Effects of clomiphene citrate for prevention of premature luteinizing hormone surge in those undergoing intrauterine insemination outcome: A randomized, double-blind, placebo-controlled trial

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Abstract

The objective of the study is to determine the effects of clomiphene citrate (CC) on preventing premature luteinizing hormone (LH) surge in infertile patients with polycystic ovary syndrome (PCOS) undergoing intrauterine insemination (IUI). This was a randomized clinical trial being performed at Shiraz Mother and Child Hospital. We included 162 women with PCOS selected for IUI cycles. Patients were randomly allocated to receive 150 mg/day CC from the 8th of the cycle though the day of human chorionic gonadotropin (hCG) injection ($n = 81$) or nothing in the same period ($n = 81$). Main outcomes included the incidence of premature LH surge, pregnancy rate, abortion and ongoing pregnancy rates, number of maturing follicles, and endometrial thickness. The incidence of premature LH surge was significantly lower in those who received CC (3.0% vs. 14.9%; $P = 0.021$). The pregnancy rate was 10 (15.1%) and 6 (8.9%) in CC and control groups, respectively ($P = 0.342$). The ongoing pregnancy rate found to be comparable between two study groups (12.1% vs. 5.9%; $P = 0.068$). Serum level of estradiol (E_2) level at the time of hCG administration was significantly higher in those who were treated with CC when compared to control (1153.5 ± 326.4 vs. 943.2 ± 215.3; $P < 0.001$). Patients who received CC also had higher number of mature follicles >18 mm when compared to controls (3.85 ± 1.3 vs. 2.94 ± 1.01; $P < 0.001$). Administration of CC from the 8th day of the cycle to the day of hCG injection in combination with Gonal-f in infertile patients with CC-resistant PCOS undergoing IUI cycles is associated with decreased incidence of premature LH surge, higher E_2 levels, and higher number of mature ovarian follicles. This protocol is safe and simple and could be considered to be cost-effective.

Key words: Clomiphene citrate, intrauterine insemination, luteinizing hormone surge, pregnancy rate

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INTRODUCTION

Intrauterine insemination (IUI) defined as insemination of sperm into female reproductive tract without sexual

intercourse is one of the assisted reproductive techniques used for treatment of those couples suffering from mild male factor infertility, minimal-to-mild endometriosis, and unexplained infertility.[1-3] IUI procedure is being widely used by the infertility centers because it is simple, noninvasive, and cost-effective. The outcome of assisted reproduction techniques including IUI and *in vitro* fertilization (IVF) is affected by several factors including age, type of infertility, past reproductive-obstetrical history, the quality of the technique, and the concentration of the inseminated semen.[2]

There is a major problem in using ovarian hyperstimulation before IUI which is the occurrence of premature luteinizing hormone (LH) surge leading to luteinization of the endometrium before maturation of the follicle. This would decrease the success rate of the IUI cycles to a great extent.[4] It has been reported that approximately 13%–36% of IUI cycles are complicated by premature LH surge.[5,6] This would increase the failure rate of IUI cycles leading to several economical and psychological burden for both patients and physicians.[6] Until now, several strategies have been introduced for overcoming and suppressing the premature LH surge in patients undergoing ovarian hyperstimulation before IUI or IVF. Currently, the standard method preventing the premature LH surge in those undergoing IUI is the administration of GnRH agonists which downregulates the pituitary gland. However, this method is associated with increased costs of treatment because of need for injection of several doses of gonadotropins. Other strategies include administration of GnRH antagonists,[7] ethinyl estradiol (E_2) plus norethindrone,[8] mifepristone,[9] and nimodipine.[10]

Clomiphene citrate (CC) is considered the drug of choice for ovulation induction, ovarian hyperstimulation and superovulation in those with unexplained infertility, polycystic ovarian syndrome (PCOS), mild endometriosis, and mild male factor before IUI in many infertility centers worldwide.[11] CC is considered to be a selective estrogen receptor modulator acting both as both agonist and antagonist of estrogen receptors based on the target tissue. It has been reported that the primary target organ of CC is pituitary gland. In the pituitary gland, the CC binds to estrogen receptors of the hypothalamus and inhibits the negative feedback of the circulating estrogen. In turn, the serum levels of LH and follicle-stimulating hormone (FSH) increases.[12]

