

# Letrozole Ovarian Stimulation improves Outcome of Endometrial Scratching prior to Intrauterine Insemination Procedure

Original  
Article

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## ABSTRACT

**Aim of the work:** To compare the outcome of intrauterine insemination (IUI) after letrozole (LET) ovarian stimulation (OS) followed by endometrial scratching (ES) versus after each intervention separately.

**Patients and Methods:** This study included 90 women with primary infertility for >2years secondary to male factor and had previous failed IUI or expectant trials. All women underwent clinical examination and transvaginal ultrasonographic (TUV) examination and hormonal profile. Enrolled couples were randomly divided into three groups: Group L received oral letrozole 5 mg from day 3 to 7 of the menstrual cycle, Group S included females subjected to ES injury and Group LS included females received LET OS followed by ES injury. For all groups, ovulation was monitored and assured using TVU for evident ovulation with a dominant follicle size >18 mm. IUI was repeated for three cycles and clinical pregnancy rate (CPR) and abortion and multiple pregnancy rates were recorded.

**Results:** The studied women gave 287 follicles of >18 mm with a mean number of follicles of 3.18/female and 1.35/cycle. Mean endometrial thickness was significantly lower, while E2 serum levels were significantly higher in women of group S compared to women of other groups. Total CPR was 41.1%/woman and 17.4%/cycle with significant difference in favor of group LS. Two patients of groups L and LE had multiple pregnancy and 5 women had abortion for a rate of 5.6%/woman and 2.3%/cycle.

**Conclusion:** Letrozole OS did better to prepare for IUI in infertile couple secondary to male subfertility. ES prior to IUI improved the chances of clinical pregnancy and could be an alternative to OS whenever OS had failed or contraindicated. ES after letrozole OS followed by IUI augments the CPR up to 53.3%/patient.

**Key Words:** Clinical pregnancy rate, Endometrial scratch, Intrauterine insemination, Letrozole ovarian stimulation, Male factor infertility.

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## INTRODUCTION

High number of infertile people requires assisted reproduction techniques (ART)<sup>[1]</sup>. The frequently used fertility treatments for couples with male subfertility include intra-uterine insemination (IUI), in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI)<sup>[2]</sup>.

IUI involves the direct intrauterine placement of sperm at time of ovulation in natural menstrual cycle or following ovarian stimulation (OS)<sup>[3]</sup>. IUI procedure increases the chance for maximum number of healthy sperm to reach the site of fertilization and efficiently performed IUI gives pregnancy rates equally good to IVF<sup>[4]</sup>. Moreover, IUI yields a higher cumulative pregnancy rate than intra-cervical insemination in both natural and stimulated cycles<sup>[5]</sup>.

Embryo implantation is a critical step in establishment of pregnancy<sup>[6]</sup>. Endometrial receptivity refers to the ability of endometrium to accept and accommodate endometrial embryo implantation during the implantation window period<sup>[7]</sup> which extends between days 5 and 10 following the luteinizing hormone surge<sup>[8]</sup>. Endometrial receptivity is an important factor affecting the rate of blastocyst implantation in ART<sup>[9]</sup> and in recurrent implantation failure of endometrial origin a displaced window of implantation leading to desynchronization between the blastocyst and the endometrium<sup>[10]</sup>.

Aromatase is a microsomal member of the cytochrome P450 hemoprotein-containing enzyme complex superfamily<sup>[11]</sup> that synthesizes estrogens by catalyzing the three consecutive hydroxylation reactions to convert C19 androgens to aromatic C18 estrogenic steroids<sup>[12]</sup>.

Aromatase is expressed in a tissue-specific manner and its activity can be demonstrated in several tissues, including the endometrium<sup>[13]</sup>, ovaries<sup>[14]</sup>, breast and estrogen-dependent breast cancer<sup>[15]</sup>.

### Hypothesis

This study hypothesized that letrozole (LET) ovarian stimulation (OS) followed by endometrial scratch (ES) before IUI improves its outcome than each intervention performed separately.

### AIM OF WORK

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To compare the outcome of IUI after letrozole OS followed by ES versus after each intervention separately.

