Early Clomiphene Citrate for Induction of Ovulation in Women with Polycystic Ovary Syndrome a randomized controlled trial

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Abstract: Objective: The aim of the current trial was to compare early (starting immediately following the progestin given to induce withdrawal bleeding) to the conventional (starting on day 2 of the withdrawal bleeding) clomiphene citrate (CC) treatment in induction of ovulation in infertile women with polycystic ovary syndrome (PCOS). Methods: The current randomized controlled trial included women with a diagnosis of PCOS. Only women who have oligo/amenorrhea were recruited. Women were then randomized into one of two groups: group I, including women who received CC for 5 days, starting on the day after the last dose of the oral progestin without waiting for the withdrawal bleeding; and group II, including women who received CC at the same dose and for the same duration, but starting on day 2 of the induced withdrawal bleeding. Transvaginal ultrasound scan (TVS) for folliculometry and measuring the endometrial thickness was performed on day 10 from the onset of the induced withdrawal bleeding, and repeated every 48 hours till a mature follicle was detected. The primary outcome was clinical pregnancy rate. Secondary outcomes included number and size of recruited follicles and endometrial thickness. Results: A total of 40 women were recruited in the current trial. Early CC was associated with a slightly higher rate of detecting at least one mature follicle when compared to early CC; this difference was, however, not significant. The mean endometrial thickness (at the day of detecting at least one mature follicle) was significantly higher in early CC group. There was no significant difference between both groups regarding the cycle day at hCG administration. The clinical pregnancy rate was slightly higher in early CC group; this difference was not significant [3 (15%) vs. 2 (10%), respectively, p=0.999]. Conclusion: For induction of ovulation in women with PCOS, early CC administration seems to be associated with significantly thicker endometrium, slightly higher ovulation and pregnancy rates, when compared to the conventional CC administration.


Key word: polycystic ovary syndrome – induction of ovulation – clomiphene citrate – early clomiphene citrate

1.Introduction:
Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women. The estimated prevalence of PCOS is 5-10% of women during the child-bearing ages \([1]\). The first-line treatment for infertile women with PCOS is induction of ovulation by the anti-estrogen clomiphene citrate (CC) \([2]\). CC is a non-steroid anti-estrogen (now classified as selective estrogen receptor modulator [SERM] \([3]\)) that interferes with the negative feedback of the endogenous estradiol on the pituitary gland and the hypothalamus, leading to increased FSH secretion \([4]\). The standard conventional method for prescribing CC is to start the treatment on day 2 or 3 of the menstrual cycle at a dose of 50-150 mg per day, for 5 days \([3]\). In women who had oligo/amenorrhea (which is frequently encountered in anovular women with PCOS), the traditional practice is to induce withdrawal bleeding by an oral (or parenteral) progestin, then to start CC on day 2 or 3 of the induced withdrawal bleeding \([8]\). The value of inducing withdrawal bleeding in amenorrheic women prior to induction of ovulation is to shed the long-estrogenized endometrium that is associated with poor receptivity and to synchronize the endometrial with the ovarian phase \([8]\). Approximately 75-80% of patients with PCOS will ovulate after CC \([1]\). Nevertheless, despite this high ovulation rate, the reported pregnancy rate is only 30-40% \([6]\). The exact explanation for the discrepancy between ovulation and pregnancy rates in anovular patients who receive CC remains unknown. The most probable explanation is failure of implantation due to diminished endometrial receptivity secondary to the anti-estrogenic effect of CC on the endometrium \([7,8,9]\). These negative effects are further magnified when we know that the half-life of CC is relatively long (5 days) of CC, which means that, with the traditional onset of treatment on day 2 or 3 of induced or spontaneous menses, those negative effects are more likely to extend into the sensitive peri-implantation period \([9]\). Accordingly, it was suggested that early onset of CC may be associated with more follicular growth and endometrial thickness, and, subsequently,
better impact on the pregnancy rate\textsuperscript{10–11}. The aim of the current study was to evaluate the efficacy of starting CC earlier in oligomenorrheic women with PCOS immediately after short-term progestin therapy without waiting for the induced withdrawal bleeding.

