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2 (63) 2022 : 001-05 • ORIGINAL RESEARCH

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

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

Design: A prospective cohort study on 60 women with infertility attending the outpatient clinic in 2 hospitals in Saudi Arabia and undergoing intrauterine insemination preceded by ovulation induction with letrozole. Our study was conducted from January 2021 to December 2021.

Methods: Patients involved in this study were subjected to ovulation induction with letrozole 5 mg beginning from day 3 of the cycle for 5, folliculometry began on day 10-12 to monitor follicular size and endometrium morphology till the trigger timing by hCG. IUI was performed after hCG administration by 36 hours. On the day of the trigger, the peri-follicular vascularity, endometrial thickness, endometrial pattern, and the number of mature follicles were measured using a 2D transvaginal doppler. Pregnancy is confirmed by a blood test.

Results: The study showed that according to the modified grading system, the distribution of all follicles studied (all >17 mm in diameter) was grade1 (n=3), grade 2 (n=11), grade 3 (n=32), grade 4 (n=14), 14 patient got pregnant while 46 cases did not get pregnant (non-pregnant group). The mean baseline level of serum FSH concentration also tended to be high in the low-grade vascularity cycle, but this result was insignificant. There was giving a pregnancy rate of 16.7%, the follicles of high-grade vascularity were associated with a high pregnancy rate (grade 4=57%), (grade 3=18.8%), then cycles with low-grade vascularity with no pregnancy occur in grade 1 and 2 vascularity group (grade 1, 2=0%) this result is significant. Six pregnant patients had an endometrial thickness of 8-10 mm, 8 patients had an endometrial thickness of 10-12 mm, and zero patients had an endometrial thickness <8 mm, In our study the pregnancy rate was 42.9% with a distinct five-line endometrial pattern, 22.9% 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

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Word count: 2169 **Tables:** 04 **Figures:** 00 **References:** 16

Received: 01.06.2022, Manuscript No. gpmp-22-55037; **Editor assigned:** 03.06.2022, PreQC No. P-55037; **Reviewed:** 17.06.2022, QC No. Q-55037; **Revised:** 24.06.2022, Manuscript No. R-55037; **Published:** 30.06.2022

INTRODUCTION

Infertility is a major problem affecting about 15 % of couples. About 20% are unexplained infertility. Unexplained infertility is a diagnosis of exclusion when there is no apparent cause after investigating the male and female factors. Immunologic factors compose 20% of causes of unexplained infertility [1].

Intrauterine insemination (IUI) has long been established as an assisted reproductive technique (ART). It is considered an empirical treatment for managing unexplained infertility, IUI is an effective, inexpensive procedure with low cost and complications [2].

The use of letrozole in ovarian stimulation in IUI cycles has shown good results regarding the pregnancy rate that varied between 6% and 22% in different studies. But with increasing concern regarding ovarian hyperstimulation syndrome and multiple pregnancies [3-5].

Gore MA, et al. [6] described that increased perifollicular blood flow can be estimated in the perifollicular period by color and pulsed Doppler, an automated measure 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 hypothesizes that those follicles containing oocytes able to produce pregnancy have a more uniform perifollicular vascular network. [6]

The extension of vascularity was graded according to the modified grading system. The grading system measured the percentage of follicular circumference in which flow was identified from a single cross-sectional slice. The grading system was as follows: G1, with vascularity ≤ 25% of follicular circumference; G2, with vascularity between 26% and 50% of follicular circumference and, G3 between 51% and 75% of follicular circumference; and G4 with vascularity >75% of the follicular circumference. The periovulatory follicles were classified as high (grades 3–4) or low grade (grades 1–2) [7,8].

Aim of Work

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

PATIENTS & METHOD

Design

A prospective cohort study on 60 women with infertility attending the outpatient clinic in 2 hospitals in Saudi Arabia and undergoing ovulation induction by letrozole and intrauterine insemination. the study was conducted from January 2021 to December 2021.