*Setting:* Tanta and Benha University Hospitals

*Design:* Comparative multicenter clinical trial

### PATIENTS AND METHODS

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The study protocol was approved by the Local Ethical Committees. All couples had primary infertility for >2years secondary to male factor and had previous failed IUI or expectant trials were eligible for evaluation. Females with hyperprolactinemia, thyroid dysfunction, Cushing's syndrome, congenital adrenal hyperplasia, adrenal tumor or ovarian tumor, autoimmune disease, malignancy, central nervous system disease, infertility for causes other than male factor infertility were excluded from the study. Also, women with intrauterine anomalies were excluded from the study. Males with hormonal abnormalities, obstructive vas lesions, processed total motile sperm count (PTMS) of <5 million<sup>[16]</sup>, infection manifested as high total leucocytic count or presence of viable microorganisms or giving growth on culture test were excluded from the study. Couples with duration of infertility of <2 years or infrequent intercourse or gave positive immunological tests were also excluded.

All women underwent clinical examination and transvaginal ultrasonographic (TVU) examination and hormonal profile including serum LH, FSH, prolactin and E2 levels for assurance of inclusion and exclusion criteria. Evaluation of male partner included clinical examination, scrotal ultrasonographic examination, semen analysis,

immunological studies and culture test, and serum hormonal profile including serum LH, FSH and testosterone.

Enrolled couples were randomly, using sealed envelopes labeled by the group title, prepared by a blinded assistant and chosen by the couples into three groups: Group L included females assigned to receive oral letrozole 5 mg from day 3 to 7 of the menstrual cycle. Group S included females subjected to ES injury. Group LS included females received oral letrozole 5 mg from day 3 to 7 of the menstrual cycle followed by ES injury. For all groups, ovulation was monitored and assured using TVU for evident ovulation with a dominant follicle size >18 mm.

Endometrial scratch technique was done as described by Maged *et al.*<sup>[17]</sup> through exposing the cervix using sterile speculum then the cervix was cleaned with sterile gauze moistened with saline. A thin catheter was introduced through the internal os then the endometrium was gently scratched by moving the catheter up and down 3 times. Patient was informed for the possibility of feeling mild cramping menstrual-like pain during the scratch and mild spotting after catheter withdrawal.

Semen was obtained after 2-3 days abstinence and sperms were prepared according to Density Gradient Sperm Wash Method and processed  $\geq 36$  hours after tracking of the dominant follicles<sup>[18]</sup>. IUI was repeated for three cycles and clinical pregnancy rate (CPR) and abortion and multiple pregnancy rates were recorded.

### Statistical analysis

Obtained data were presented as mean $\pm$ SD, numbers and percentages. The results were analyzed using paired t-test and Chi-square test (X2 test). Statistical analysis was conducted using the IBM SPSS (Version 23, 2015) for Windows statistical package. *P value* <0.05 was considered statistically significant.

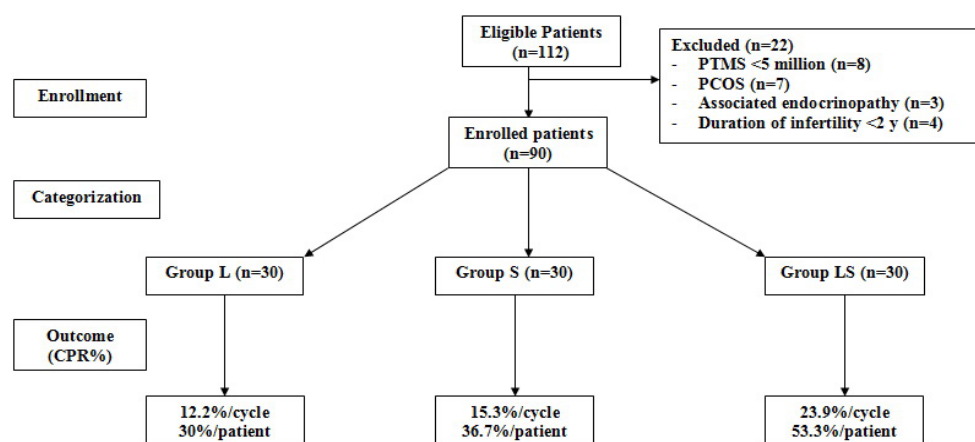
### RESULTS

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The study included 112 women with primary infertility for >2years secondary to male factor and had previous failed IUI or expectant trials; 22 women were excluded and 90 women were included in the study (Fig. 1). There was non-significant (*p*>0.05) difference between studied groups as regards data of enrolled women as shown in Table 1.