2. Methods
The current randomized controlled trial was conducted at Ain Shams University Maternity Hospital during the period between May 2009 and February 2010. The study protocol was designed in accordance to the Declaration of Helsinki for the Ethical Principles of Medical Research, and was approved by the Ethical Research Committee at Obstetrics and Gynecology Department, Faculty of Medicine, Ain Shams University. Participating women had to sign an informed consent after thorough explanation of the purpose and procedures of the study. The study included women presenting to the Infertility Outpatient Clinic at Ain Shams University Maternity Hospital, with a diagnosis of PCOS. Diagnosis of PCOS was based on the PCOS Consensus of the European Society of Human Reproduction and the American Society of Reproductive Medicine (ESHRE/ASRM), as presence of at least two of the following three criteria: hyperandrogenism (biochemical or clinical [in the form of hirsutism and/or acne]); chronic anovulation (oligo/amenorrhea); sonographic features of polycystic ovary; after exclusion of other etiologies (e.g. congenital adrenal hyperplasia, androgen-secreting tumors and Cushing syndrome) \textsuperscript{11}. The sonographic feature of PCOS include the presence of ≥ 12 follicles in each ovary measuring 2-9 mm in diameter and/or increased ovarian volume (> 10 ml), regardless of follicle distribution or ovarian stromal echogenicity. One ovary fulfilling these criteria is sufficient to define positive sonography for PCOS. In the current trial, only women who have oligo/amenorrhea were recruited. Women above 35 years old, having a body mass index (BMI) calculated as weight (in kilograms) divided by squared height (in squared meters) above 35 kg/m\textsuperscript{2}, who have abnormal husband’s semen analysis (according to the WHO criteria) \textsuperscript{12}, abnormal tubal testing (through hysterosalpingogram [HSG]), those who had other endocrine disorders (e.g. hyperprolactinemia or thyroid disorders), and those who have not fulfilled the basic infertility work up were not recruited in the trial. All eligible women should have had a negative serum pregnancy test before recruitment. All recruited women received oral progestin (medroxy-progesterone acetate 5 mg) [Provera\textsuperscript{®}, Pharmacia Pharmaceuticals, Pfizer Egypt] twice per day for 5 days to induce withdrawal bleeding. Women were then randomized into one of two groups: group I, including women who received clomiphene citrate (CC) [Clomid\textsuperscript{®}, Sanofi Aventis, Egypt] at a dose of 50 mg twice per day for 5 days, starting on the day after the last dose of the oral progestin without waiting for the withdrawal bleeding; and group II, including women who received CC at the same dose and for the same duration, but starting on day 2 of the induced withdrawal bleeding. A blood sample was taken from each recruited woman, on cycle day 2 or 3 of the induced withdrawal bleeding, to check serum levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH). Transvaginal ultrasound scan (TVS) for folliculometry and measuring the endometrial thickness was performed on day 10 from the onset of the induced withdrawal bleeding, and repeated every 48 hours till a mature follicle was detected. A mature follicle was defined as a follicle with a mean diameter ≥ 18 mm \textsuperscript{13}. When at least one mature follicle was detectable, human chorionic gonadotropin (hCG) [Chorionon\textsuperscript{®}, IBSA, Switzerland] was intramuscularly given at a dose of 10,000 IU. TVS was repeated 48 hours after hCG injection to confirm successful ovulation [through disappearance or decrease in follicle diameter, irregularity of the follicle walls and/or appearance of a free fluid in cul-de-sac \textsuperscript{14}]. All recruited women who had successful ovulation received luteal phase support in the form of progesterone vaginal suppositories [Prontogest\textsuperscript{®}, Marcryl Pharmaceuticals, Egypt], at a dose of 400 mg once per day for 14 days. Serum pregnancy test was performed 16 days after hCG injection. Transvaginal scan was performed after 2-3 weeks in women who had a positive serum pregnancy test to ensure viability and location of pregnancy. The primary outcome was clinical pregnancy rate, defined as sonographically detectable intrauterine gestational sac with detectable embryonic pulsations. Secondary outcomes included number and size of recruited follicles and endometrial thickness at the time of hCG injection.

Statistical Analysis
Statistical analysis was performed using SPSS\textsuperscript{®} for Windows\textsuperscript{®} version 15.0. Data was described as range, mean and standard deviation (for numeric parametric variables); or number and percentage (for categorical variables). Difference between two independent groups was estimated using independent student’s t-test (for numeric parametric variables); or chi-squared or the relative risk [RR] and its 95% confidence interval (for categorical variables). Significance level was set at 0.05.