Previous studies have shown that CC administration before ovarian hyperstimulation suppresses the LH surge in those undergoing IVF[13] and IUI[14] cycles. It is hypothesized that antiestrogenic effects of CC may prevent the LH surge which affects the results of IUI cycles negatively while preserving the positive effects on ovarian follicles development. As data regarding the use of CC for this purpose is limited in the literature, we performed this study to determine the effects of CC on preventing premature LH surge in infertile patients with PCOS undergoing IUI.

MATERIALS AND METHODS

Study population

This was a randomized clinical trial being performed in Mother and Child Hospital, a tertiary health-care center affiliated with Shiraz University of Medical Sciences, during a 16-month period from August 2013 to December 2014. We included 162 patients aging 18–35 years diagnosed to have CC-resistant PCOS suffering from infertility who referred to infertility clinics of Mother and Child hospital for medical assistant. The study protocol was approved by the Institutional Review Board of Shiraz University of Medical Sciences, and the approval of the Ethics Committee was achieved before the start of the study. All the participants' parents gave their informed written consent.

PCOS was defined according to the Rotterdam European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine PCOS consensus workshop.[15] All patients had at least two of the three following criteria: (I) chronic anovulation, (II) clinical and/or biochemical evidence of androgen excess, and (III) polycystic-appearing ovaries on transvaginal ultrasound. None of the participants had taken any medication that could influence carbohydrate metabolism for 2 months before the onset of the study, including oral contraceptives (OCPs). We defined resistance to CC as the absence of ovarian response for three consecutive menstrual cycles on transvaginal ultrasound after receiving 150 mg of CC per day from the 5th to the 9th day of each cycle. Diabetes was excluded with a fasting glucose of <120 mg/dL. Patients with Cushing's syndrome, hyperprolactinemia, diabetes mellitus, thyroid disease, or other endocrinopathies, have been excluded from the study. Those participants who had kidney or liver diseases and those who were smoker or had breast cancer were also excluded from the study. None of the participants received OCPs, steroid hormones, or any medications that interfere with lipid metabolism, ovarian and pituitary and hypothalamic function, or insulin sensitivity in the past 3 months before the study. All patients followed almost the same exercise and diet protocols during the study period. All participants were nonsmokers and had normal physical activity and none drank alcoholic beverages. None of the patients with PCOS had a history of previous abdominal surgeries.

Infertility was defined as 1 year of unprotected intercourse without conception. Semen analysis (for the partners), hormonal assay including thyroid-stimulating hormone, prolactin (to rule out hypophyseal adenomas), day 3 FSH, day 3 LH (to rule out ovarian dysfunction such as premature ovarian failure), and hysterosalpingogram (HSG) were performed for all the patients to find the etiology of the

infertility. All women had normal tests of renal and hepatic function, normal complete blood counts, normal HSG, and negative pregnancy tests. We excluded those who had autoimmune disorders, endocrinopathies, cirrhosis, and other chronic liver diseases. Only those with mild endometriosis were included in the study while those with moderate to severe endometriosis were excluded from the study. The men had at least two semen analyses and microbiological tests before any treatment. Normal semen analyses were defined by the threshold values of the World Health Organization:[16] volume 1.5 mL, concentration 15×10^6/mL, progressive motility 40%, and typical morphology 14%.

Study design and assays

A total number of 162 women were screened regarding eligibility for the study. All the patients underwent a complete history, and physical examination and all the positive findings were recorded in the questionnaire. The study protocol as well as side effects and benefits was all explained to all the patients, and informed written consents were obtained before the study.