**Table 1:** Patients' data recorded at time of study enrollment

Data	Group L	Group S	Group LS	P=	
Age (years)	28.5±3	28.3±2.6	28.6±3.7	0.933	
Duration of infertility (years)	4.8±0.8	4.5±1.1	4.4±1.2	0.358	
BMI parameters	Body weight (kg)	79±8.3	78±9.7	80.7±9.3	0.515
	Body height (cm)	168.2±1.8	169.6±2.9	168.7±2.8	0.104
	BMI (kg/m <sup>2</sup> )	27.9±2.9	27±3.4	28.4±3.2	0.326
Number of previous cycles	2±0.7	2.2±0.6	2.1±1	0.616	
Baseline serum hormonal levels	FSH (mIU/ml)	6.1±2.4	5.96±3	6.35±2.8	0.859
	LH (mIU/ml)	8.22±2.3	7.87±1.89	8.03±2	0.808
	Prolactin (ng/ml)	29.72±23.4	30.3±20.1	31.27±19.3	0.930
	E2 (IU/ml)	54.14±16.4	56.6±16.9	59.2±15.3	0.477

**Fig. 1:** Flow chart of the study

The studied women underwent 213 IUI cycle; 40 had three, 43 had two and 7 had only one cycle with non-significant ( $p>0.05$ ) difference between studied groups as regards patients' distribution between number of cycles and median number of performed cycles. Studied women gave 287 follicles of >18 mm with a mean number of

follicles of 3.18/female and 1.35/cycle. Mean number of follicles and follicle/cycle was significantly ( $p<0.05$ ) lower in women of group S than other groups. Mean endometrial thickness was significantly ( $p=0.003$ ) lower and E2 serum levels were significantly higher ( $p=0.0022$ ) in women of group S compared to women of other groups (Table 2, Fig.2).

**Table 2:** IUI cycle data of studied patients

Data	Group L	Group S	Group LS	P=	
No of cycles completed	3 cycles	16 (53.4%)	14 (46.7%)	10 (33.3%)	0.632
	2 cycles	12 (40%)	14 (46.7%)	17 (56.7%)	
	One cycle	2 (6.6%)	2 (6.6%)	3 (10%)	
	Total	74	72	67	
No of follicles >18 mm	Median (IQR)	3 (2-3)	2 (2-3)	2 (2-3)	0.345
	Total	3.3±1	2.8±0.8	3.4±1.2	0.035
Endometrial thickness (mm)	Follicle/cycle	1.3 (1-1.5)	1 (1-1.3)	1.5 (1-1.7)	0.015
	Total	9.1±1.4	7.7±1.3	9±1.5	0.003
E2 (IU/ml)	222.3±54.9	260.7±46	216±57.2	0.0022	
Procedural complications	Cramping pain	0	2 (6.6%)	3 (10%)	
	Spotting	0	2 (6.6%)	4 (13.2%)	

