ORIGINAL ARTICLE

Impact of letrozole on ultrasonographic markers of endometrial receptivity in polycystic ovary syndrome women with poor endometrial response to clomiphene citrate despite adequate ovulation

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KEYWORDS
PCOS; Clomiphene citrate; Letrozole; Thin endometrium; Receptivity; Doppler

Abstract  Objectives: To investigate the endometrial effects of letrozole in PCOS women with poor endometrial response (endometrium thickness ≤ 7 mm) to clomiphene citrate (CC) despite adequate ovulation, using the ultrasonographic markers of endometrial receptivity.

Study design: Ambidirectional cohort study.

Patients and methods: Sixty women with anovulatory PCOS having endometrial thickness less than 7 mm despite adequate ovulation with CC underwent ovulation induction with Letrozole (5 mg/day from cycle day 3 to 7) for one treatment cycle. Main outcome measures: Comparison of the endometrial thickness (ET) and pattern, uterine artery and spiral artery, resistance index (RI) and pulsatility index (PI) between the current letrozole and previous CC stimulated cycles.

Results: In the current letrozole cycle compared with the previous CC cycles, there were significantly greater midcycle endometrial thickness (8.97 ± 1.32 vs. 5.7 ± 1.2, respectively; P < 0.05), multilayered endometrial pattern (93.33% vs. 50%, respectively; P < 0.05) and rate of detection of subendometrial blood flow. Both RI and PI of spiral arteries in the letrozole cycle (0.63 ± 0.05 and 1.12 ± 0.06, respectively) showed significantly lower impedance compared to the previous CC cycle (0.75 ± 0.09 and 1.42 ± 0.13, respectively) (P < 0.05). Pregnancy rate per cycle was 20% (12/60) in the letrozole cycle, all in women with endometrial thickness ≥ 7 mm.

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1110-5690 © 2014 Production and hosting by Elsevier B.V. on behalf of Middle East Fertility Society.
Letrozole is an effective second-line treatment in women with inadequate endometrial response to CC, as letrozole increased endometrial thickness trilaminar pattern and improved endometrial perfusion.

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1. Introduction

Polycystic ovary syndrome (PCOS) is a complex, heterogeneous endocrine disorder in the reproductive-age women, with an incidence of 5–10% (1). Infertility due to anovulation affects 75% of women with PCOS (2). Since the 1960s, clomiphene citrate (CC) has been the first-choice ovulation inducer in PCOS anovulatory infertility. Clomiphene citrate is an orally active nonsteroidal drug with mixed estrogen agonist/antagonist properties. Clomiphene citrate stimulates ovulation by competitively inhibiting the estrogen (E) binding to the hypothalamic estrogen receptors (ER), thereby releasing the hypothalamus from the negative inhibition of endogenous (E), leading to increase in gonadotropin pulse frequency, which consecutively induces ovulation (3). Clomiphene citrate has the advantages of being highly effective in inducing ovulation, while relatively safe, low-priced and orally administered. The lower pregnancy rate (30–40%) in relation to the ovulation rate (60–85%), and the reported miscarriage rate (13–25%), in the CC-stimulated cycles, may be attributed to the antiestrogenic effects of CC on cervical mucus and endometrium (4). Several studies revealed that CC reduces endometrial receptivity, as it impaired endometrial development and uterine blood flow resulting in endometrial thinning in 15–50% of patients with subsequent implantation failure and induces early pregnancy loss due to luteal phase defect (3,5).

Letrozole, a selective reversible third-generation aromatase inhibitor, has the potential to be used for ovulation induction with demonstrable endometrial sparing effect. Letrozole induces ovulation by inhibiting the conversion of androgens to estrogen, creating an estrogen-deficient environment; mimic the central reduction of negative feedback through which CC works (6). Several studies revealed that letrozole may be superior or at least equal to CC in ovulation and pregnancy rates in women with anovulatory PCOS and inadequate clomiphene response. Compared to CC, letrozole is cleared from circulation more rapidly due to shorter half-life; associated with monofollicular growth, lower preovulatory estradiol (E2) levels, and thicker endometrium. Letrozole does not deplete estrogen receptors and is devoid of any antiestrogenic peripheral actions; therefore, it has no adverse effect upon the endometrial receptivity and cervical mucous quality (7).