The patients were randomly divided into two groups based on a computer random digit generator using their registration number. On the 3rd day of the menstrual cycle, a basal ultrasonography was performed for each patient, and a blood sample was withdrawn to perform the hormonal assays. Highly purified recombinant FSH (rFSH) (Gonal-f, Serono, Hellas, Puregon, Greece) was injected intramuscularly in a dosage of 75 IU/day from the 3rd day of the cycle to the day of the human chorionic gonadotropin (hCG) injection. Those who were assigned to clomiphene group received 150 mg/day of CC (Razak Drug Laboratory, Tehran, Iran) from the 8th day of the cycle which was continued until the day of hCG injection ($n = 81$). Those who were assigned to the control group received nothing but the rFSH until the day of hCG injection ($n = 81$).

Transvaginal sonography was performed on the day 11th of the cycle, and according to the size and number of stimulated follicles, rFSH was continued till at least two dominant follicles with the size of >18 mm were seen. Then, 10,000 units of hCG (Choriomon, IBSA, Switzerland) were injected intramuscularly, if serum E_2 level was below 2000 pg/ml. The endometrial thickness (ET) was measured the same day at its greatest diameter perpendicular to the midsagittal plane at the fundal region of the uterus. Serum levels of LH were also measured on the day of hCG injection. Those E_2 levels of >2000 pg/ml were further excluded from the study because of the risk of developing ovarian hyperstimulation syndrome (OHSS). We also omitted those that did not develop an appropriate follicle (18 mm).

IUI was performed 36 h after hCG administration. Swim-up method (A method used to isolate motile spermatozoa from washed sample of at least 10 million motile sperm) by a single expert using a 6–8 French IUI catheter (BioRad, Berkeley, California). Each patient underwent one cycles of IUI. For this purpose, a speculum was used to visualize the cervix, the mucus was removed with a cotton swab, the catheter was gently introduced into the endometrial cavity, and the sample was slowly injected. β-hCG was checked if the patient experienced 1 week missed period. Moreover, pregnancy was documented by transvaginal sonography, at 6–7 weeks of gestation. The main outcome measures were clinical pregnancy rate defined as positive β-hCG and documentation by ultrasonography, premature LH surge (≥10 mIU/mL on the day of hCG injection), number of mature follicles, and ET and serum levels of E_2.

Statistical analysis

Based on 80% power and with α-coefficient 0.05 to detect significant differences between corresponding variables ($P = 0.05$, two-sided), 66 patients were required for each study group. For compensating for nonevaluable patients, we included 81 patients in each study group. The statistical software package SPSS for Windows, version 16.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis. The paired *t*-test was used to compare results within groups, the independent *t*-test to compare results between the groups, and the Chi-square test to compare proportions. Data were reported as mean ± standard deviation. $P < 0.05$ was considered statistically significant.

RESULTS

A total of 168 patients with CC-resistant PCOS selected for undergoing IUI cycles were screened for eligibility of which 6 were excluded from the study. Thus, 162 patients were allocated to two study groups with 81 in CC group and 81 in the control group. In the clomiphene group, 7 (8.6%) patients developed OHSS and 8 (9.8%) had inadequate response. In the control group, 8 (9.8%) patients developed OHSS and 6 (7.4%) had inadequate response. Thus, the final number of patients was 133 with 66 in the clomiphene group and 67 in the control group [Figure 1]. There was no significant difference between the two study groups regarding the baseline characteristics [Table 1].

