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Clomiphene stair-step protocol for ovulation induction in women with polycystic ovarian syndrome

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Abstract

The objective of this study is to evaluate the efficacy of the clomiphene stair-step protocol to induce ovulation in women with polycystic ovarian syndrome (PCOS) compared to traditional protocol. This single center randomized controlled trial was undertaken. A 140-patients who met all of the inclusion criteria were divided into two main groups and induction of ovulation for both protocols was performed. Follow up of follicular maturation is done by transvaginal ultrasound. The time to ovulation with the stair step protocol was 21-28 days as compared with the traditional protocol which was 42 -70 days. The dose dependent ovulation rate was 43 % at 100 mg with the stair step protocol compared with 25.3 % with the traditional regimen and the ovulation rate at 150 mg was 21.6% with the stair step protocol compared with 14.7% with the traditional protocol while the clomiphene citrate resistance rate was higher with the traditional protocol 38.7 % as compared to the stair step method which was 20 % which are statistically significant. This randomized controlled trial suggests that clomiphene stair step protocol decreases the time to ovulation and may improve ovulation rates in clomiphene-resistant women.

Keywords: Polycystic ovarian syndrome (PCO); Stair-step and traditional protocol; Transvaginal ultrasonography

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Introduction

Until recently, there has been no universally accepted definition for polycystic ovarian syndrome (PCO). In 2003, an international consensus group proposed that the diagnostic criteria for PCOS are ovarian dysfunction evidenced by oli37gomenorrhea or amenorrhea and clinical evidence of androgen excess (e.g., hirsutism and acne) in the absence of other conditions that can cause these same signs and symptoms [1]. At a recent joint ESHRE/ASRM (European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine) consensus meeting a refined definition of the PCOS was agreed: namely the presence of two out of the following three criteria:

1. Oligo-and/or anovulation;
2. Hyperandrogenism (clinical and/or biochemical);
3. Polycystic ovaries

The morphology of the polycystic ovary, has been redefined as an ovary with 12 or more follicles measuring 2–9 mm in diameter and increased ovarian volume (>10 cm^3) on transvaginal ultrasound [1, 2]. Classically clomiphene citrate (CC) is the first approach to induce ovulation in patients with PCOS. Although 70-80% of PCOS women can ovulate by the treatment with CC, only 40% of the PCOS women become pregnant. Women who do not ovulate with increasing doses of CC are described as being CC-resistant and remain a major challenge in gynecologic endocrinology [3]. The second ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group in Thessaloniki, Greece in 2007 concluded that clomiphene citrate is the first-line agent for ovulation induction, followed by gonadotropins or laparoscopic ovarian diathermy [4]. Over the last years, there have been different regimens to treat patients who fail to ovulate with the initial dose of clomiphene citrate. Each one of these regimen have advantage and disadvantage, these include: A simple dose increase of clomiphene citrate in the next cycle after progestin withdrawal [5], and co treatment with metformin [6]. A recent, large, prospective, randomized, multi-center trial does not support the hypothesis that metformin, either alone or in combination with CC, improves the rate of live birth in women with PCOS [7]. Metformin alone or combined with clomiphene is associated with bothersome gastrointestinal side effects and a small risk of lactic acidosis [8]. The expert panel said metformin should be limited to PCOS patients with glucose

intolerance, as current evidence does not support the routine use of metformin in ovulation induction.

Unfortunately, debate continues as to which infertility PCOS patients; if any, truly will gain from metformin [9]. Other modality of treatment includes the use of aromatase inhibitors, gonadotropin therapy, which is very expensive and require intensive monitoring with serum estradiol and ultrasound assessments to minimize the risks of multiple pregnancy, and ovarian hyper stimulation [10]. Laparoscopic ovarian drilling which is an invasive technique carry the risk of postoperative adhesion formation as well as the other risks of laparoscopic surgery. Additionally, theoretical risks of diminished ovarian reserve and premature ovarian failure remain to be well investigated [11]. However, all of these approaches require months of treatment to determine whether the patient is nonresponsive to ovulation induction therapy, and some are expensive and invasive. Hurst *et al* in 2009 [12] described a novel clomiphene stair-step protocol that it is hoped to reduce time to ovulation in women with polycystic ovary syndrome.

