GinPolMedProject ©
2 (63) 2022 : 001-06 • ORIGINAL RESEARCH

Perifollicular vascularity as a potential variable affecting outcome in stimulated intrauterine insemination letrozole treated cycles: A study using transvaginal power doppler

Mohamed I Taema*
Department of Obstetrics and Gynecology, Faculty of Medicine, Ain Shams University Hospital, Abbassia, Cairo, Egypt

AUTHORS' CONTRIBUTION: (A) Study Design · **(B)** Data Collection . **(C)** Statistical Analysis · **(D)** Data Interpretation · **(E)** Manuscript Preparation · **(F)** Literature Search · **(G)** Funds Collection

SUMMARY

Aim: This is a clinical trial study that included 44 women with infertility attending the fertility unit of University hospital and undergoing ovulation induction with letrozole and intrauterine insemination. The study was conducted from October 2020 to June 2021.

Inclusion criteria: Normal folliculometry and ultrasound finding, infertile women aged 20-35 years, infertility more than 1 year (after 12 month of regular intercourse), Body mass index between 20-30 kg/m², Normal prolactin level, follicular stimulating hormone on day 3 <12 IU/ml, thyroid stimulating hormone, Tubal patency ensured by hystrosalpingy graph or by laparoscope, Total sperm count of husband ≥ 10 million.

Methods: Patients involved in this study were subjected for Ovulation induction began on the third day of the menstrual cycle for 5 days, with 5 mg of letrozole added Hmg 75 IU on day, On day 10-12 of the menstrual cycle, transvaginal ultrasound was performed for monitoring follicular and endometrial development and was repeated every 2 days to decide time of hCG administration, When one or more follicles are measured 17 mm. When ovum reach 18 mm in diameters, hCG 5,000 IU was administered. Approximately 36 hours after hCG administration, the rupture of follicles was confirmed by transvaginal ultrasound and then a single IUI was performed. On the day of HCG, trans-vaginal 2D power Doppler ultrasound was performed at Ultrasound Unit in zagazig University Hospital by the same operator after the patients had emptied their bladders, measurements included:, peri-follicular vascularity, It also included endometrial volume and thickness and endometrial pattern, number of mature follicles, and then pregnancy is confirmed by blood test.

Results: The study showed that according distribution modified grading system grade 1, n=1, grade 2, n=4 grade 3, n=12 grade 4, n=5 the distribution of all follicles studied (all >17 mm in diameter), 5 patient get pregnant, mean diameter in relation of diameter are not significant (the pregnant group) while 17 cases did not get pregnant (non-pregnant group). The mean base line serum FSH concentration also tended to be high in low grade vascularity cycle, but this difference was not significant. Primary infertility found to be 12 patients (55%), while secondary infertility found to be 10 patients (45%). There were 5 pregnancy giving pregnancy rate 22%, the follicles of high grade vascularity were associated with high pregnancy rate (grade 4=60%), (grade 3=16.6%),than cycles with low grade vascularity with no pregnancy occur in grade 1 and 2 vascularity group (grade 1, 2=0%) this result is significant .5 pregnant patients, 2 patients had endometrial thickness 8-10 mm, 3 patients had endometrial thickness 10-12 mm, zero patient had endometrial of 8 mm, In our study the pregnancy rate was 40% with distinct five line endometrial pattern, 23% with hazy five line Endometrial pattern, and 0% with no endometrial layering.

Conclusion: Perifollicular vascular perfusion appears to be an important factor in determining the outcome in stimulated IUI cycles, and may have clinical implications in assisted reproduction techniques.

Keywords: Fertility; Ovulation; Perifollicular vascularity; Letrozole

Address for correspondence:

Dr. Mohamed I Taema
Department of Obstetrics and Gynaecology, Faculty of Medicine, Ain Shams University, Abbassia, Cairo, Egypt
E-mail: research.med@yandex.com

Word count: 2981 **Tables:** 09 **Figures:** 00 **References:** 16

Received: 21.02.2022, Manuscript No. gpmp-22-55037; **Editor assigned:** 24.02.2022, PreQC No. P-55037; **Reviewed:** 04.03.2022, QC No. Q-55037; **Revised:** 29.04.2022, Manuscript No. R-55037; **Published:** 29.06.2022

INTRODUCTION

Infertility is a major problem affecting about 14% of couples. There are many factors affecting couple fertility, roughly half of fertility problems with a diagnosed cause are due to male factors, and about half due to female factors. However, about one in five cases of infertility has no clear diagnosed cause. Unexplained infertility is still somewhat nebulous diagnosis of exclusion. Immunologic factors have been proposed to be involved in as many as 20% of otherwise unexplained cases of infertility [1].

