Ovarian Response can be Predicted in Women with PCOS who have Ovulation Induction with Letrozole

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ABSTRACT

Objective: To compare the clinical, metabolic, and hormonal characteristics of patients with and without selected dominant follicles in infertile women with PCOS who used letrozole for ovulation induction.

Study Design: A descriptive cohort study.

Place and Duration of Study: Department of Obstetrics and Gynaecology of Bagcilar Research and Training Hospital, Istanbul, Turkey, from October 2019 to November 2021.

Methodology: Eighty-eight female patients with PCOS, who underwent ovulation induction by giving 5 mg/day letrozole, were screened for demographic characteristics, laboratory values, and dominant follicle development. Those who were given letrozole as the first treatment agent, those who took clomiphene citrate (CC) and started letrozole the following month, and those who were treated with letrozole and given letrozole again were recorded separately. Seventy-five patients responded to letrozole and developed a dominant follicle; 13 patients did not develop a dominant follicle. Threshold values were determined for statistically significant parameters between patients with and without dominant follicles.

Results: Follicle development occurred in 85.2% of the women. A statistically significant variable in clinical and metabolic values, between ovulating and non-ovulating groups could not be found. There was a significant difference between the two groups for the serum AMH value, total testosterone value, and FSH level. The authors found that follicle response was higher in those with AMH values less than 11.89 ng/mL, FSH levels higher than 6.25 Iu/L, and total testosterone less than 0.96 ng/mL. In this study, the pregnancy rate was found to be lower than in the literature (11%).

Conclusion: Among the women with PCOS who had ovulation induction with letrozole, follicle development was higher in women with lower FSH, androgen and AMH values.

Key Words: *Letrozole, Aromatase inhibitor, Androgens, Ovulation induction, AMH.*

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INTRODUCTION

Twenty five percent of infertile couples have ovulation problems[1] with polycystic ovary syndrome (PCOS) being the most common cause of ovulation problems, at rates ranging 70-85%.[2]

Although letrozole was previously used in animal experiments for ovulation induction (OI),[3] it started being used for ovulation induction in PCOS patients in the early 2000s.[4] In subsequent studies, it has been suggested that it is more effective than clomiphene citrate (CC) in PCOS patients.[5] Its use as an OI agent is controversial in cases of endometriosis and infertility.[6]

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In the literature, the ovulation rate in ovulation induction treatments with letrozole varies between 62% and 84%. The pregnancy rate with letrozole is 27%. The 2018 ACOG recommendation (Committee Opinion No. 738) is to use letrozole as the first choice for women with infertile PCOS and OI in unexplained infertility.[7-9]

Data on whether there will be an ovulation response with letrozole in infertile patients diagnosed with PCOS are limited. However, in some studies, some patients were unresponsive to letrozole.

The objective of this study was to compare the clinical, metabolic, and hormonal characteristics of patients with and without selected dominant follicles in infertile women with PCOS who used letrozole for ovulation induction.

METHODOLOGY

This retrospective study was initiated at the Department of Obstetrics and Gynaecology of Bagcilar Research and Training Hospital, Istanbul, Turkey, with the approval of the ethics committee, dated 23/12/2021, and decision number 1327. Between October 2019 and November 2021, 88 infertile women

with PCOS aged 20-37 years, who were followed up and treated in the infertility outpatient clinic of a tertiary centre, were recruited. The diagnoses of PCOS were made according to the Rotterdam criteria.[2]

Women with endocrine disease, autoimmune disease, patients with ovarian cysts, those who previously used oral contraceptives, and patients who received ovulation induction following assisted reproductive technology treatment were excluded from the study.

The age, body mass indexes (BMI), and previous pregnancy statuses of all the women were recorded. FSH, LH, E2, 17 OHP, DHEA-SO4, total testosterone, AMH, and HOMA-IR tests were recorded on one of the second and fourth days of menstruation. Data were compared as dominant follicle in the developing and non-developing groups.

An Esaote® brand Mylab X7 model ultrasonography device with vaginal probe ultrasonography (TVUSG) was applied to each patient and both ovaries were evaluated for the presence of antral follicles and cysts prior to starting the medicine. Letrozole was administered orally at 5 mg daily between the third and seventh days of the menstrual cycle.[10] Following the end of the drug administration, TVUSG control was performed every two days. The newly detected anechoic, thin-walled structure of 18 mm and above was recorded as the dominant follicle. Dominant follicle follow-up was extended until the twenty-first day of maximum menstruation. Two hundred and fifty microgram/0.5 mL choriogonadotropin alfa was administered subcutaneously to women who developed dominant follicles. Intrauterine insemination (IUI) was performed 34-36 hours later in all patients who developed follicles. IUI was performed using a Medbar catheter under outpatient conditions without any difficulties. BHCG was requested for women who did not menstruate on the expected menstrual date, and menstruating women and non-pregnant women were checked with TVUSG. If letrozole was started again, 5 mg per day was started.