Receptive endometrium is fundamental for the successful implantation of an embryo. Receptive endometrium is endometrium adequately primed for implantation and its growth is regulated by steroid hormones, various growth factors and cytokines. A good blood supply toward the endometrium is essential for these factors to reach the endometrium. Ultrasonography evaluation of the endometrial morphology (thickness and pattern) and Doppler assessment of blood flow toward the endometrium and the subendometrial region, measured during the preovulatory period provide non invasive tool for the evaluation of endometrial receptivity (8,9). Moreover, conventional 2D and 3D power Doppler sonography had comparable efficacy for the prediction of endometrial receptivity and pregnancy outcome (8).

Based upon these considerations, this study was designed to investigate the endometrial effects of letrozole in PCOS women with poor endometrial response (i.e., endometrium thickness ≤7 mm) to CC despite adequate ovulation, using the ultrasonographic markers of endometrial receptivity.

2. Patients and methods

This ambidirectional cohort study was conducted at the Department of Obstetrics and Gynecology, Benha University Hospitals, and private practice settings, Alkalubia, Egypt from September 2012 to January 2014. The study protocol was approved by the Local Ethics Committee. All participants gave their written informed consent before their inclusion in the study. The study included 60 women with anovulatory PCOS, who had endometrial thickness less than 7 mm measured on the day of hCG injection, despite adequate ovulation (i.e. mid-luteal serum progesterone ≥5 ng/ml) with CC. Diagnosis of PCOS was based on the Rotterdam criteria (2003 ESHRE/ASRM consensus), (10) whereby the diagnosis of PCOS requires the presence of two of three criteria, i.e., oligomenorrhea and/or anovulation, clinical and/or biochemical signs of hyperandrogenism, and/or polycystic ovaries on ultrasound. Other inclusion criteria were: (i) age of women between 18 and 35 years at the time of screening; (ii) period of infertility >2 years; (iii) basal serum follicle stimulating hormone level (FSH) <10 mIU/mL in the early follicular phase; (iv) all women had bilateral tubal patency proved by hysterosalpingography or laparoscopy and their partners satisfied the normal parameters of semen analysis according to the modified WHO criteria (11); and (v) good physical and mental health. Those with (i) history of laparoscopic ovarian drilling or ovarian cystectomy; (ii) endocrinopathies such as hyperprolactinemia, congenital adrenal hyperplasia, thyroid disease and clinically suspected Cushing’s syndrome; (iii) uterine pathology such as leiomyoma, adenomyosis, or congenital uterine anomalies; (iv) androgen-secreting neoplasm; (v) chronic cardiovascular, hepatic, renal or pulmonary disease; (vi) hypersensitivity or contraindications to Letrozole; and (vii) users of metformin, gonadotropins, hormonal contraception or diet regimen within the last 6 months were excluded from the study. Letrozole cycle was started two months after last CC treatment cycle to eliminate any post-treatment effect of CC.

The patients received Letrozole oral tablets (Femara 2.5 mg tablet; Novartis Pharma Services, Switzerland) 5 mg daily from cycle day 3 to 7 of the spontaneous or progestin induced cycle. Only one complete treatment cycle was offered to each woman. Starting from cycle day 9, follicular growth monitoring (number and mean diameter) by transvaginal sonography...
was done every other day. When there was at least one follicle with a diameter $\geq 18$ mm, 10,000 IU/mL of human chorionic gonadotropin (hCG; Choriomon; IBSA, Switzerland) was given intramuscularly to trigger ovulation and timed intercourse (i.e. 24-36 h after hCG injection) was advised. Serum E2 was measured on the day of hCG administration.

On the day of hCG administration, transvaginal sonography examination was performed with the patient in the lithotomy position and had an empty bladder, using a 7.5 MHz vaginal probe with Doppler facility (Voluson 730 PRO V, GE Healthcare, USA). All ultrasound scans were performed by the same investigator to avoid interobserver variability. All patients were studied between 8.00 and 10.00 A.M. to exclude the effects of circadian rhythmicity on the uterine blood flow and rested for 20 min before Doppler scan to minimize the effects of exercises on uterine Doppler indices. The endometrial thickness was measured in the midsagittal plane in the fundus of the uterus (point of maximal thickness) from the echogenic interface at the junction of the endometrium and myometrium. Two types of endometrial pattern were identified as either a multilayered (pattern I) or a nonmultilayered (pattern II) endometrium (12). A multilayered endometrium presented as a triple-line pattern in which prominent outer and central hyperechogenic lines were seen with hypoechogenic or black areas between these lines. A nonmultilayered endometrium consisted of homogeneous endometrial patterns characterized by either hyperechogenic or isoechoic endometrium with absent or a nonprominent central echogenic line.