Intrauterine insemination (IUI) with or without ovarian stimulation are an empirical treatment line that has been used in management of infertility, there is increasing role of expectant management, effectiveness of less invasive procedures and growing concern about the complications and costs associated among patients as well as service providers [2].

Intrauterine insemination (IUI) has long been established as an alternative to other forms of assisted reproductive techniques (ART). The use of letrozole in IUI has been shown to improve significantly the pregnancy rate. However, the risk of both ovarian hyperstimulation syndrome and multiple pregnancies is significantly increased, despite this; IUI has cost savings and is less invasive compared with other forms of ART in particular for non-tubal infertility [3].

Letrozole is a third generation non-steroidal reversible aromatase inhibitor. It can suppress oestradiol level up to 95% to 99% after administration according to pharmacodynamics and pharmacokinetics studies, When aromatization of androgens to estrogens is inhibited, a reduction of circulating estrogens causes modifications in the hypothalamic–pituitary–ovary axis, including release of the hypothalamic–pituitary axis from estrogenic negative feedback and FSH secretion is increased, with the resultant stimulating effect on the growth of ovarian follicles, thus use of letrozole in IUI has been shown to improve significantly the pregnancy rate [3].

The reported pregnancy rates per COH/IUI cycle in different parts of the world usually varied between 7% and 22%, there is also a lack of consensus regarding the optimum numbers of COH/IUI that patients should attempt before proceeding to IUI [4].

One of the earliest studies by Azim A and Oktay K

[5] using letrozole as a fertility drug looked at 12 women with inadequate response to clomiphene citrate. Ovulation on letrozole occurred in 9 of 12 cycles and 3 patients conceived, a later study by the same investigators compared the effects of letrozole to those of clomiphene citrate, and this time 19 women were studied. Ten women received clomiphene citrate and nine women received letrozole. This study was unable to demonstrate any difference in the number of women who ovulated, the number of eggs that developed in each woman, or the thickness of the uterine lining during treatment, letrozole is associated with a thicker uterine lining and a lower miscarriage rate.

Gore MA, et al. [6] described that increased perifollicular blood flow can be measured in the perifollicular period using color and pulsed Doppler, automated estimation of blood volume around the ovarian follicles, the blood volume does not differ between follicles containing an oocyte and those with no oocyte in the aspirate, or a non-fertilizable oocyte, he hypothesize that those follicles containing oocytes able to produce pregnancy have a more uniform perifollicular vascular network.

The extension of vascularity was graded using a modified grading system. The grading system consisted of assessing the percentage of follicular circumference in which flow was identified from a single cross-sectional slice. The grading system was as follows: F1, with vascularity ≤ 25% of follicular circumference; F2, with vascularity between 26% and 50% of follicular circumference and, F3 between 51% and 75% of follicular circumference; and F4 with vascularity >75% of follicular circumference. The periovulatory follicles were categorized as high (grades 3–4) or low grade (grades 1–2) [7,8].

Aim of Work

The aim of this work was to study the effect of letrozole on perifollicular vascularity and outcome in cases of intrauterine insemination.

PATIENTS

The Study was cross section clinical trial for patient undergoing IUI treatment cycles were recruited in this study carried out at infertility Unit in University Hospital with unexplained infertility attending the fertility unit of University hospital and undergoing ovulation induction with letrozole and intrauterine insemination, the study was conducted from October 2020 to June 2021, included 44 cases.

Inclusion criteria

All women underwent IUI treatment including:

- Infertile women aged 20-35 years.
- Infertility more than 1 year (after 12 month of regular intercourse).
- Body mass index between 20-30 kg/m²
- Normal prolactin level, follicular stimulating hormone on day 3 <12 iu/ml, thyroid stimulating hormone.
- Tubal patency ensured by hystrosalpingy graph or by laparoscope.
- Total sperm count of husband ≥ 10 million.

Exclusion criteria

- Total sperm count of husband <10 million
- Ovarian cyst, closed tube.
- Organic pelvic adhesions or severe endometriosis stage III-IV by laparoscope.
- Previous IUI treatment last 3 months.
- Liver disease or kidney disease.