Although TVUSG was performed until the twenty-first day of maximum menstruation, it was accepted that there was no follicle development in patients who did not have 18 mm or larger follicles. Development of the dominant follicle was considered as the outcome measure.

Mean Standard Deviation, Median, and IQR values were given in descriptive statistics for the continuous data, and number and percentage values were given in the discrete data. The Shapiro-Wilk test was used to examine the conformity of the continuous data to the normal distribution. In the comparison of biomarker values between developing and non-developing follicles, an independent samples t-test was used on the data showing a normal distribution. The Mann-Whitney U test was used for the data that did not show a normal distribution in the comparison of biomarker values between those with and without follicles. The authors performed the student's t-test for FSH and DHEA, and the Mann-Whitney U-test for the others. The strength of the hormone values in the follicle development was evaluated by the area under the ROC Curve (AUC). The best cut-off point was calculated using Youden's Index. The diagnostic performance of the hormone values was demonstrated by sensitivity, specificity, PPV, and NPV values. Chi-square and Fisher's Exact tests were used for group comparisons (cross tables) of the discrete variables.

The IBM SPSS version 20 (Chicago, IL, USA) program was used in the evaluations with $p < 0.05$ being accepted as the statistical significance limit.

RESULTS

A total of 88 patients aged 20-37 years (mean 28 ± 4 years) were included in the study. More than half of the patients (56%) were under the age of 30. The mean BMI of the patients was 27 ± 5 (kg/m²), and nearly half (41%, 36 women) were of normal weight. Approximately two-thirds (72%, 63 patients) of the patients were primary infertile (Table I).

Follicle development was detected in 75 (85%) of the patients using letrozole. Pregnancy occurred in approximately 13% (10 - patients) of these.

The clinical features of the patients were grouped according to whether the infertility was primary or secondary, and the order of drug use in the treatment month.

A quarter of the patients (23.9%, 21 women) used letrozole in the first treatment cycle, approximately 38% (33 women) used letrozole after CC, and the remaining 39% (34 women) used letrozole after letrozole. There was no difference between the use of letrozole and the rates of follicle development between the primary infertile and the secondary infertile patients ($p = 0.751$). The follicle development rates of patients who used letrozole as the first drug, patients who used letrozole after CC, and those who used letrozole after letrozole was similar again ($p = 0.095$).

There was no difference between the follicle development rates of patients younger than 30 years of age and patients aged 30-37 years ($p = 0.287$). The follicle development rates of normal BMI, overweight, and obese patients were found to be similar ($p = 0.081$).

The AMH values of the patients who developed follicles were found to be significantly lower than those who did not develop follicles (median 5.28 ng/mL and 16.8 ng/mL, respectively, $p < 0.001$). AUC was found significant in ROC analysis for AMH values ($p < 0.001$) and the threshold value for the AMH value was determined to be 11.89 ng/mL. While the rate of follicle development was 97% in patients with an AMH value less than 11.89 ng/mL, the rate of follicle development was 48% in patients with a higher AMH value ($p < 0.001$, Tables II, III, Figure 1).

The FSH values of the patients with follicles were found to be higher than those without follicles (mean 7.04 IU/L and 5.82 IU/L, respectively, $p = 0.02$). In the ROC analysis, the threshold value for FSH was found to be 6.25 IU/L in those who developed follicles with the rate of follicle development being 94% at values above this ($p < 0.01$, Table III).

Table I: Comparison of age, BMI, pregnancy, and hormone cutoff values and follicle development rates of those with and without follicles.