When a longitudinal view of the uterus was obtained, power Doppler ultrasonography with a 7.5 MHz probe in the two-dimensional (2-D) mode was used to determine the presence or absence of blood flow in the endometrial and subendometrial regions. Subendometrial region is defined sonographically as a thin hypoechogenic layer at the myometrial–endometrial junction. The blood flow velocity waveforms were obtained from the spiral arteries with the highest color intensity within the subendometrial region of the upper two-thirds of the uterus by placing the Doppler gate over the color area and activating the pulsed Doppler function, and the lowest values for resistance to flow were recorded. No correction was made for the insonation angle; as the angle of insonation for the small spiral arteries could not be determined. The pulsatility index (PI) and resistance index (RI) of the spiral arteries were calculated electronically when 3–5 similar, consecutive waveforms of good quality were obtained. Simultaneously in each examination; the uterine vessels were visualized using color Doppler, and the ascending branch was identified lateral to the cervix, at the level of the internal os before they entered the uterus. The blood velocity waveform was obtained by placing the Doppler gate on the target vessel. The mean values of bilateral uterine RI and PI and those of two points of spiral arteries were used for statistical analysis.

Ovulation was confirmed when midluteal (day 21 of the cycle) serum progesterone was $\geq 5$ ng/mL measured by RIA using the antibody coated-tube method (Coat-A-Count; Diagnostic Product Corporation, Los Angeles, USA). Serum $\beta$-hCG was determined 2 weeks after hCG injection for the diagnosis of biochemical pregnancy. Sonographic evidence of an intrauterine gestational sac at 6 week gestation was considered an evident of a clinical pregnancy.

Primary outcome measures were the comparison between the previously reported CC cycles and the current letrozole cycles for the endometrial thickness and pattern, uterine artery and spiral artery Doppler indices measured on the day of hCG administration. However, the secondary outcome measures were the ovulation and pregnancy rates.

2.1. Sample size calculation

Sample size was calculated provided that mid-cycle endometrial thickness was the primary outcome measure. Sample size of 60 patients was calculated by Stats To Do computer program with the following parameters: Probability of Type I Error ($\alpha$) was 0.05, Power ($1 - \beta$) was 0.8, Difference Between Two Means To Be Detected was 0.52, Expected Background Standard Deviation was 1.

2.2. Statistical analysis

Data obtained were statistically analyzed using The Statistical Package for Social Sciences (SPSS, Chicago, USA) software version 15.0 for Windows. Results were expressed as mean ± SD, numbers and percentages. Means were compared using the paired Student’s $t$ test while categorical data were compared using the Z test and the Chi-square test with Yates’ continuity correction when appropriate. A $p$ value of less than 0.05 was considered statistically significant.

3. Results

Table 1 shows demographic and characteristic data of participants (i.e. data of the previous CC stimulated cycles) at the time of study enrollment as regards the mean age, body mass index, hormonal profile and mean period and type of infertility.

Table 2 shows that, in the letrozole cycles, the midcycle endometrial thickness and multilayered endometrial pattern were significantly higher, time to ovulation was similar, but the number of mature follicles and midcycle serum E2 levels were significantly lower compared with the previous CC cycles. The detection rate for subendometrial blood flow was significantly ($P < 0.05$) higher in the letrozole cycles than in the CC cycles. The RI and PI values of the spiral arteries showed significantly lower impedance in the letrozole cycles compared to the previous CC cycles. However, a non significant difference in the uterine artery Doppler indices (RI, PI) was reported.

In the subsequent letrozole cycles, endometrial thickness was still less than 7 mm in four women and nonmultilayered endometrial pattern was still present in four women.

In the subsequent letrozole cycles all women were ovulatory and 12 women were pregnant hence the pregnancy rate was 20%.

4. Discussion

The current study relied on the evaluation of the endometrial effects of letrozole on PCOS women with poor endometrial response to CC despite adequate ovulation, using the ultrasonographic markers of endometrial receptivity on the day of hCG administration. In the current study, poor endometrial response was defined as endometrial thickness less than
When endometrium was prepared with estrogen replacement, endometrial biopsy showed maturational arrest with endometrial thickness of <7 mm, however, an inphase endometrium was detected when endometrial thickness reached 7 mm (13). Also, many authors reported that the endometrial thickness of <7 mm on the day of hCG injection is independently coupled with inferior clinical pregnancy rate in IVF/ICSI patients, although some studies reported pregnancies with endometrial thickness of 6 mm (14). Meanwhile there is no consensus as regards the minimum endometrial thickness required for spontaneous pregnancy.