The aim of this study is to determine the efficacy of this protocol compared to traditional one in terms of reducing time to ovulation and increasing rate of ovulation.

Method

Study objectives

To determine the efficacy of stair-step clomiphene protocol for ovulation induction in women with polycystic ovarian syndrome (PCOS) compared to traditional protocol.

Overall study design

This was a randomized clinical trial using toss a coin as randomization technique carried out on sub-fertile women attending the Fertility Clinic of AL Sadder teaching hospital at Al- Najaf city, Iraq from May 2010 - May 2011. 170 infertile women were selected at that time for study participation after their written consent as shown in fig.1.

Participant, recruitment and randomization

The diagnostic criteria adopted for PCOS was according to Rotterdam ESHRE/ASRM sponsored PCOS consensus workshop group, 2004 based on the presence of two of the following three criteria: 1) Oligo-and/or anovulation; 2) hyper-androgenism clinical and/or biochemical; 3) Polycystic ovaries, an ovary with 12 or more follicles measuring 2–9mm in diameter and increased ovarian volume (>10 cm^3) on transvaginal ultrasound [1]. Out of 170 patients, 30 patients were lost in the study

and only 140 patients completed the study. Clinical, ultrasonographic and endocrine screening performed before initiation of clomiphene citrate medication.

Inclusion criteria

Female in the reproductive age who is sub-fertile with the diagnosis of polycystic ovarian syndrome, serum FSH≤10 mIU/ml with spontaneous menses or positive bleeding response to progestagen withdrawal.

Exclusion criteria

Male factor (moderate to severe) infertility, bilateral tubal blockage diagnosed by hystrosalpingographgy or laparoscopy and patients on metformin or other medication. Tv USS screening included: baseline ultrasound using a 7.5 MHz vaginal probe of Siemens ultrasound at day 2 of cycle before starting the initial dose of clomiphene to identify pretreatment ovarian cysts and confirm ovarian morphology for polycystic ovarian syndrome. Assessment of the total follicular number and size for both ovaries, and measurement of endometrial thickness. Follow up done by transvaginal Ultrasound scan (tvUSS) done by our team as part of the management for monitoring of follicular number and their size and for measurement of endometrial thickness.

Endocrine screening included early follicular serum assays at day 2 of cycle for FSH, LH, prolactin, progesterone, estradiol and free testosterone before initiation of CC therapy. Blood samples were obtained by venipuncture and serum was stored at (–20°C); Hormones were measured using enzyme linked immuno-sorbent assay (ELISA) kits (Accu-Bind). Clomiphene citrate ovulation induction protocol: 140 patients who met all of the inclusion criteria were divided into two main groups; 65 patients for the stair step protocol and 75 patients for the traditional protocol. Induction of ovulation for both protocols was performed as following: The stair-step protocol group: 50 mg clomiphene given for 5 days beginning on day 2 after spontaneous or progestagen-induced withdrawal bleeding. Tv USS done at days 11-14. When there is no response (no follicle >10 mm), 100 mg clomiphene is initiated immediately for 5 days, and U/S is repeated 1 week after the first tvUSS at day 21. If there is no response, another 150 mg clomiphene is initiated immediately for 5 days and U/S is performed 1week after the second U/S (day 28).

Ovulation for the stair-step cycles was confirmed by folliculometry (follicle tracing) by tvUSS. Mature follicle is considered to measure between18-24mm. HCG (10000IU) was administered intramuscularly when follicle size was ≥18 mm in

diameter and the patient was advised to have intercourse after 36 hours and on two subsequent days.

The traditional protocol group

Clomiphene medication was initiated at day two after spontaneous or progestagen-induced withdrawal bleeding. The starting dose was 50 mg/day for 5 consecutive days. In case of absent response, the patient was treated with 10 mg medroxyprogesterone acetate (MPA) for10 days. Daily doses of clomiphene citrate were increased by 50 mg in the next cycle up to 3 treatment cycle. In each cycle monitoring of follicular growth was done by tvUSS at day 11-14 of each cycle. First ovulation was used as the endpoint and the duration of follow-up was three treatment cycles (up to 150mg). Ovulation was assessed by tvUSS monitoring of follicle growth. Mature follicle is considered to measure between18-24mm. HCG (10000IU) administered intramuscularly when follicle size was ≥18 mm in diameter and the patient was advised to have intercourse after 36 hours and on two subsequent days.