Administrative design:

Explanation of study and the type of maneuver for each participant after which a written consent for approval was taken, Ethical consideration will be done.

METHODS

Initial evaluation

Steps of performance and techniques used:

1. History taking
2. General, abdominal and pelvic examination.
3. Investigations: FSH, prolactin TSH, complete blood count, hystrosalpingo graphy, and hystrosalpingo graphy, semen analysis.

Technique

- Patient stimulated on day 3 to day 7 with letrozole 5 mg.
- Folliculometry: Follicular growth was measured by serial using transvaginal ultrasound scan with power and color Doppler facilities. Perifollicular vascularity was assessed on day of HCG administration by TVS power Doppler u/s D^2, the power Doppler box was positioned on each ovarian follicle and the cross-section image of follicle with the maximum colour indication in the follicular circumference is frozen and vascularity is graded (according to F1-F4 grading system) [9].

1. Grade 1: <25% of circumference
2. Grade 2: 26-50%
3. Grade 3: 51-75%
4. Grade 4: >75%, grade 1 and 2 considered as low grade vascularity grade 3 and 4 were high grade, if more than one grade mean is considered [7].

- HCG 5000 IU was injected intramuscular and then IUI was carried out after 30-36 hours after HCG

injection, HCG injection when less than 3 mature follicles is seen, HCG injection is canceled in present of >3 follicles with diameter ≥ 17 mm.

Day of IUI:

- Semen specimen was collected in site, and will be prepared within 1 hour semen dilute 1:2 with Ham's medium and centrifuge at 250 × g for 10 min and then the pellets will be suspended and dilute in 1:1 with medium and centrifuge for 10 min and re-suspended and placed in incubator at temperature 37°C in 5% CO_2 in air for 30 min, and transferred to insemination catheter and inseminated intrauterine [9].
- Progesterone support is given for 14 days after IUI is done.
- Pregnancy test is done after 14 days, it is +ve when serum B-HCG >10ml u/ml.

Outcome measures

Outcome of each case is determined according to:

1) Primary outcome: Response ovarian stimulation, number of follicles and size, perifollicular vascularity grases, endometrial pattern and thickness

2) Secondary outcome: a) Pregnancy b) Not-pregnant.

Statistical analysis

Data were analyzed with SPSS version 18.0. The normality of data was first tested with Shapiro-Wilk test.

RESULTS

Initially 56 case were included, but 12 cases was excluded, 10 cases non responder, 2 case HCG administration was canceled because of risk of hyper stimulation syndrome, a total of 44 women undergoing consecutive COS-IUI cycles were analyzed in our study.

Parameters included in this study age, type of infertility, duration, indication of IUI treatment, number and diameter of follicles, FSH prolactin BMI and LH level, vascularity grade, endometrium pattern and thickness.

The results of the study showed that according distribution modified grading system grade 1, n=2, grade 2, n=8, grade 3, n=24 grade 4, n=6 the distribution of all follicles studied (all >17 mm in diameter) in day of HCG administration. There was difference in the age between the mean age was higher within non-pregnant group compared with pregnant group, also duration of infertility was higher in group of follicle ≥ 17 mm in non-pregnant compared with pregnant one (**Tab. 1.**) Infertility due to ovulatory disorder found to be 59%, and 22% due to male factor, and unexplained infertility found to be 18% (**Tab. 2.**).

The mean base line serum FSH, prolactin, LH, concentration also tended to be high in non-pregnant group, but this difference was not significant (**Tab. 3.**).

The median number of pre-ovulatory follicles (>17 mm in diameter) on the day of HCG among the pregnant patients was 2 (± 1.6) and in non-pregnant cases was 1.79 (± 1.2). In cycles with a single pre-ovulatory follicle (>17 mm in diameter) the pregnancy rate (0%) was significantly lower than in cycles with more follicles (28% and 42% respectively with two or three dominant follicles), mean diameter in relation of diameter are not significant (the pregnant group) (**Tab. 4.**).

The follicles of high grade vascularity were associated with high pregnancy rate (grade 4=60%), (grade 3=16.6%), than cycles with low grade vascularity with no pregnancy occur in grade 1 and 2 vascularity group (grade 1, 2=0%) this result is significant (**Tab. 5.**).