Receptive endometrium is essential for the implantation of an embryo. Endometrial thickness, trilaminar pattern, and Doppler assessment of blood flow toward the endometrium are the essential detriments of endometrial receptivity (8,9). The current study reported that the midcycle endometrial thickness is significantly greater in the letrozole cycles compared to the previous CC cycles. This agrees with studies that compare CC with letrozole as 1st line treatment in PCOS (14–16). Also, this agrees with Selim and Borg (16) and Jang and Jee (17), who reported thicker endometrium in the letrozole cycles compared to the previous CC cycles in which thin endometrium was identified. On the other hand, Kar (18) reported a nonsignificant difference in the endometrial thickness between the two treatment modalities; however Badawy et al. (19) reported a significantly greater endometrial thickness in the CC cycles. Some authors have postulated that higher doses of letrozole might impair endometrium growth during the follicular phase of the cycle (6). However, consequent data reject this idea even with letrozole doses up to 7.5 mg and when used as long as 10 days (7). Since four women in our study had endometrial thickness less than 7 mm despite the use of letrozole, additional investigations will be required to clarify the etiology.

In the current study, the trilaminar 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 and Jee (17). Trilaminar 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%) (20). The beneficial endometrial effects in the letrozole

![Table 1](image1.png)

Table 1 Demographic and characteristic data of participants (i.e. data of the previous CC cycle) at the time of study enrollment.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Previous CC cycle (n = 60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>27.7 ± 2.7 (23–32)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.3 ± 5.9</td>
</tr>
<tr>
<td>Type of infertility</td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>58.8% (20/34)</td>
</tr>
<tr>
<td>Secondary</td>
<td>41.2% (14/34)</td>
</tr>
<tr>
<td>Duration of infertility, years</td>
<td>4.2 ± 2.4</td>
</tr>
<tr>
<td>Hormonal profile</td>
<td></td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td>7.17 ± 2.9</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>5.5 ± 2.3</td>
</tr>
<tr>
<td>Midcycle E2 (pg/ml)</td>
<td>472.1 ± 71.0</td>
</tr>
<tr>
<td>Midluteal progesterone (ng/ml)</td>
<td>14.5 ± 5.3</td>
</tr>
<tr>
<td>Data are presented as mean ± SD, ranges, numbers and percentage. BMI, body mass index; kg/m², kilogram per square meter; FSH, follicle-stimulating hormone; LH, luteinizing hormone.</td>
<td></td>
</tr>
</tbody>
</table>

![Table 2](image2.png)

Table 2 Comparison between the previous clomiphene citrate and current letrozole cycles as regards endometrial thickness and pattern, rate of detection of subendometrial blood flow, uterine artery and spiral artery Doppler indices, serum estradiol and number of follicles ≥ 18 mm in diameter on the day of hCG administration.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Previous CC cycle (n = 60)</th>
<th>Letrozole cycle (n = 60)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day of hCG administration</td>
<td>12.50 ± 1.10 (11–15)</td>
<td>12.35 ± 1.05 (10-14)</td>
<td>0.446</td>
</tr>
<tr>
<td>Serum E2 (pg/ml)</td>
<td>472.1 ± 71.0 (335.9–601.8)</td>
<td>213.3 ± 40.8 (135.2–291.4)</td>
<td>P &lt; 0.001*</td>
</tr>
<tr>
<td>Number of follicles ≥ 18 mm in diameter</td>
<td>2.47 ± 1.19 (1–5)</td>
<td>1.3 ± 0.56 (1–2)</td>
<td>P &lt; 0.001*</td>
</tr>
<tr>
<td>Endometrial thickness (mm)</td>
<td>5.7 ± 1.2 (4.5–6.9)</td>
<td>8.97 ± 1.32 (5.8–11.4)</td>
<td>P &lt; 0.001*</td>
</tr>
<tr>
<td>Endometrial pattern</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonmultilayered</td>
<td>30(50%)</td>
<td>4(6.67%)</td>
<td>P &lt; 0.001*</td>
</tr>
<tr>
<td>Multilayered (triple-line)</td>
<td>30(50%)</td>
<td>56(93.33%)</td>
<td>P = 0.287</td>
</tr>
<tr>
<td>Uterine artery RI</td>
<td>0.81 ± 0.04</td>
<td>0.82 ± 0.06</td>
<td>P = 0.150</td>
</tr>
<tr>
<td>Uterine artery PI</td>
<td>2.01 ± 0.52</td>
<td>2.15 ± 0.54</td>
<td>P = 0.00034</td>
</tr>
<tr>
<td>Detection rate of subendometrial blood flow (%, %)</td>
<td>33/60 (55%)</td>
<td>51/60 (85%)</td>
<td>P = 0.00034</td>
</tr>
<tr>
<td>Spiral artery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant index (RI)</td>
<td>0.75 ± 0.09</td>
<td>0.63 ± 0.05</td>
<td>P &lt; 0.001*</td>
</tr>
<tr>
<td>Pulsatility index (PI)</td>
<td>1.42 ± 0.13</td>
<td>1.12 ± 0.06</td>
<td>P &lt; 0.001*</td>
</tr>
</tbody>
</table>