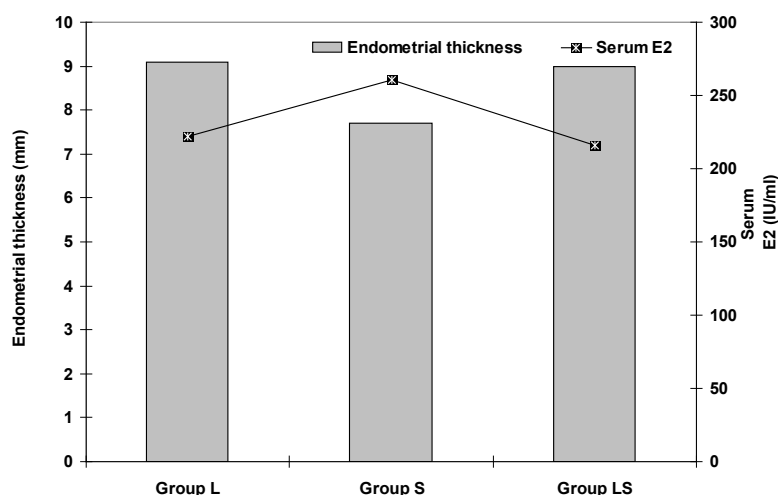


Fig. 2: Mean endometrial thickness and serum E 2 levels estimated after os

Clinical pregnancy was detected in 37 women for a total CPR of 41.1%/woman and 17.4%/cycle with significant (p=0.037 and 0.043, respectively) difference in favor of LS group. No patient developed ectopic pregnancy, but two patients of letrozole groups had multiple pregnancy. Unfortunately, during the 1st trimester 5 women had abortion for a rate of 5.6%/woman and 2.3%/cycle (Table 3, Fig. 3).

Table 3: IUI cycle outcome data of studied patients

	Group L	Group S	Group LS	P=
Total clinical pregnancy	9	11	17	0.162
Clinical pregnancy/cycle	12.2%	15.3%	25.4%	0.043
Clinical pregnancy/patient	30%	36.7%	56.7%	0.037
Ectopic pregnancy rate	0	0	0	0
Multiple pregnancy rate	1 (11.1%)	0	1 (6.3%)	0.675
Abortion rate	1 (11.1%)	2 (18.2%)	2 (12.5%)	0.881

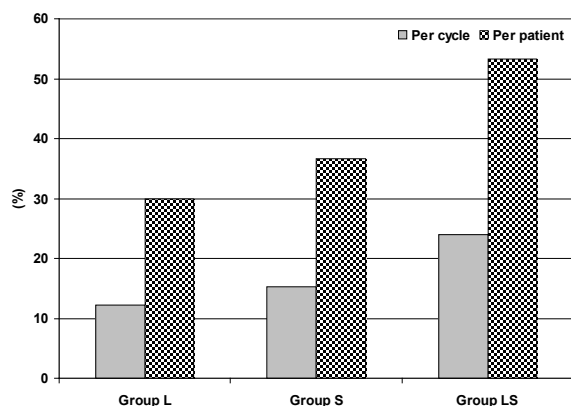


Fig. 3: Clinical pregnancy rates of studied patients

## DISCUSSION

The current study relied on ovarian stimulation (OS) for preparation of patients of the two groups (Group L and LS) prior to IUI. In line with this policy to prepare women especially whose husbands were subfertile so as to increase the chance for these women to get pregnant, Peeraer *et al.*<sup>[19, 20]</sup> documented that OS with low-dose human menopausal gonadotrophin is superior to clomiphene citrate (CC) in IUI cycles with respect to CPR and Farquhar *et al.*<sup>[21]</sup> found IUI with OS is a safe and effective treatment with higher cumulative live birth rate (LBR) for women with unexplained infertility and an unfavorable prognosis for natural conception.

Letrozole (LET), an aromatase inhibitor, was used for OS to prepare patients of groups L and LS prior to IUI. In support of the choice of letrozole for OS, Bjelica *et al.* (22) detected statistically significant difference in endometrial thickness, 1st IUI cycle and 3-IUI cycle CPR between letrozole over CC combined with metformin in moderately obese patients with polycystic ovary syndrome (PCOS) who are resistant to stimulation with CC alone. Also, El Hachem *et al.*<sup>[23]</sup> detected no differences in LBR/ IUI cycle and cumulative LBR between CC and letrozole, but multiple pregnancy rates was lower with letrozole.

Recently, Franik *et al.*<sup>[24]</sup> out of literature review reported that letrozole appears to improve live birth and pregnancy rates in subfertile women with anovulatory PCOS, compared to CC with similar rates of OHSS, miscarriage or multiple pregnancy rates. Also, Huang *et al.*<sup>[25]</sup> performed 14,519 IUI cycles and found LBR were significantly higher in IUI cycles stimulated by CC (8.9%), letrozole (9.4%) and gonadotropins (9.5%) versus 6.2% with natural-cycle IUI and the cumulative 3-IUI cycles LBR was 25.7%, 26.2%, and 23.7% with the use of CC,

letrozole, and gonadotropins, respectively, versus 18.4% with natural-cycle IUI.