Patient characteristics	Total (n = 88)	Dominant follicle developed (n = 75)	Dominant follicle did not develop (n = 13)	p-value
Age (years)				
<30	49 (55.7%)	40 (81.6%)	9 (18.4%)	0.287
30-37	39 (44.3%)	35 (89.7%)	4 (10.3%)	
BMI (kg/m²)				
18-25 normal	36 (40.9%)	34 (94.4%)	2 (5.6%)	0.081
25-30 overweight	27 (30.7%)	22 (81.5%)	5 (18.5%)	
>30 obesity	25 (28.4%)	19 (76%)	6 (24%)	
Infertility status				
Primary infertility	63 (71.6%)	53 (84.1%)	10 (15.9%)	0.751
Secondary infertility	25 (28.4%)	22 (88%)	3 (12%)	
Cycle				
First drug	21 (23.9%)	18 (85.7%)	3 (14.3%)	0.095
After CC	33 (37.5%)	25 (75.8%)	8 (24.2%)	
After Letrozole	34 (38.6%)	32 (94.1%)	2 (5.9%)	

• *Chi-Square / Fisher's Exact test.*

Table II: Comparison of hormone values of patients with and without follicles.

	Follicle did not develop (n = 13)	Follicle developed (n = 75)	p-value
AMH Median (IQR)	16.8 (8.4)	5.3 (5.2)	<0.001*
FSH Mean ± SD	5.8 ± 2.0	7.0 ± 1.6	0.019**
LH Median (IQR)	7.80 (3.58)	5.90 (3.60)	0.082*
E2 Median (IQR)	44.30 (22.95)	39.00 (20.10)	0.668*
17-OHP Median (IQR)	1.02 (1.83)	1.78 (2.17)	0.621*
DHEA Mean ± SD	240.7 ± 144.6	218.8 ± 88.3	0.605**
T.TEST. Mean ± SD	0.96 ± 0.3	0.58 ± 0.2	<0.001**
HOMA	2.43 (3.86)	1.89 (1.44)	0.070*

** Mann Whitney U test; ** Student's t-test.*

Table III: ROC curves of hormone values in follicle development and diagnostic performance.

	AUC 95% CI	p	Threshold	Sensitivity 95% CI Specificity 95% CI	PPV NPV
AMH	0.84 (0.69-0.99)	<0.001	<11.89	0.87 (0.77-0.92) 0.84 (0.57-0.96)	0.97 (0.90-0.99) 0.52 (0.41- 0.63)
FSH	0.68 (0.51-0.85)	0.041	>6.25	0.64 (0.52-0.74) 0.77 (0.49-0.92)	0.94 (0.86-0.97) 0.27 (0.18-0.37)
Total testosterone	0.83 (0.68-0.99)	<0.001	<0.96	0.95 (0.87-0.98) 0.69 (0.42-0.87)	0.95 (0.87-0.98) 0.69 (0.58-0.78)

The total testosterone values were also lower in those who did not develop follicles. (p <0.001). The rate of follicle development was 95% in patients with total testosterone values less than 0.96 ng/mL (p <0.001, Table III). The LH, E2, 17-OHP, DHEA, HOMA-IR values of the patients with and without follicles were not significantly different in patients with and without follicles (p >0.05).

DISCUSSION

According to the results of this study, 85% of the patients developed dominant follicles from ovulation induction with letrozole; pregnancy was achieved in 10 (13%) of these patients. A higher basal serum FSH level was found in patients with dominant follicles compared to those without follicles. In addition, patients with dominant follicles had lower AMH and total testosterone levels.

In studies in the literature, the rate of ovulation with CC in women with PCOS is between 56% and 73%, while the rate of ovulation with letrozole is between 73.08% and 93.1%.[9] In this study, there were more than 85% dominant follicle development in patients with PCOS who received letrozole.

It is known in the literature that normal basal FSH level is not useful in predicting the clinical pregnancy rate.[11] It is generally accepted that ovarian reserve is low only when the FSH level exceeds 10-12 IU/L.[12] In this study, there were lower follicle responses when FSH was below 6.25 IU/L, which is inconsistent with the literature.

Although AMH is a test that shows the ovarian reserve of women of reproductive age, it does not reflect oocyte health or the probability of conception.[13] AMH elevation is used to predict the response in patients using CC.[14] In literature, the threshold values between 3.2 ng/mL and 12.38 ng/mL were determined in the prediction of CC resistance in women with PCOS.[15] Although many studies are showing that the ovarian response to CC is reduced with high AMH, there is no defined threshold value for letrozole.[14] In this study, the women with AMH <11.9 ng/mL developed significantly more dominant follicles with the use of letrozole.