The incidence of LH surge among all the patients was 12 (9.1%). In those who received CC, the incidence of premature LH surge was recorded to be 2 (3.0%) whereas it was 10 (14.9%) in the control group. The incidence of premature LH surge was significantly lower in those who received CC in comparison to controls ($P = 0.021$). In the same way, the serum levels of LH in the day of hCG injection were significantly lower in those who received CC when compared to controls (7.6 ± 2.4 vs. 9.4 ± 2.1; $P = 0.006$). The pregnancy rate determined by positive β-hCG was 12.1%. The pregnancy rate was found to be 10 (15.1%) and 6 (8.9%) in CC and control groups, respectively. The
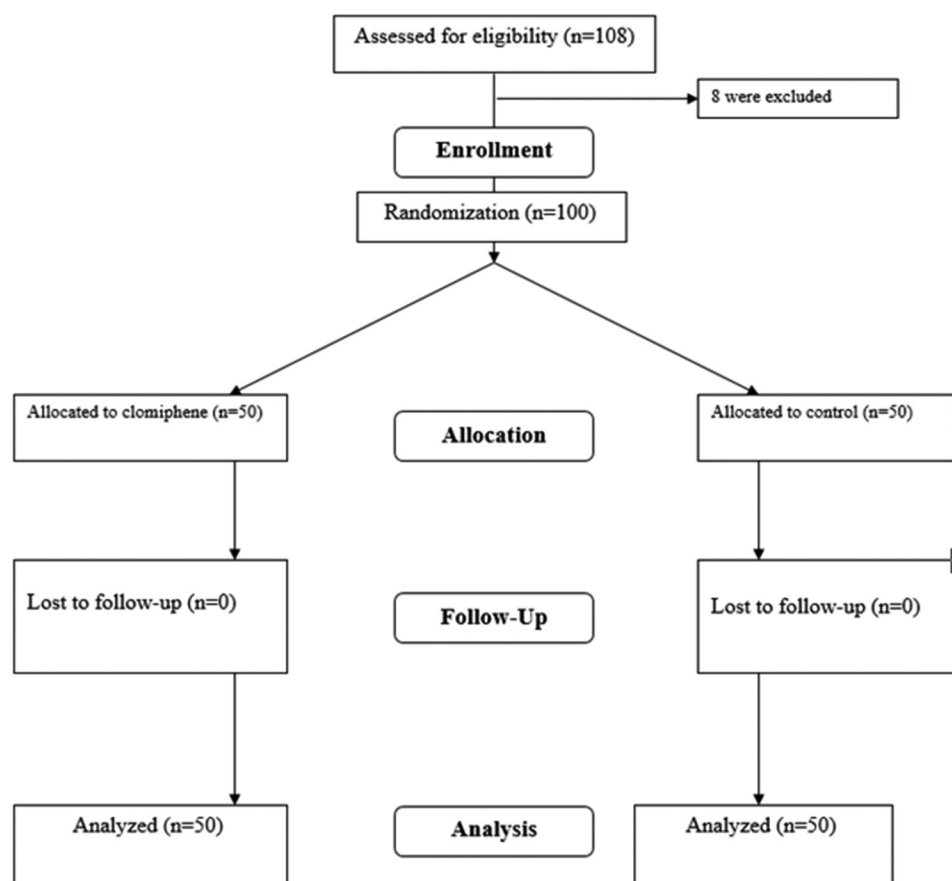
Figure 1: CONSORT flowchart of the study

Table 1: Baseline characteristics of 100 infertile patients undergoing *in vitro* fertilization in two study groups

	Estradiol group (*n*=50)	Control group (*n*=50)	*P*
Age (years)	26.4±2.1	26.1±2.3	0.352
Infertility duration (years)	5.54±2.7	5.35±1.9	0.125
Weight (kg)	63.1±11.4	67.1±7.8	0.022
Height (cm)	161.2±4.5	161.7±2.3	0.395
BMI (kg/m²)	24.3±4.4	25.6±3.2	0.047
Infertility type (%)			
Primary	53 (80.3)	54 (80.6)	0.999
Secondary	13 (19.7)	13 (19.4)	
Previous IUI (%)	27 (40.9)	37 (55.2)	0.119
Day 3 LH (mIU/mL)	7.4±6.5	7.3±5.6	0.473
Day 3 FSH (mIU/mL)	7.2±3.8	6.8±2.5	0.185
TSH (µg/dL)	3.6±1.8	3.9±2.1	0.215
Prolactin (mg/dL)	15.7 ± 6.7	15.4 ± 5.8	0.306