3. Results
A total of 40 women were recruited in the current trial. The mean age of included women was
26.05 ± 4.92 years (range: 20 – 35 years). The mean weight was 80.63 ± 8.99 Kg (range: 61 – 97 Kg). The mean body mass index (BMI) was 29.77 ± 2.01 Kg/m² (range: 26.4 – 34.2 Kg/m²). Of the included 40 women, 29 (72.5%) had primary infertility, while 11 (27.5%) had secondary infertility. The mean duration of infertility in included women was 3.83 ± 2.61 years (range: 1 – 12 years). Oligo/amenorrhea was reported by all included women; hirsutism by 20 (50%) women; and sonographic criteria for PCO were found in 29 (72.5%) women. The mean basal serum FSH in included women was 5.22 ± 1.89 mIU/ml (range: 1.5 – 9.4 mIU/ml). The mean basal serum LH was 8.62 ± 2.79 mIU/ml (range: 3.2 – 18.2 mIU/ml). There were no significant differences between women of both groups regarding these initial characteristics (Table-1).

### Table-1 Difference between Groups regarding Initial Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Group I [Early CC Group] (n=20)</th>
<th>Group II [Late CC Group] (n=20)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>25.45 ± 3.66</td>
<td>26.65 ± 5.02</td>
<td>0.393*</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79.55 ± 7.54</td>
<td>81.7 ± 9.31</td>
<td>0.427*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.34 ± 1.77</td>
<td>30.19 ± 2.49</td>
<td>0.246*</td>
</tr>
<tr>
<td>Primary infertility</td>
<td>15 (75%)</td>
<td>14 (70%)</td>
<td>0.723**</td>
</tr>
<tr>
<td>Duration of infertility (years)</td>
<td>4.13 ± 2.91</td>
<td>3.52 ± 2.02</td>
<td>0.450*</td>
</tr>
<tr>
<td>Criteria for Diagnosis of PCOS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oligo/amenorrhea</td>
<td>20 (100%)</td>
<td>20 (100%)</td>
<td>NE</td>
</tr>
<tr>
<td>Hirsutism</td>
<td>11 (55%)</td>
<td>9 (45%)</td>
<td>0.527**</td>
</tr>
<tr>
<td>Sonographic PCO</td>
<td>14 (70%)</td>
<td>15 (75%)</td>
<td>0.723**</td>
</tr>
<tr>
<td>Basal serum FSH (mIU/ml)</td>
<td>5.35 ± 2.16</td>
<td>5.08 ± 1.27</td>
<td>0.633*</td>
</tr>
<tr>
<td>Basal serum LH (mIU/ml)</td>
<td>8.82 ± 3.73</td>
<td>8.42 ± 2.58</td>
<td>0.696*</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD; or number (percentage)

BMI body mass index [calculated as weight (kg) divided by squared height (m²)]

PCOS polycystic ovary syndrome

FSH follicle stimulating hormone

LH luteinizing hormone

* Analysis using Independent Student’s t-Test

** Analysis using Chi-Squared Test

NE not estimable due nullity in both groups

Early CC was associated with a slightly higher rate detecting at least one mature follicle when compared to late CC; this difference was, however, not significant [RR 1.2, 95% CI (0.7 to 2.1), p=0.525, NNT=10]. The mean endometrial thickness (at the day of detecting at least one mature follicle) was significantly higher in early CC group [10.42 ± 1.93 mm vs. 8.7 ± 1.77 mm, respectively, p=0.006]. There was no significant difference between both groups regarding the cycle day at hCG administration. The clinical pregnancy rate was slightly higher in early CC group; this difference was not significant [3 (15%) vs. 2 (10%), respectively, p=0.999; RR 1.5, 95% CI (0.3 to 8.1), NNT = 20] (Table-2).

### Table-2 Difference between Groups regarding Outcomes

<table>
<thead>
<tr>
<th></th>
<th>Group I [Early CC Group] (n=20)</th>
<th>Group II [Late CC Group] (n=20)</th>
<th>P</th>
<th>RR/MD (95% CI)</th>
<th>NNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of ≥ one mature follicle</td>
<td>12 (60%)</td>
<td>10 (50%)</td>
<td>0.525*</td>
<td>1.2 (0.7 to 2.1)</td>
<td>10</td>
</tr>
<tr>
<td>Endometrial thickness†</td>
<td>10.42 ± 1.93</td>
<td>8.7 ± 1.77</td>
<td>0.006**</td>
<td>1.7 (-0.5 to 2.9)</td>
<td>-</td>
</tr>
<tr>
<td>Cycle day at hCG injection</td>
<td>15.42 ± 1.31</td>
<td>15.9 ± 1.52</td>
<td>0.292**</td>
<td>-0.48 (-1.4 to 0.4)</td>
<td>-</td>
</tr>
<tr>
<td>Clinical pregnancy rate</td>
<td>3 (15%)</td>
<td>2 (10%)</td>
<td>0.999*</td>
<td>1.5 (0.3 to 8.1)</td>
<td>20</td>
</tr>
</tbody>
</table>