The Inclusion criteria of women indicating IUI (Sperm count of husband ≥ 10 million, normal hysterosalpingography (HSG), normal hormonal female profile. The women selected are those aged 20-35 years and BMI between 20-30 kg/m².

The Exclusion criteria are any sperm count <10 million, closed Fallopian tubes by HSG, or advanced stage of endometriosis as diagnosed by laparoscopy or cases of ovarian cysts and previous failed IUI cycles.

All patients participating in this study were subjected to full medical, gynecological, and surgical history taking. General, local, and ultrasound examinations were performed for all 60 patients. Hormonal profiles were done including day 2 FSH, LH, prolactin, and TSH. Hysterosalpingography and husband semen analyses were evaluated.

Patients involved in this study were subjected to ovulation induction with letrozole 5 mg beginning from day 3 of the cycle for 5 days, folliculometry began on day 10-12 to monitor follicular size and endometrium morphology till the trigger timing by hCG. HCG 10000 IU was injected intramuscularly, and HCG injection was canceled if there are more than 3 follicles with a diameter ≥ 17 mm. IUI was performed after hCG administration by 36 hours. On the day of the trigger, the peri-follicular vascularity, endometrial thickness, endometrial pattern, and the number of mature follicles were measured using a 2D transvaginal doppler. To study the perifollicular vascularity, the sonographer used the power Doppler box to position each growing ovarian follicle and capture the cross-section image of the follicle with the maximum color indication. The vascularity was grading used in the same way as Bhal et al., study. Where grade 1 when vascularity is less than 25% of circumference, grade 2 between 26-50%, grade 3 between 51-75%, and grade 4 greater than >75%, grade one and grade two were considered as low-grade vascularity while grade three and grade four were considered high grade [7].

Day of IUI

The semen specimen was collected and was prepared within one hour, the semen was diluted in Ham's medium 1:2. It was centrifuged for 10 min and then the pellets were suspended and diluted in 1:1 with medium and centrifuged for ten minutes and then re-suspended and placed in the incubator at temperature 37°C in 5% Carbon dioxide in the air for 30 minutes, then transferred to the insemination catheter to be inseminated intrauterine [9]. The patients were given luteal support with progesterone for 14 days and a pregnancy test was performed at the end of two weeks.

Outcome measures

1) Primary outcome: the perifollicular vascularity grades of growing follicles, number, and size of follicles, endometrial thickness, and patterns
2) Secondary outcome: Pregnancy rate

Sample size justification

The study included the women who met the inclusion criteria during the period from January 2021 to December 2021.

Statistical analysis

The collected data were revised, coded, tabulated, and studies Statistical Package for Social Science (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp). The tests used were Student's t-test (for numeric parametric variables), Mann-Whitney's U test, Fisher exact test (for numeric non-parametric variables), and chi-squared test (for categorical variables). P value <0.05 was considered significant.

RESULTS

Initially, 74 cases were included in the study, 14 cases were excluded as follows; 10 cases were nonresponders to ovarian stimulation and 4 cases were canceled because of the risk of hyperstimulation syndrome. A total of 60 women were finally enrolled in our study.

The mean age was 30.46 ± 4.60 years, the mean BMI was 26.70 ± 4.02 kg/m², the duration of infertility in years was 5.17 ± 2.60, and Primary infertility was present in 33 patients while secondary infertility was present in 27 patients (data not tabulated).

Infertility due to ovulatory disorder was found to be 58.3% and 21.7% due to male factor, and unexplained infertility was found to be 18.3% (Data not tabulated.).

Tab. 1. shows the different hormonal and number of follicles in the US on the day of the HCG trigger. Among the 60 patients enrolled in the study 14 patients got pregnant (23.33%).

The different percent's of the grading system of ovulatory follicles induced by letrozole were as follows; G1 3 cases (5.0%), G2 11 cases (18.3%), G3 32 cases (53.3%) and G4 14 cases (23.3%) so high-grade vascularity follicles were present in 46 cases (G3+G4). (Data not tabulated.)