For both protocols follow up of the follicular growth has been made by frequent tvUSS monitoring, which have the advantage of providing direct information about the size and number of follicle and measurement of endometrial thickness during clomiphene medication. The tvUSS monitoring also allow us increasing the dose of clomiphene citrate in the same cycle in PCOS patient who do not respond to CC initially in the stair-step protocol. Responders were defined as patients who ovulate during CC therapy independent of the dose administered. The number of treatment cycles and the CC dose in which the first ovulation occurred was recorded. Non-responders were patients who did not ovulate despite receiving maximum CC dose of 150 mg/day. The protocols for each ovulation induction method are summarized in table-1.

Reference group

A 170-infertile women were selected for study participation after their written consent, all of them received a detailed explanation of their treatment, follow up by frequent examination, they were advised to contact by telephone in case they need any further explanation. The methods of assessment of our result done by assessing the ovulation rate by tvUSS which was done by the researcher and the pregnancy rate done by assessing the pregnancy test 10-14 days after the ovulation. The study was performed in accordance to Helsinki declaration and ethical approval was obtained from Kufa University in Najaf.

Data handling

Statistical package for social science (SPSS) Version18. Statistical software for window was used to analyses data. Independent-sample t-test was used to detect the significant differences between each two groups of continuous variables. Non parametric data tested by Chi squared test ($\chi 2$). $P<0.05$; $P<0.001$ were considered to be significant at 5% and 1% respectively.

Result

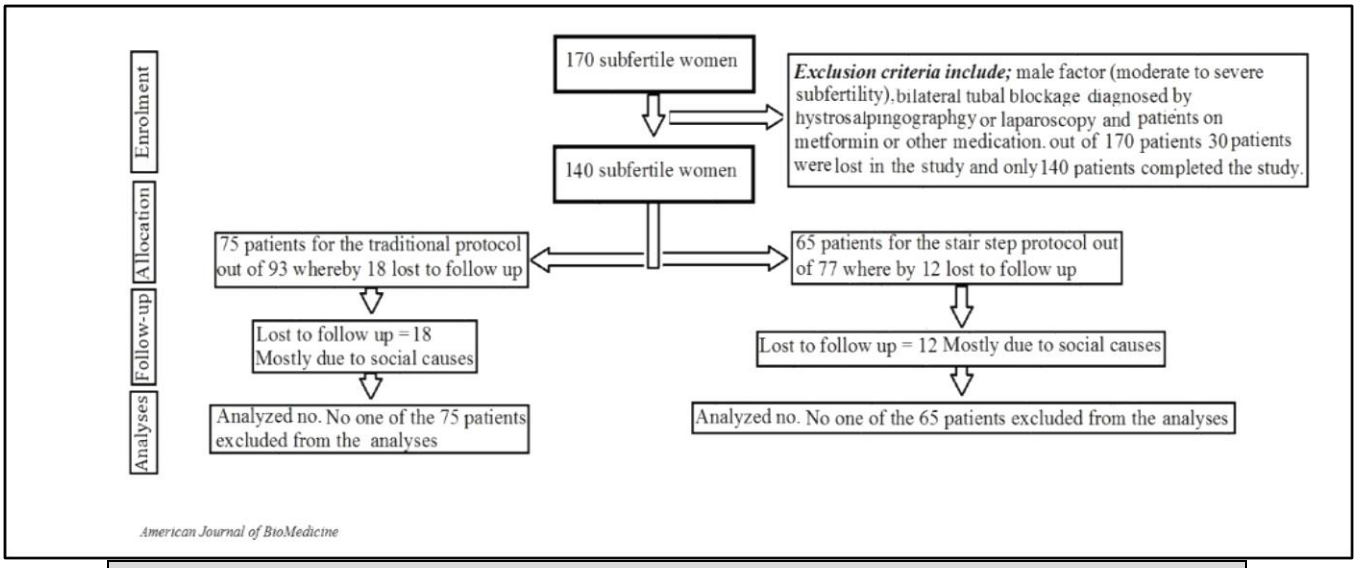

Figure 1.
Cosort flow chart

Screening parameters	**Stair step protocol (65)**	**Traditional (75)**	***P* value**
Age (yr)	27.65±5.634	28.58±5.979	0.366
Duration of infertility (yr)	5.67±2.277	6.15±3.595	0.392
Primary inf.	38(69.09%)	59(78.67%)	0.166
Secondary inf.	17(30.91%)	16(21.33%)	
BMI (gm/m^2	27.85±5.229	29.17±5.462	0.166

Table 2.
Clinical baseline patient characteristics of the two treatment group.