BMI: Body mass index, FSH: Follicular-stimulating hormone, IUI: Intrauterine insemination, LH: Luteinizing hormone, TSH: Thyroid-stimulating hormone

pregnancy rate was not significantly different between the two study groups ($P = 0.342$) [Table 2]. A total number of 5 (3.75%) abortions were recorded. The abortion percentage of pregnancy rate was comparable between the two study groups (20.0% vs. 33.3%; $P = 0.095$). The ongoing pregnancy rate found to be comparable between two study groups (12.1% vs. 5.9%; $P = 0.068$). Serum level of E_2 at the time of hCG administration was significantly higher in those who were treated with CC when compared to control (1153.5 ± 326.4 vs. 943.2 ± 215.3; $P < 0.001$). Patients who received CC also had higher number of mature follicles > 18 mm when compared to controls (3.85 ± 1.3 vs. 2.94 ± 1.01; $P < 0.001$). The ET on the day of insemination was comparable between the two study groups (8.24 ± 1.4 vs. 8.31 ± 1.6; $P = 0.807$). Table 2 compares the results of IUI cycles between those who received CC and controls.

DISCUSSION

In this randomized clinical trial, we tried to investigate the efficacy of CC in preventing premature LH surge in those infertile patients suffering from PCOS undergoing IUI. We revealed that administration of CC from the 8th day of the cycle to the day of hCG injection in combination with Gonal-f during ovarian stimulation prevents premature LH surge. The results of the present study clearly demonstrate that administration of CC provides better follicular maturation when compared to Gonal-f alone leading to lower incidence of premature LH surge. Although the

Table 2: The outcomes of intrauterine insemination cycles in infertile patients with polycystic ovary syndrome treated with clomiphene citrate and controls

	Clomiphene (*n*=66)	Control (*n*=67)	*P*
Premature LH surge (%)	2 (3.0)	10 (14.9)	0.021
Pregnancy rate (%)	10 (15.1)	6 (8.9)	0.342
Abortion rate (%)	2 (20.0)	2 (33.3)	0.095
Ongoing pregnancy rate (%)	8 (12.1)	4 (5.9)	0.068
LH (mIU/mL)*	7.6±2.4	9.4±2.1	0.006
E_2 (pmol/L)*	1153.5±326.4	943.2±215.3	<0.001
Number of follicle >18 mm	3.85±1.3	2.94±1.01	<0.001
Endometrial thickness (mm)	8.24 ± 1.4	8.31 ± 1.6	0.807

*On day of hCG administration. hCG: Human chorionic gonadotropin, E_2: Estradiol, LH: Luteinizing hormone

pregnancy rates were comparable between two study groups, it could be concluded that CC administration in combination with Gonal-f is better than Gonal-f alone for preventing premature LH surge and increasing the number of matured follicles.

The hypothesis behind this study was that CC has antiestrogenic effects that increase the maturation of ovarian follicles.[13] CC also suppresses the hypothalamus and inhibits the pituitary gland from secreting great amounts of LH during ovarian stimulation.[14] We observed that the serum levels of E_2 were significantly higher in those who received CC when compared to controls. This demonstrates that the antiestrogenic effects of CC could not affect the stimulatory properties of gonadotropins, and thus, the negative results are mineralized. In the same way, CC administration led to increased number of mature follicles indicative of better ovarian stimulation. In addition, we observed that the ET was comparable between two study groups which are indicative of favorable effects of estrogen. In other words, antiestrogenic effects of CC do not affect the endometrium negatively.

The pregnancy rate in the current study was 12.1% which was 15.1% in the clomiphene group and 8.9% in the control group. The pregnancy rates in the current study are inconsistent with previously reported rates (8%–18%) for conventional IUI studies per stimulated cycle.[17-19] However, previous studies which had used antagonist protocol for preventing LH surge reported much higher pregnancy rates (20%–35%).[20] The lower pregnancy rate in our study and similar studies[9,19,20] could be because of using minimal ovarian-stimulating protocols. The pregnancy rate was significantly lower when compared to that of Alborzi *et al*.[21] who reported a pregnancy rate per patient of 38.2%.

However, the pregnancy rate per cycle was 8.6% which is comparable to our study.