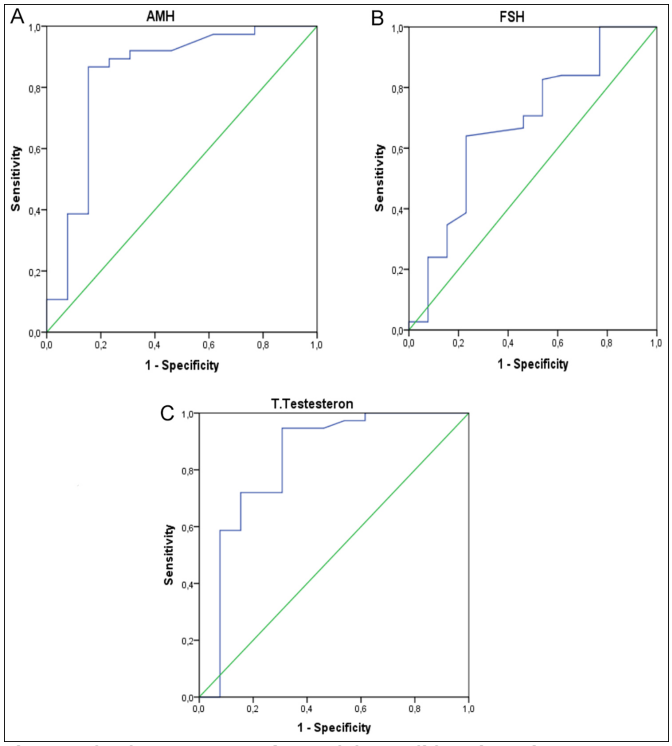

Figure 1 (a-c): ROC curve of AMH (a) FSH (b) and total testosterone (c) values in follicle formation.

According to a meta-analysis published in 2019, higher live birth rates than CC were found with the use of letrozole in women with PCOS with high total testosterone levels.[6] The present authors did not make a comparison between letrozole and CC in this study, but there was a high follicle response at low total testosterone levels, in line with the literature.

In this study, although the ovulation rates were consistent with the literature, the pregnancy rates were found to be lower than those in the literature. According to Nakajo *et al*. in a retrospective study comparing letrozole and CC, the pregnancy rate was found to be 21.8% in the letrozole 5 mg/day group.[16] In the present study, the pregnancy rate was 13.3% in the patients whose follicles were obtained. The reason for the low pregnancy rate may be the application of the IUI method, the difference in coitus frequency before and after IUI, endometrial thickness, and the endometrial stage not being taken into account. In addition, the small number of cases makes it difficult to evaluate the pregnancy rate.

In this study, a difference was found in terms of follicle development between women who took letrozole as the first drug, starting with letrozole after CC, and starting letrozole after letrozole. However, the rate of follicle selection (86%) in women who used letrozole as the first treatment option was found to be higher than in women who started letrozole after CC (76%). The highest follicle response was in patients who received letrozole followed by letrozole (94%). In the comparison between the groups, a statistically significant difference was found between the patients who received letrozole as the first drug and the patients who used letrozole after letrozole in terms of follicle response. However, patients using letrozole after CC had a lower response than patients using letrozole after letrozole. Letrozole is a short--acting agent, while CC is a long-acting agent. The low follicle response in women using letrozole after CC can be explained by the long duration of action of CC.

Despite all these successful results, letrozole has not been approved for use in ovulation induction in Turkey or around the world. Shakeel *et al*. from Pakistan in 2022 mention that professionals dealing with infertility are familiar with the use of this drug off-label. Letrozole should be given if it is mutually discussed with the patient and consent is given.[17]

In this study, approximately one-third of the patients were secondary infertile patients who had previously been pregnant. However, previous pregnancy history did not change the response to letrozole.

CONCLUSION

The study showed that letrozole is an effective ovulation induction agent with a high follicle response in all age groups and all weight groups in infertile women with PCOS. However, elevated AMH and total testosterone decrease the follicle response to letrozole.

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ETHICAL APPROVAL:
This retrospective study was performed with the permission of the clinical research ethics committee of Istanbul Medipol University, dated 23/12/2021, and numbered 1327. We obtained ethic approval prior to the initiation of the research work.

PATIENTS' CONSENT:
Informed consent was obtained from patients to publish data for this study.

COMPETING INTEREST:
The authors declared no competing interest.

AUTHORS' CONTRIBUTION:
OKA: Created and planned the working hypothesis, worked on data collection, statistical analysis, and interpretation.
HG: Worked on data evaluation, interpretation, literature search, and manuscript control.

REFERENCES

1. Carson SA, Kallen AN. Diagnosis and Management of Infertility: A review. *JAMA* 2021; **326(1)**:65-76. doi: 10.1001/jama.2021.4788.
2. Rotterdam ESHRE/ASRM-Sponsored PCOS consensus work-

shop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod* 2004; **19(1)**:41-7. doi: 10.1093/humrep/deh098.

