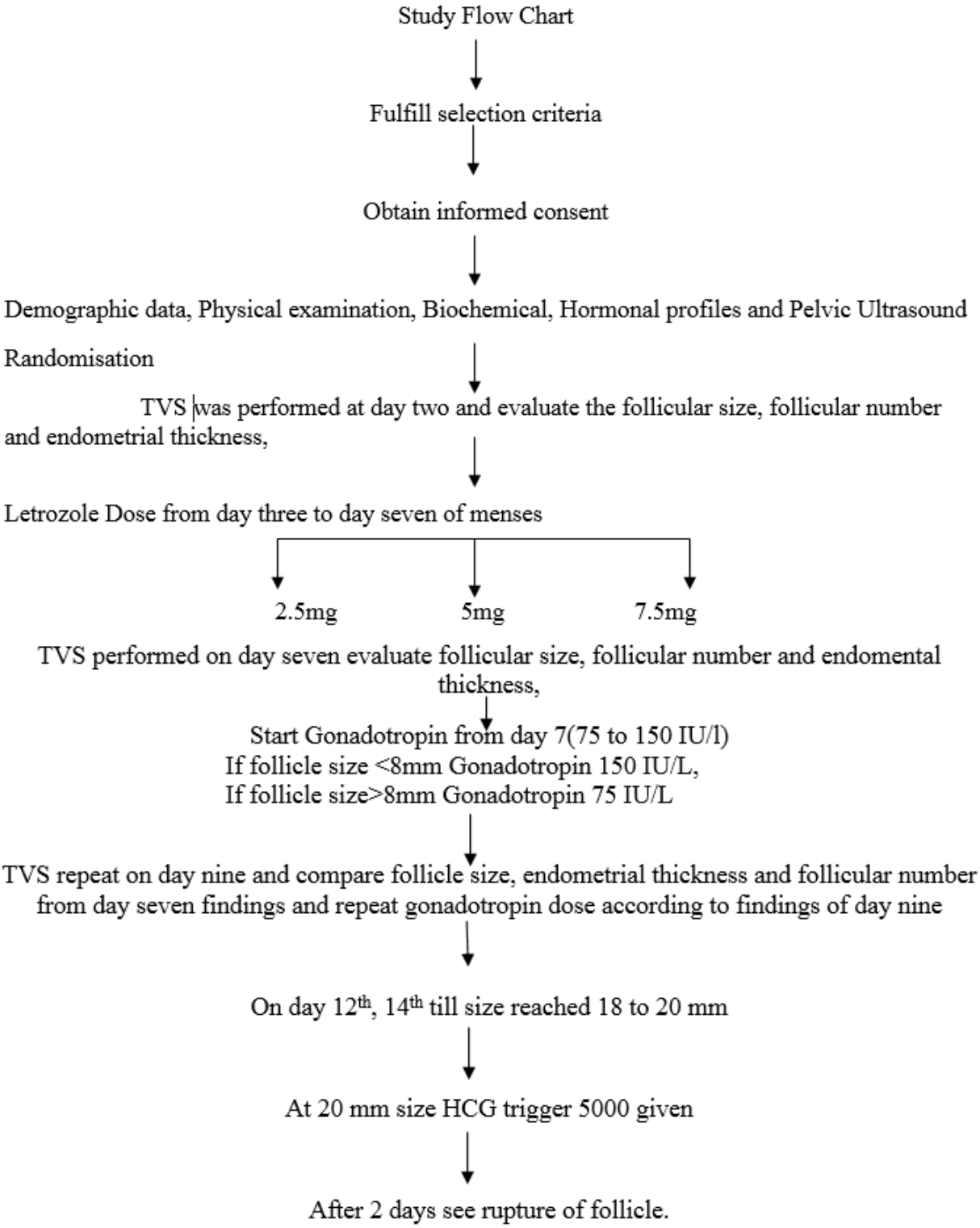

Fig 1: Study flow chart

Table 1: Mean age, BMI and duration of infertility among the study groups

Variables	Mean	±SD
Age		
60 patients with 2.5mg	31.12	4.11
60 patients with 5mg	30.85	3.78
60 patients with 7.5mg	31.98	3.90
BMI		
Dose of Letrozole		
60 patients with 2.5mg	27.45	3.19
60 patients with 5mg	27.31	3.67
60 patients with 7.5mg	27.11	3.98
Anova test	1.02	
p value	0.69	
Duration of infertility (Years)		
60 patients with 2.5mg	3.90	1.42
60 patients with 5mg	4.31	1.24
60 patients with 7.5mg	4.34	1.29
Anova test	0.92	
p value	0.75	

Table 2: FSH, LH and FSH/LH ratio in mIU/ml among the study population on day 3 in menses

Variables	Mean in mIU/ml	±SD
FSH		
60 patients with 2.5mg	4.77	0.43
60 patients with 5mg	4.95	0.24
60 patients with 7.5mg	4.94	0.29
Anova test	2.37	
p value	0.49	
LH		
60 patients with 2.5mg	5.14	0.33
60 patients with 5mg	5.26	0.24
60 patients with 7.5mg	5.36	0.28
Anova test	2.80	
p value	0.24	
FSH/LH		
60 patients with 2.5mg	0.94	0.08
60 patients with 5mg	0.94	0.12
60 patients with 7.5mg	0.90	0.11
Anova test	2.92	
p value	0.06	

Table 3: Comparison of follicle size and ET in mm at different intervals among the study groups

Day of menses	Dose of Letrozole						Anova test	p value
	2.5mg		5mg		7.5mg			
Follicle Size	Mean in mm	±SD	Mean in mm	±SD	Mean in mm	±SD		