We found that the abortion rate was comparable between two study groups resulting in comparable ongoing pregnancy rates between those receiving CC and controls (20.0% vs. 33.3%). These findings are consistent with Badawy *et al*.[22] who found comparable miscarriage rates between those receiving letrozole or CC for induction of ovulation (14.4% vs. 16.2% in letrozole and CC groups). Another study demonstrated that abortion rates are similar between those PCOS patients receiving metformin and CC for ovulation induction (10% vs. 10%; *P* > 0.05).[23]

Our results are inconsistent with the previous report from Egypt. Al-Inany *et al*.[14] studied the effectiveness of CC in preventing premature LH surge in 230 infertile women undergoing IUI cycles. The patients suffered from mild male factor and unexplained infertility. They assigned the patients to receive a combination of CC and Gonal-f or Gonal-f alone. They reported that the number of patients who had a premature LH surge was significantly lower in the CC group when compared to control group (5.45% vs. 15.89%). In addition, the mean E_2 levels and the number of mature follicles were also significantly higher in the CC group. The number of canceled cycles, ET, or clinical pregnancy rate was comparable between groups.[14]

Another study by Allegra *et al*.[7] assessed if the administration of GnRH antagonists can develop the success rate of controlled ovarian stimulation (COS)/IUI treatments, through the premature LH surge inhibition. A total number of 104 patients were participated and randomly divided into two groups: in Group A (*n* = 52), rFSH was administered with GnRH antagonist cetrorelix and in Group B (*n* = 52), the patients were given rFSH alone in a way like Group A. The primary outcome measure was clinical pregnancy rate per couple. They found that the pregnancy rate per patient was 53.8% in Group A and 30.8% in Group B (*P* = 0.017). The incidence of premature LH surge was 7% in Group A and 35% in Group B (*P* < 0.0001). They pointed out that those patients receiving GnRH antagonists had considerably lower serum levels of LH and progesterone. Finally, the use of GnRH antagonists in those undergoing COS/IUI was accompanied with better pregnancy rate and lower incidence of premature LH surge.[7]

The premature LH surge should be prevented to obtain several advantages. Suppressing the premature LH surge reduces the requirements for timing of hCG injection and insemination leading to less demand for extensive monitoring. In the same way, the rate of cycle cancellation would be lower, and there would be more flexibility in performing IUI cycles.[24] Improving the IUI outcome regarding the pregnancy rate could be considered as another advantage which is not common among all the

studies. Several studies have indicated that using antagonist protocols improve the pregnancy rate extensively[25,26] while some other could not demonstrate this improvement.[14,25] A recent meta-analysis of randomized controlled trials demonstrated a significantly higher probability of a clinical pregnancy following the use of an antagonist protocol in women undergoing IUI (odds ratio: 1.56, 95% confidence interval 1.05–2.33).[24] This is because during these protocols, we can continue the administration of gonadotropins until a greater number of ovulatory follicles are recruited. In addition, it is well established that premature LH surge is associated with poor oocyte quality and decreased fertilization and implantation rates.[26,27]

We note some limitations to our study. First, all the patients in our study underwent one cycle of IUI. In the standard method, each patient is supposed to undergo up to three cycles of IUI until getting pregnant. However, our patients underwent only one cycle of IUI, and this may be responsible for low pregnancy rate in this study. Second, we did not determine the cost of each cycle by CC and other protocols. Thus, we cannot comment on the resource saving effects of CC to reduce the cost of treatment with favorable results. Future cost-effectiveness trials are highly recommended for shedding light on this issue. Third, the number of patients in the current study was limited, and the power of the study was calculated to be 80%. Future studies with larger study populations are recommended.

CONCLUSIONS

Administration of CC from the 8th day of the cycle to the day on hCG injection in combination with Gonal-f in infertile patients with CC-resistant PCOS undergoing IUI cycles is associated with decreased incidence of premature LH surge, higher E_2 levels, and a higher number of mature ovarian follicles. This protocol is safe and simple and could be considered to be cost-effective. Further trials are needed to confirm the results of this single study.

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Conflicts of interest
There are no conflicts of interest.

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