3. Bhatnagar AS, Batzl C, Häusler A, Nogués V. The role of estrogen in the feedback regulation of follicle-stimulating hormone secretion in the female rat. *J Steroid Biochem Mol Biol* 1993; **47(1-6)**:161-6. doi: 10.1016/0960-0760(93)90070-d.
4. Mitwally MFM, Casper RF. Aromatase Inhibition: A novel method of ovulation induction in women with polycystic ovary syndrome. *Reprod Technol* 2000; **10(5)**:244-7.
5. Legro RS, Brzyski RG, Diamond MP, Coutifaris C, Schlaff WD, Casson P, *et al*. Letrozole versus clomiphene for infertility in the polycystic ovary syndrome. *N Engl J Med* 2014; **371(2)**:119-29. doi: 10.1056/NEJMoa1313517.
6. Wang R, Li W, Bordewijk EM, Legro RS, Zhang H, Wu X, *et al*. First-line ovulation induction for polycystic ovary syndrome: An individual participant data meta-analysis. *Hum Reprod Update* 2019; **25(6)**:717-32. doi: 10.1093/humupd/dmz029.
7. Yu Q, Hu S, Wang Y, Cheng G, Xia W, Zhu C. Letrozole versus laparoscopic ovarian drilling in clomiphene citrate-resistant women with polycystic ovary syndrome: A systematic review and meta-analysis of randomised controlled trials. *Reprod Biol Endocrinol* 2019; **17(1)**:17. doi: 10.1186/s12958-019-0461-3.
8. ACOG Committee Opinion No. 738: Aromatase inhibitors in gynecologic practice. *Obstet Gynecol* 2018; **131(6)**:1. doi: 10.1097/AOG.0000000000002640.
9. Sakar MN, Oglak SC. Letrozole is superior to clomiphene citrate in ovulation induction in patients with polycystic ovary syndrome. *Pak J Med Sci* 2020; **36(7)**:1460-5. doi: 10.12669/pjms.36.7.3345.
10. Casper RF, Mitwally MFM. Use of aromatase inhibitor for ovulation induction in women with polycystic ovarian syndrome. *Clin Obstet Gynecol* 2011; **54(4)**:685-95. doi: 10.1097/GRF.0b013e3182353d0f.
11. Jain T, Soules MR, Collins JA. Comparison of basal follicle-stimulating hormone versus the clomiphene citrate challenge test for ovarian reserve screening. *Fertil Steril* 2004; **82(1)**:180-5. doi: 10.1016/j.fertnstert.2003.11.045.
12. Fang T, Su Z, Wang L, Yuan P, Li R, Quyang N, *et al*. Predictive value of age-specific FSH levels for IVF-ET outcome in women with normal ovarian function. *Reprod Biol Endocrinol* 2015; **13**:63. doi: 10.1186/s12958-015-0056-6.
13. Cedars MI. Evaluation of female fertility – AMH and ovarian reserve testing. *J Clin Endocrinol Metab* 2022; **107(6)**:1510-19. doi: 10.1210/clinem/dgac039.
14. La Marca A, Broekmans F, Volpe A, Fauser B, Macklon N. Anti-mullerian hormone (AMH): What do we still need to know? *Hum Reprod* 2009; **24(9)**:2264-75. doi: 10.1093/humrep/dep210.
15. Gulşen MS, Ulu I, Kopuk SY, Kıran G. The role of anti-Mullerian hormone in predicting clomiphene citrate resistance in women with polycystic ovarian syndrome. *Gynecol Endocrinol* 2019; **35(1)**:86-89. doi: 10.1080/09513590.2018.1499085.
16. Nakajo Y, Shibuya Y, Yuri S.S, Miyatani S, Honda M, Kyono K. Pregnancy and prenatal outcome following ovulation induction with aromatase inhibitor Letrozole and clomiphene citrate (CC). *Fertil Steril* 2010; **94(4)**:156. doi: 10.1016/j.fertnstert.2010.07.623.
17. Shakeel S, Iffat W, Qamar A, Nesar S, Butt F, Siddiqui SN, *et al*. Assessment of knowledge, attitude, and practice of obstetricians and gynecologists toward off-label medicine use in female reproductive health issues. *Front Public Health* 2022; **10**:829339. doi: 10.3389/fpubh.2022. 829339.