Grade 1 found to be 4%, grade 2 was 18%, grade 3 found to be 55%, and grade 4 was 23% (**Tab. 6.**). 10 pregnant patients, 4 patients had endometrial thickness 8-10 mm, 6 patients had endometrial thickness 10-12 mm, zero patient had endometrial of 8 mm, we found significant relation between endometrial thickness and pregnancy rates, highest (38%) with endometrial thickness ranging from 10 to 12 mm ($p < 0.05$), however not all pts with thicker endometrium had positive outcome, In case endometrial thickness was 6-8 mm, it showed a significantly no pregnancy rates (**Tab. 7.)**. Statistically higher pregnancy rate (40%) was reported in women with distinct five line appearance ($p < 0.05$). Patients with no endometrial layering had poor pregnancy rate (0%) and 23% pregnancy was seen with hazy five line appearance (**Tab. 8. and Tab. 9.**).

DISCUSSION

Many studies have been conducted to evaluate the role of various ultrasound parameters in predicting pregnancy during ART, but little information exists in the literature with regard to their role in predicting subsequent pregnancy outcomes [10].

Our study was attempted to assess the effect of

Tab. 1. Demographic data of studied population.

Parameter		Total	Pregnant	Non-pregnant	P-value
Number of patient		44	10	34	-
Age in years (Mean ± SD)		29.57 ± 4.47	29.07 ± 4.25	30.08 ± 4.69	0.443
BMI(Kg//m^2) (Mean ± SD)		25.92 ± 3.9	25.59 ± 3.4	26.29 ± 4.5	0.396
Duration of infertility(years) (Mean ± SD)		5.02 ± 2.52	4.87 ± 2.07	5.18 ± 2.98	0.439
Type of infertility	Primary	24	4	20	0.557
	Secondary	20	6	14	

Tab. 2. Comparison between pregnant and non-pregnant patients according to indication of IUI.

Indication of IUI	Total %	Pregnant (10)	Non-pregnant (34)
Ovulatory disorder	26 (59%)	8 (30%)	18 (70%)
Male factor	10 (22%)	4 (40%)	6 (60%)
Unexplained	8 (18%)	0 (0%)	8 (100%)

Tab. 3. Basal investigation of studied population.

Parameter	Total (44)	Pregnant	Non-pregnant	P-value
Day 3 FSH (IU/ml) (Mean ± SD)	6.57 ± 0.77	6.22 ± 0.96	6.92 ± 0.59	0.943
LH (mIU/ml) (Mean ± SD)	7.6 ± 0.3	7.6 ± 2.0	7.6 ± 4.1	0.996
PROLACTIN(mIU/ml) (Mean ± SD)	12.2 ± 7.5	10.4 ± 5.8	14.0 ± 8.2	0.193
Ovarian volume (cm^3) (Mean ± SD)	6.35 ± 2.1	6.1 ± 1.6	6.6 ± 2.6	0.739
Basal endometrium mm (Mean ± SD)	5.46 ± 0.6	5.31 ± 0.9	5.25 ± 0.7	0.173

Tab. 4. Comparison between pregnant and non-pregnant patients according to no of follicles and mean diameter.

Parameters			Pregnant	Non- pregnant	P value
Follicles on day of HCG	No. of follices more than 17 mm	One follicle	0 (0%)	16 (100%)	0.737
		Two follicles	4 (28%)	10 (72%)	
		Three follicles	6 (42%)	8 (58%)	
	Mean diameter of follicles in mm (Mean ± SD)		20.1 ± 1.5	19.7 ± 1.2	0.53

Tab. 5. Grading of follicles according to perifollicular vascularity.

Parameter	Grade	Pregnant	Non-pregnant	P value
Perifollicular vascularity	Grade 1	0 (0%)	2 (100%)	< 0.001
	Grade 2	0%	8 (100%)	
	Grade 3	4 (17%)	20 (83%)	
	Grade 4	6 (60%)	4 (40%)	

Tab. 6. Percent of grading system of follicles induced ovulation by letrozole.

Perifollicular vascularity	Number	Percent %
G1	2	-4%
G2	8	-18%
G3	24	-55%
G4	10	-23%

Tab. 7. Comparison between pregnancy and non-pregnancy according to endometrial thickness.

Endometrial Thickness	Pregnant(10)	Non- pregnant(34)	P-value
10-12 mm	6 (38%)	10 (62%)	< 0.05
8-10 mm	4 (22%)	14 (78%)	
Less than 8 mm	0 (0%)	10 (100%)	

Tab. 8. Comparison between pregnant and non-pregnant according to endometrial layering.