3rd day@	2.1[a]	0.3	2.4[a]	0.2	2.6[a]	0.4[a]	2.79	0.06
7th day@	4.4[a]	0.6	5.9[b]	0.5	6.8[c]	0.6	16.78	<0.01*
t test	18.21		16.72		29.81			
p value	<0.01*		<0.01*		<0.01*			
ET								
3rd day@	3.53[a]	0.23	3.61[a]	0.38	3.65[a]	0.35	0.98	0.43
7th day@	8.56[a]	0.32	8.67[a]	0.51	9.95[b]	0.62	8.72	0.02*
t test	16.79		15.99		20.57			
p value	<0.01*		<0.01*		<0.01*			

*: statistically significant, @: TukeyHsd Post hoc Test, values with different letters indicate statistically significant difference

Table 4: Comparison of follicular number among the study groups at day 3 and 7 of menses

Follicular Number	Dose of Letrozole						Chi square test	p value
	60 patients with 2.5mg		60 patients with 5mg		60 patients with 7.5mg			
Day 3	No	%	No	%	No	%		
8	42	70	41	68.33	37	61.67		
9	11	18.33	10	16.67	12	20	1.98	0.21
10	7	11.67	9	15	11	18.33		
Day 7								
5	39	65	36	60	31	51.67		
6	12	20	13	21.67	15	25	3.47	0.07
7	9	15	11	18.33	14	23.33		

Table 5: Comparison of Gonadotropin doses among the study groups

Gonadotropin dose	Dose of Letrozole						Fisher exact test	p value
	60 patients with 2.5mg		60 patients with 5mg		60 patients with 7.5mg			
	No	%	No	%	No	%		
1	0	0	0	0	27	45		
2	0	0	0	0	21	35		
3	5	8.3	29	48.3	12	20		
4	5	8.3	25	41.7	0	0	3.94	0.11
5	25	41.7	3	5	0	0		
>5	25	41.7	3	5	0	0		