Tab. 2. showed that the high-grade vascularity follicles were associated with a high pregnancy rate (grade 4=57.1%), (grade 3=18.8%), while no pregnancy occurred with those with low-grade vascularity follicles, these results were statistically significant. **Tab. 3.** Shows the Comparison between pregnant and non-pregnant patients according to endometrial thickness revealed a significant relationship between endometrial thickness and pregnancy rates, the highest (36.4%) with endometrial thickness ranging from 10 to 12 mm (p <0.05). **Tab. 4.** shows the relation between perifollicular vascularity and endometrial pattern

and fecundability rate. A statistically higher pregnancy rate (42.9%) was reported in women with distinct five-line appearances (p <0.05). Patients with no endometrial layering had poor pregnancy rates (0%) and 22.9% of pregnancy was seen with a hazy five-line appearance (**Tab. 3**. and **Tab. 4.**).

DISCUSSION

The results of the current study and their comparison to similar studies.

Ultrasound parameters have been conducted in various studies to predict pregnancy outcomes during ART [10]. The current study aimed to assess the effect of letrozole-stimulated IUI cycles regarding perifollicular vascularity and finally their effect on successful pregnancy outcomes.

In the current study, pregnancy occurred in 14 patients (23.33%) of the total 60 infertile women with letrozole-induced IUI cycles. This was higher than other studies where their rate was about 12-20% [11-15].

In the current study, the perifollicular vascularity was attributed to a higher pregnancy rate, where there was no pregnancy achieved with low-grade perifollicular vascularity (grade 1 or grade 2). The pregnancy rate was 18.8% with grade 3 perifollicular vascularity and 57.1% with grade 4 perifollicular vascularity. These results are in accordance with the study of Bhal, et al. [7]. Ragni G, et al. [3] confirm that perifollicular vascularity could predict the likelihood of conception in patients undergoing ovarian stimulation and IUI cycles where the pregnancy rates in their study were 14.1% in low-grade, 10.0% in medium grade, and 11.8 percent in high-grade perifollicular vascularity groups.

Several researchers have studied the effect of endometrial pattern and thickness as a predictor of pregnancy outcome with conflicting findings. Endometrial thickness was measured in our study with results showing that no pregnancy was reported with endometrial thickness less than 8 mm, while 6 patients get pregnant when the

Tab. 1. Basal investigation of studied group.

Parameter		Total (60)
Day 3 FSH (IU/ml) (Mean ± SD)		6.63 ± 0.78
LH (mIU/ml) (Mean ± SD)		7.67 ± 0.30
PROLACTIN (mIU/ml) (Mean ± SD)		12.31 ± 7.57
Ovarian volume (cm^3) (Mean ± SD)		6.41 ± 2.12
Basal endometrium (mm) (Mean ± SD)		5.51 ± 0.61
No. of follicles more than 17 mm	One follicle	22 (36.7%)
	Two follicles	19 (31.7%)
	Three follicles	19 (31.7%)
Using: t-Independent Sample t-test ; p-value >0.05 is insignificant; *p-value <0.05 is significant; **p-value <0.001 is highly significant		

Tab. 2. Grading of follicles according to perifollicular vascularity.

Parameter	Grade	Pregnant (n=14)	Non- pregnant (n=46)	x^2	p-value
Perifollicular vascularity	Grade 1 (n=3)	0 (0.0%)	3 (100.0%)	13.583	0.004*
	Grade 2 (n=11)	0 (0.0%)	11 (100.0%)		
	Grade 3 (n=32)	6 (18.8%)	26 (81.3%)		
	Grade 4 (n=14)	8 (57.1%)	6 (42.9%)		
Using: x^2: Chi-square test; *p-value <0.05 is significant					

Tab. 3. Comparison between pregnancy and non-pregnancy according to endometrial thickness.