Endometrial pattern and subendometrial blood flow detection were expressed as number (percent), all other values are given as mean ± SD (Rang); t, paired t test; v², Chi-square test; RI, resistance index; PI, pulsatility index; hCG, human chorionic gonadotrophin. *Significant = P < 0.05.
cycle compared to the previous failed CC cycle may be attributed to: (i) the enhancing effect of letrozole on endometrial vasculature (21); (ii) letrozole, unlike CC, has no endometrial antiestrogenic effects, as it does not deplete estrogen receptor; and (iii) letrozole effect decreases during the late follicular phase as it is more rapidly cleared from the circulation due to a shorter half-life (48 h) compared to CC that may take up to 2 months due to its prolonged half-life (2 weeks) (6,7).

Cacciatore et al. (22) reported that, the uterine artery PI > 3.0 or an absence of the uterine artery end-diastolic velocities have been associated with poor implantation rate. In the current study there was no significant difference between uterine artery Doppler indices between the current letrozole and previous CC cycles on the day of hCG administration. The uterine artery impedance has limited value in assessing endometrial receptivity as most of the blood passing through the uterine arteries supply the myometrium and not the endometrium. Also, it reflects the impedance of the whole uterine vascular bed that may be affected by factors, such as adenomyosis and fibroids. Therefore, evaluation of vascularization around the endometrium is more reasonable for assessing endometrial receptivity (23). Kupesic and Kurjak were the first who studied blood flow velocity waveform changes in the subendometrial spiral artery and demonstrated that they may be useful indicators of endometrial receptivity (8,24). In our study, we found that there were a significantly higher rate of detection of blood flow signals in the subendometrial region and a significantly lower impedance of both the RI and PI of the subendometrial spiral arteries in the letrozole cycle compared to the previous CC cycle. These results agree with Selim who studied blood flow velocity waveform changes in the subendometrial spiral artery and demonstrated that they may be useful indicators of endometrial receptivity (23).

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All study subjects were ovulatory during previous CC and subsequent letrozole cycles. This agrees with studies revealed that letrozole may be superior or at least equal to CC in ovulation rates in women with anovulatory PCOS and inadequate clomiphene response (7). Pregnancy was reported in 12 women (20%) during subsequent letrozole cycle, all with endometrial thickness ≥ 7 mm. This agrees with studies reported that endometrial thickness should be 7 mm or more for successful implantation (14).

Our study has some limitations: (i) non-blinded; (ii) endometrial status in the natural cycle was not described; and (iii) endometrial growth is a dynamic process and it continued behind the time of hCG injection so it is better to repeat investigations in the mid luteal phase but we thought that as letrozole has a short half-life, its effects on the endometrium might be maximized in the late follicular phase. However, we believe the results are of interest since there are few studies evaluating the impact of letrozole on ultrasonographic markers of endometrial receptivity in PCOS women with poor endometrial response to clomiphene citrate.

5. Conclusion

Letrozole is an effective second-line treatment in anovulatory PCOS women with inadequate endometrial response to CC despite adequate ovulation, as letrozole improved uterine receptivity by improving the endometrial thickness, trilaminar pattern and blood flow. However, larger, randomized, controlled studies are required to confirm these results.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

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