FSH 1 dose = 150 IU

Discussion

Despite several studies that have shown the effectiveness ofletrozole for ovarian stimulation, there have been very fewstudies to determine the optimal dose. Most studies have useda 2.5 mg dose (Mitwally and Casper, 2005; Atay *et al.*, 2006) [9, 10]; others have used a range from 2.5–5 mg (Begum *et al.*, 2006) [11], while at least one study has used a 7mg dose (Al-Fozan *et al.*, 2004) [12]. One study (Al-Fadhli *et al.*, 2006) [13] has compared two of these doses, 2.5 mg versus 5 mg, and found that 5 mg of letrozole was associated with more follicles and a higher pregnancy rate. However, there are rare studiesto date that have compared all of the three commonly used doses. This study reports on the different responses elicited by administration of 2.5 mg, 5mg and 7.5 mg of letrozole in patients within explained infertility.

In our study, mean FSHon day 3 was 4.77±0.43 IU/ml, 4.95±0.24IU/ml and 4.94±0.29 IU/ml among the subjects with 2.5mg, 5mg and 7.5mg letrozole dose respectively with statistically insignificant difference. Approximately similar results were revealed by A Badawy *et al.* [14].

In the present study, mean follicle size increased to 4.4, 5.9 and 6.8 among the subjects with 2.5mg, 5mg and 7.5mg letrozole dose respectively. Maximum follicle size was increased in subjects having letrozole 7.5mg dose with statistically significant difference as p<0. Hananel Holzer *et al.* [15] in their study reported that 5 mg of letrozole daily was associated with a higher number of follicles and a higher pregnancy rate. Available evidence suggests a dose-response with letrozole, with higher doses producing more mature follicles and higher ovulation rates [16]. In the initial such study, 5mg daily produced a higher number of ovulations than 2.5mg [13]. A second study,

comparing 2.5mg, 5mg, and 7.5 mg, found the number of mature follicles to be significantly greater as the dose increased (1.0, 1.4, and 3.4, respectively) [14]. A study suggested by Elizabeth A. Pritts *et al*., [16] found that there may be utility in increasing the dose even further, beyond 7.5mg/day to as much as 12.5mg/day. Predicted ovulation number was greater for increasing doses of the drug, and endometrial thickness was unaffected. Thus, when the patient's goal for number of predicted ovulations is not met with lower doses of the drug, it seems reasonable to explore their response to a dosage of 10–12.5mg daily.

In our study, maximum ET was increased in subjects having letrozole 7.5mg dose. A Badawy *et al.,* [14] in their study that maximum ET was increased in subjects having letrozole 7.5mg dose which is similar to the present study. Leila Pourali *et al.,* [17] gave 5mg dose of letrozole to the study subjects and reported that endometrial thickness before and after letrozole dose was 3.7mm and 8.5mm respectively.

Justification for not exceeding doses of 2.5–7.5 mg has been based on the concept that these doses reduce estradiol levels 88–98%. At a dose of 2.5 mg daily, it takes 2–4 days for maximal suppression to occur. Steady-state plasma levels do not occur for as long as 2 months. Thus, there is reason to believe that higher-dose short-term administration may be more effective at inducing endogenous FSH release, resulting in greater follicular development. In addition, estradiol suppression may not be the only effect of value. Letrozole has been noted to inhibit other aspects of the steroidogenic pathway, including a reduction in synthesis along the cortisol pathway. Thus, intraovarian androgen accumulation may be disproportionately greater than the reduction in estrogen. Androgen has been well demonstrated in the primate to stimulate early follicular growth by augmenting follicular FSH expression and to stimulate endocrine and paracrine factors that synergize with FSH to promote folliculogenesis. The limitation of this study was short period of follow-up which cannot detect pregnancy outcome and also the teratogenic effects of these drugs.

Conclusion

We have shown that letrozole, used in doses greater than those commonly employed, can produce enhanced follicular growth without detrimental effects upon the endometrium. Further study is clearly needed, including basic investigation into estradiol and androgen levels with these doses in reproductive age women. Nevertheless, we believe that high doses of this drug can and should be employed, particularly in women inadequately responsive to lower doses. In addition, we believe randomized trials comparing high-dose to low-dose administration would help determine the optimal starting dose for this medication in women of varying diagnoses.