Variables		Total (n=60)	Pregnant (n=14)	Non-pregnant (n=46)	x^2	p-value
Endometrial Thickness	10-12 mm	22 (36.7%)	8 (36.4%)	14 (63.6%)	6.386	0.041*
	8-10 mm	24 (40.0%)	6 (25%)	18 (75%)		
	Less than 8 mm	14 (23.3%)	0 (0.0%)	14 (100%)		
Endometrial layering	No layering	11 (18.3%)	0 (0.0%)	11 (100.0%)	x^2:14.048	<0.001**
	Hazy five line	35 (58.3%)	8 (22.9%)	27 (77.1%)		
	Distinct five line	14 (23.3%)	6 (42.9%)	8 (57.1%)		
Endometrial thickness in mm on day of HCG (Mean±SD)			10.22±1.48	9.02±1.61	t:2.485	0.016*
Using: x^2: Chi-square test; *p-value <0.05 is significant; **p-value <0.001 is highly significant						

Tab. 4. Relation between perifollicular vascularity and endometrial pattern and fecundability rate.

	No endometrial Layering	Hazy Five Layer endometrium	Distinct Five Line endometrium	x^2	P-value
Grade 1 (n=3)	3 (100.0%)	0 (0.0%)	0 (0.0%)	10.376	0.006*
Grade 2 (n=11)	3 (27.3%)	3 (27.3%)	5 (45.5%)	2.248	0.325
Grade 3 (n=32)	5 (15.6%)	26 (81.3%)	1 (3.1%)	18.449	<0.001**
Grade 4 (n=14)	0 (0.0%)	6 (42.9%)	8 (57.1%)	8.802	0.012*
#Fecundability: Ability that single cycle will result in pregnancy. Using: x2: Chi-square test; p-value >0.05 is insignificant; *p-value <0.05 is significant					

endometrial thickness was 8-10 mm and 8 patients got pregnant with endometrial thickness 10-12 mm.

In the study of Friedler S, et al. [4] concerning endometrial thickness, they included 25 reports including 2665 ART; they found a statistically significant difference in mean endometrial thickness between conception and non-conception cycles in 1203 cycles, but not in 331 cycles. They concluded that an endometrial thickness of 6 mm had a high negative predictive value (NPV) for pregnancy.

In the present study, the multilayer endometrial pattern was significantly higher in pregnant and non-pregnant patients. These results are in agreement with the study of Jang and Jee [16], where they found that a multilayer endometrial pattern has a low PPV (positive predictive value) for pregnancy (33.1%). In contrast, the absence of this multilayered pattern has a negative predictive value (NPV, 85.7%), decreasing the chance of conception.

In the present study, the pregnancy rate was 42.9% with a distinct five-line endometrial pattern and 22.9 % with a hazy five-line endometrial pattern. It was 0% with no endometrial layering. This was in accordance with a review of Friedler S, et al. [4] who found a statistically significant difference in the endometrial patterns of conception and non-conception cycles in 2892 cycles.

STRENGTHS AND LIMITATIONS OF OUR STUDY

The study's main strength was that the procedures were done by two same-level gynecologist consultants regarding stimulation and IUI. In contrast, sonography was done by other radiologist consultants so that statistical bias was reduced. The limitation of the study is the relatively small number of patients.

CLINICAL IMPLICATIONS OF THE STUDY

Perifollicular vascularity results could allow us to decide whether to cancel 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 applying this form of prospective management of treatment cycles.

Recommendation for further research; further randomized studies on a large number of patients are needed to study the effects of letrozole-induced IUI-cycles on perifollicular vascularity and subsequent pregnancy outcome.

CONCLUSION

Perifollicular vascularity appears to be an important factor in determining the outcome of stimulated IUI cycles, and may have a clinical impact on assisted reproduction techniques. As there were no pregnancies in women with low-grade perifollicular vascularity, identifying grades of vascularity would be valuable early in cycles (before HCG/IUI).

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