The current study detected beneficial effects of OS prior to IUI manifested as significantly higher number of follicles and follicle/cycle with significantly higher endometrial thickness and lower E2 serum levels in women received OS (groups L and LS) compared to women who underwent IUI without OS (group S). These data supported that previously reported<sup>[19, 20, 22, 23]</sup> and go in hand with the recently detected in literature<sup>[21, 24, 25]</sup> concerning the use of OS prior to IUI.

Endometrial scratch (ES) was used to augment the possibility of success of IUI trial in women with (Group LS) and without (Group S) OS; such assumption go in hand with multiple previous studies<sup>[26, 27, 28, 29, 30]</sup> assured the beneficial effect of ES and documented that endometrial injury can improve implantation rate, CPR and LBR in couples with unexplained infertility especially those with recurrent implantation failure after ART cycles. Recently, Viera-Molina *et al.*<sup>[1]</sup> considered endometrial scratching as an effective surgical intervention for infertile women who require ART.

The proposed possibility to improve outcome of IUI using ES was evident where the CPR/cycle and per patient was non-significantly higher in patients of group ES than in patients of group L (15.4% vs. 12.2% and 36.7% vs. 30%, respectively). These findings spot light on the near equality of the results obtained by either OS or ES alone, but was in favor of ES. Similarly, Ashrafi *et al.*<sup>[31]</sup> tried to determine the effect of ES on pregnancy rate (PR) in women with IUI failure versus no intervention and reported chemical PR of 10.7% vs. 2.7%, respectively. These findings point to a fact that application of ES solo is better than OS alone, and so ES alone could be used whenever OS failed, contraindicated or inaccessible.

However, the complementary beneficial effect of both OS and ES (group LS) was superior to the effect of each intervention separately where CPR/cycle and per patient were significantly higher than in group L, but non-significantly higher than in group S.

These findings go in hand with Maged *et al.*<sup>[17]</sup> who reported significantly higher cumulative PR in patients received OS and ES than those had OS alone (39% vs. 18.2%) with significantly higher PR at the second and third trials in combination group. Also, Goel *et al.*<sup>[32]</sup> detected significantly higher CPR with combination of OS and ES than with OS alone in women with male factor or unexplained infertility underwent IUI and concluded that ES can be used as a low cost-effective tool to improve clinical pregnancy and ongoing pregnancy rate in stimulated IUI cycles. Thereafter, Wadhwa and Mishra<sup>[33]</sup> found ES in conjunction with controlled OS is a cost-effective and easy technique which may improve CPR in previous controlled

OS failure cycles and Vitagliano *et al.*<sup>[34]</sup> suggested that ES performed once, preferably during the follicular phase of the same cycle of IUI with flexible aspiration catheters, may improve clinical pregnancy and ongoing pregnancy rates in stimulated IUI cycles.

Concerning the timing of ES, women of group LS received 5-day OS from D3-D7 of menstrual cycle and than ES was performed, in line with this timing, Wadhwa and Mishra<sup>[32]</sup> compared pregnancy outcome by ES in early (D2-D4) and late follicular phases (D7-D9) of the same stimulation cycle versus no intervention and found CPR per protocol analysis 13.46% and 19.57% with early and late ES, respectively, versus 15.69% with no intervention.

## CONCLUSION

Letrozole OS did better to prepare for IUI in infertile couple secondary to male subfertility. Endometrial scratch prior to IUI improved the chances of clinical pregnancy and could be an alternative to OS whenever OS had failed or contraindicated. ES after letrozole OS followed by IUI augments the CPR up to 53.3%/patient. Wider scale study for application of these results for males with sperm count <5 million are recommended to evaluate these results for this patients as trial to replace the more complicated and costly ART procedures.

## CONFLICTS OF INTEREST

There are no conflicts of interest.

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