References

1. Dankert T, Kremer JA, Cohlen BJ, Hamilton CJ, Pasker-de Jong PC, Straatman HT, *et al.* A randomized clinical trial of Clomiphenee citrate versus low dose recombinant FSH for ovarian hyperstimulation in intrauterine insemination cycles for unexplained and male subfertility. Hum Reprod. 2007; 22:792-797.
2. Hembram M, Biswas R, Jain A. A study of controlled ovarian stimulation with clomiphene citrate or letrozole in combination with gonadotropins and IUI in unexplained infertility. J Hum Reprod Sci. 2017; 10:173-7.
3. Mitwally MF, Casper RF. Aromatase inhibitors for the treatment of infertility. Expert OpinInvestig Drugs. 2003; 12:353-371.
4. Shrivastav P. Aromatase inhibitors -their role in treatment of infertility. In: The Art and Science of Assisted Reproductive Techniques (A.R.T). Edited by GautamAllahbadia, RitaBasuray Das, Rubina Merchant. India, 2003, 47-49.
5. Dickey RP, Holtkamp DE. Development, pharmacology and clinical experience with clomiphene citrate. Hum Reprod Update. 1996; 2:483506.
6. Homburg R, Insler V. Ovulation induction in perspective. Hum Reprod update. 2002; 8:449-462.
7. Bisagni G, Cocconi G, Scaglione F, Fraschini F, Pfister CH, Trunet PF, Letrozole. A new oral non-steroidal aromastase inhibitor in treating postmenopausal patients with advanced breast cancer. A pilot study. Annals of Oncology. 1996; 7(1):99-102.
8. Buzdar AU, Robertson JF, Eiermann W, Nabholtz JM. An overview of the pharmacology and pharmacokinetics of the newer generation aromatase inhibitors anastrozole, letrozole, and exemestane. Cancer: Interdisciplinary International Journal of the American Cancer Society. 2002; 95(9):2006-16.
9. Mitwally MF, Biljan MM, Casper RF. Pregnancy outcome after the use of an aromatase inhibitor for ovarian stimulation. Am J Obstet Gynecol. 2005; 192:381-6.
10. Atay V, Cam C, Muhcu M, Cam H, Karateka. A Comparison of L and CC in women with PCOS undergoing ovarian stimulation J Int Med Res. 2006; 34:73-76.
11. Begum MR, Ferdous J, Begum A, Quadir E. Comparison of efficacy of aromatase inhibitor and clomiphene citrate in induction of ovulation in polycystic ovarian syndrome. Fertility and sterility. 2009; 92(3):853-7.
12. Al-Fozan H, Al-Khadouri M, Tan SL, Tulandi T. A randomized trial of letrozole versus clomifene citrate in women undergoingsuperovulation. Fertility and Sterility 2004; 82:1561-63.
13. Al-Fadhli R, Sylvestre C, Buckett W. A randomized trial of superovulation with two different doses of letrozole. Fertility and Sterility. 2006; 85:161-64.
14. Badawy A, Metwally M, Fawzy M. Randomized controlled trial of three doses of letrozole for ovulation induction in patients with unexplained infertility. Reproductive biomedicine online. 2007; 14(5):559-62.
15. Holzer H, Casper R, Tulandi T. A new era in ovulation induction. Fertility and sterility. 2006; 85(2):277-84.
16. Pritts EA. Letrozole for ovulation induction and controlled ovarian hyperstimulation. Current Opinion in Obstetrics and Gynecology. 2010; 22(4):289-94.
17. Pourali L, Ayati S, Tavakolizadeh S, Soleimani H, Sani FT. Clomiphene citrate versus letrozole with gonadotropins in intrauterine insemination cycles: A randomized trial. International Journal of Reproductive Bio Medicine. 2017; 15(1):49.