Parameter		Pregnant	Non-pregnant	P-value
Endometrial layering	No layering	0 (0%)	8 (100%)	< 0.05
	Hazy five line	6 (23%)	20 (77%)	
	Distinct five line	4 (40%)	6 (60%)	
Endometrial thickness in mm on day of HCG (Mean ± SD)		10.13 ± 1.47	8.94 ± 1.60	< 0.001

Tab. 9. Relation between perifollicular vascularity and endometrial pattern and fecundability rate.

Endometrial Pattern	No Layering	Hazy Five Layer	Distinct Five Line	P value
Grade I	2 (100%)	0 (0%)	0 (0%)	<0.05
Grade II	2 (25%)	2 (25%)	4 (50%)	<0.05
Grade III	4 (25%)	14 (58%)	6 (17%)	<0.01
Grade IV	0 (0%)	4 (40%)	6 (60%)	<0.01
* Fecundability: Ability that single cycle will result in pregnancy.				

letrozole on perifollicular vascularity in IUI with regard to successful outcome (pregnant/non-pregnant) using colour Doppler transvaginal ultrasound so that the outcome of subsequent COS-IUI cycles could be more accurately predicted than before.

In our study pregnancy occurred in 10 patients (22%) of total 44 infertility with letrozole induced IUI cycles.

However, the success rate in some studies was lower than ours, about 12% [11], as regards the diagnosis of infertility, the highest pregnancy rate (33%) was achieved in women with ovulatory disorders, our study we found ovulatory disorder 45% with pregnancy rate 10%, male factor found to be 27% with pregnancy rate of 33%, and unexplained with 27% pregnancy rate 33% [11].

In present study we found that 55% of patients had primary infertility, and 45% patients had secondary infertility, other number of primary and secondary infertility was seen in Shamila S and Sasikala SL [12] (82.48%), (17.52%), Samal S, et al. [13] (62%), (38%).

Women with endometriosis have not been able to conceive. The second highest pregnancy rate was among patients with unexplained infertility (23%). According to Farimani and Amiri MF [14], women with ovulatory problems had the greatest pregnancy rate (23.1%), while those with endometriosis have the lowest (7.7%).

Despite the fact that most studies showed greater pregnancy rates in patients with unexplained infertility and ovulatory problems and considerably lower pregnancy rates in patients with endometriosis, IUI should not be used in patients with severe endometriosis [15].

In present study it was found that, 0 (0%) patients conceived when there was presence of one pre ovulatory follicle of >16 mm, 4 patients conceived with 2 follicles (28%), 6 (42%) patients conceived when follicles was 3.

In our research, we discovered that PR was 0% in induction with letrezol in IUI cycles with single pre-ovulatory follicles, which was significantly lower than PR in cycles with more follicles.

Perifollicular vascularity was linked to a higher pregnancy rate, with no pregnancy achieved when perifollicular vascularity was grade 1 or grade 2. With grade 3 perifollicular vascularity, the pregnancy rate was 19%, but with grade 4 perifollicular vascularity, the pregnancy rate was 68% (p 0.001).

The pregnancy rate was low in cycles with low-grade perifollicular vascularity, and it was greater in cycles with grade 3 and grade 4 perifollicular vascularity, according to Bhal PS, et al. [7]. Follicular vascularity did not appear to predict the likelihood of conception in women undergoing moderate COS and IUI cycles, according to Ragni G, et al. [3]. The pregnancy rate was 14.1, 10.0, and 11.8 percent, respectively, in the low-, medium-, and high-grade vascularity groups.

A variety of research has looked at endometrial thickness and pattern as a predictor of outcome, with mixed findings. While some research organizations discovered a link between endometrial thickness and pregnancy rate, others did not. Endometrial thickness was measured in our study. No pregnancy was reported when endometrial thickness was less than 8 mm, 10 patient get pregnant 6 when endometrial thickness more than 10 mm (37%), 4 endometrial between 10-8 mm (22%).

Friedler S, et al. [4] discovered in their study of literature survey (concerning endometrial thickness, which included 25 reports comprising 2665 assisted reproduction treatment cycles) that the difference in mean endometrial thickness of conception and non-conception cycles was statistically significant in 1203 cycles, but not in 331 cycles. They discovered that an endometrial thickness of 6 mm had a high NPV for recurrent pregnancy.

The multilayer endometrial pattern was significantly greater in the current letrozole cycle compared to the previous failed CC cycle. These results agree with the study of Jang EJ and Jee BC [16], multilayer endometrial pattern has a low positive predictive value (PPV) for pregnancy (33.1%), while the nonexistence of a multilayered pattern does not exclude conception but renders it improbable (NPV, 85.7%).