Data presented as number (percentage); or mean ± SD

hCG human chorionic gonadotropin

* Analysis using Chi-Squared Test

** Analysis using Independent Student’s t-Test

† measured at detecting at least one mature follicle

MD (95% CI) mean differenced and its 95% confidence interval

NNT number needed to treat
4. Discussion

The current trial showed that earlier CC administration immediately after the 5-days progestin treatment was associated with significantly higher mean endometrial thickness, slightly higher rates of detectable mature follicles and slightly higher clinical pregnancy rate, when compared to the conventional CC administration (starting on day 2 or 3 of the induced withdrawal bleeding). CC was shown to adversely affect the endometrial receptivity, by its direct anti-estrogenic effect on the endometrium. In a prospective controlled study conducted on 33 women with PCOS compared to a similar number of fertile women, all sonographic parameters (including Doppler-ultrasound-measured endometrial and sub-endometrial blood flow) were significantly impaired in women with PCOS when compared to control women; in women who receive CC when compared to women who have non-stimulated cycles among the PCOS group; and in women who had favorable pregnancy outcome when compared to women who had no pregnancy outcome among the PCOS group [6]. In another later study, a direct, yet invasive measurement of endometrial status, which was endometrial biopsy, was histologically examined in the late luteal phase in women with PCOS receiving CC, in comparison to control regularly ovulating women (whose husbands had abnormal sperms). The rates of out-of-date endometrial biopsies (according to the classical Noyes criteria) was 16% and 3%, respectively (p<0.001). The authors concluded that this adverse impact of CC on the endometrium may explain, at least in part, the large difference between ovulation and pregnancy rates in such women [8]. Administration of CC earlier than conventionally made was suggested to prolong the time to implantation, and, therefore, may allow the endometrium to ‘recover’ from CC-induced suppression. Moreover, it was suggested that recruitment of a new ovulating follicle in regularly ovulating women is directed by a selective increase in FSH that begins approximately 2 days before the onset of menses [18]. This was confirmed by sensitive FSH bioassay, which showed an increase in FSH bioactivity as early as the midluteal phase [18]. Owing to all these observations, it was suggested that earlier CC administration would have a better impact on the endometrial receptivity, and thus may narrow the gap between ovulation and pregnancy rates. In a randomized controlled trial conducted on 78 infertile Iranian women with PCOS (144 cycles), CC was administered at a daily dose of 100 mg per day either on days 1 through 5, or on days 5 through 9. The mean number of follicles and the maximum follicular size were greater in the latter group; whereas, ovulation rates were slightly higher, and the pregnancy rates were significantly higher in the former group [72.8% vs. 70.8%, p=0.78; and 40.5% vs. 19.5%, p =0.04; respectively and respectively]. Although both groups received CC in the follicular phase, earlier CC administration was associated with better outcome [10]. In agreement with the results of the current trial, a recent very similar randomized controlled trial, conducted by Badawy et al., randomized 212 women (438 cycles) with PCOS, into two groups similar to those of the current study. The ovulation rate was slightly higher in the early CC group, but not to a significant level [59.1% vs. 51.9%, respectively, p =0.136]. The pregnancy rate was also higher in the early CC group, but, again, not to a significant level [20.9% vs. 15.7%, respectively, p =0.327] [11]. The current trial has two major limitations; the first was the obviously small sample size; the second was administration of CC for a single cycle in each recruited woman. The trial was just a ‘non-inferiority’ study to preliminarily investigate whether an early CC administration would have a better efficacy regarding endometrial thickness and pregnancy rate. Indeed, the higher ovulation and pregnancy rate in the early CC group, reported by Badawy et al. [11], and in the current trial, did not reach a statistical significant level for both trials being underpowered. Large randomized trials with adequately calculated sample sizes are needed to validate the results of both trials.

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References


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