In our study the pregnancy rate was 40% with distinct five line endometrial pattern, 23% with hazy five line endometrial pattern, and 0% with no endometrial layering.

Pregnancy rate was higher in distinct five line endometrial appearance. Friedler S, et al. [4] in their study found that the difference in the endometrial patterns of conception and non-conception cycles was statistically significant in 2892 cycles; however in 844 cycles no such significance was noted [4].

CONCLUSION

Perifollicular vascular perfusion appears to be an important factor in determining the outcome in stimulated IUI cycles, and may have clinical implications in assisted reproduction techniques. As there were no pregnancies in women with low-grade vascularity, the identification of these cycles would be valuable early in cycles (before HCG/IUI).

Perifollicular vascularity results could allow cancellation of treatment after careful counseling and cancellation of the cycles could be cost-effective both financially and emotionally. However more longitudinal data would be needed before this form of prospective management of treatment cycles could be applied clinically.

REFERENCES

1. **Zhu JL, Basso O, Obel C, et al.** Infertility, infertility treatment, and congenital malformations: Danish national birth cohort. *BMJ*. 2006;333(7570):679.
2. **Kamath MS, Bhave P, Aleyamma TK, et al.** Predictive factors for pregnancy after intrauterine insemination: A prospective study of factors affecting outcome. *J Hum Reprod Sci.* 2010;3(3):129.
3. **Ragni G, Anselmino M, Nicolosi AE, et al.** Follicular vascularity is not predictive of pregnancy outcome in mild controlled ovarian stimulation and IUI cycles. *Hum Reprod.* 2007;22(1):210-4.
4. **Friedler S, Schenker JG, Herman A, et al**. The role of ultrasonography in the evaluation of endometrial receptivity following assisted reproductive treatments: A critical review. *Hum Reprod Update*. 1996;2(4):323-35.
5. **Azim A, Oktay K.** Letrozole for ovulation induction and fertility preservation by embryo cryopreservation in young women with endometrial carcinoma. *Fertil Steril.* 2007;88(3):657-64.
6. **Gore MA, Nayudu PL, Vlaisavljevi*ƒ* V, et al.** Prediction of ovarian cycle outcome by follicular characteristics, stage 1. *Hum Reprod.* 1995;10(9):2313-19.
7. **Bhal PS, Pugh ND, Gregory L, et al.** Perifollicular vascularity as a potential variable affecting outcome in stimulated intrauterine insemination treatment cycles: A study using transvaginal power Doppler. *Hum Reprod.* 2001;16(8):1682-9.
8. **Yousefi B, Azargon A.** Predictive factors of intrauterine insemination success of women with infertility over 10 years. *J Pak Med Assoc.* 2011:165-8.
9. **Russell LD, Rogers BJ**. Improvement in the quality and fertilization potential of a human sperm population using the rise technique. *J Androl.* 1987;8(1):25-33.
10. **Ng EH, Chan CC, Tang OS, et al.** Endometrial and subendometrial vascularity is higher in pregnant patients with livebirth following ART than in those who suffer a miscarriage. *Hum Reprod.* 2007;22(4):1134-41.
11. **Zhao Y, Vlahos N, Wyncott D, et al.** Impact of semen characteristics on the success of intrauterine insemination. *J Assist Reprod Genet.* 2004;21(5):143-8.
12. **Shamila S, Sasikala SL**. Primary report on the risk factors affecting female infertility in South Indian districts of Tamil Nadu and Kerala. *Indian J Community Med.* 2011;20(25):25-30.
13. **Samal S, Dhadwe K, Gupta U, et al.** Epidemiological study of male infertility. *Indian Med Gaz.* 2012;5:174-80.
14. **Amiri MF**. Analysis of prognostic factors for successful outcome in patients undergoing intrauterine insemination. *Acta Medica Iranica.* 2007:101-6.
15. **Khalil MR, Rasmussen PE, Erb K, et al.** Homologous intrauterine insemination. An evaluation of prognostic factors based on a review of 2473 cycles. *Acta Obstet Gynecol Scand.* 2001;80(1):74.
16. **Jang EJ, Jee BC.** The efficacy of letrozole in women with a poor endometrial response to clomiphene citrate. *Clin Exp Reprod Med.* 2010;37(1):73-81.