Screening parameters	Stair step protocol (65)	Traditional (75)	*P* value
LH (mIU/ml)	8.67±5.471	7.59±4.251	0.227
FSH (mIU/ml)	5.39±1.547	5.43±2.29	0.910
LH : FSH ratio	1.804±1.201	1.78±1.304	0.917
Prolactin (ng/ml)	11.19±6.664	11.90±6.204	0.534
Progesterone (ng/ml)	0.64±0.398	0.79±0.519	0.068
E2 (pg/ml)	24.72±17.949	24.11±13.251	0.824
Free Testosterone (pg/ml)	2.39±0.617	2.56±0.641	0.075

Table 3.

The Hormonal baseline patient characteristics of the two treatment groups

CC Dose	Stair step protocol (n 65)	Traditional protocol (n75)	P value
50mg	10 (15.4%)	16 (21.3%)	0.366
100mg	28 (43%)	19 (25.3%)	0.042
150mg	14 (21.6%)	11 (14.7)	0.043
Non responder	13 (20%)	29 (38.7%)	0.016

Table 4.

Rate of ovulation between the stair step and the traditional protocol.

parameters	Stair step protocol (n 65)	Traditional protocol (n75)	P value
Endometrial thickness (cm)	1.01±0.169	0.832±0.153	0.000
No. of follicle	1.62±0.634	1.28±0.544	0.043
Size of follicle (mm)	20.69±2.112	19.78±1.504	0.022
Pregnancy rate	8(12.54)	6 (8%)	0.000

Table 5.

Mean endometrial thickness, no. and size of follicle and pregnancy rate for both protocols after cc administration at time of ovulation.

Categories	No. (%)
Endometrial thickness 7 -9mm I0-13mm	 5(35.7%) 9(64.3%)
No. of Follicles One follicle Two follicle	 3(21.4%) 11(78.6%)
Size of Follicles 17-19mm 20-23mm	 2(14.3%) 12(85.7%)
Ovulating Dose 100mg 150mg	 9(64.3%) 5(35.7%)
Multiple pregnancy	0%

Table 6.

Endometrial thickness, no. and size of follicles, and ovulating dose for all pregnant women in both protocols (No.14).

Clinical baseline patient characteristics of the two treatment group

From the total study group of 140 patients treated with clomiphene citrate medication, 65 women of them were included in the stair step protocol and 75 patients for the traditional protocol. Their mean age was 27.65±5.634, 28.58±5.979, BMI 27.85±5.229, 29.17±5.462, duration of infertility was 5.67±2.277, 6.15±3.595 for both group respectively. 38(69.09%) suffered from primary infertility for the stair step protocol and for the traditional protocol it was 59(78.67%), as summarized in table-2.

The endocrine screening parameters at day 2 of cycle for the stair step and the traditional protocols

For the stair step protocol mean FSH level were 5.39±1.547, LH 8.67±5.471, prolactin 11.19±6.664, progesterone 0.64±0.398, estradiol 24.72±17.949 and free testosterone 2.39±0.617. For the traditional protocol mean FSH level 5.43±2.29, LH 7.59±4.251, prolactin 11.90±6.204, progesterone 0.79±0.519, estradiol 24.11±13.251 and free testosterone was 2.56±0.641. LH: FSH ratio for the stair step and the traditional protocol was1.804±1.201 and 1.78±1.304 respectively. This is shown in table No. 3. For both protocol there were no statistical significant differences among the two groups with respect to clinical and hormonal parameters as shown in tables-2, 3.

Ovulation rate between both protocols

A significantly higher ovulation rate 43% % was observed with the stair-step protocol at a clomiphene dose of 100mg compared with the ovulation rate of 25.3% with same dose in the traditional regimen. At 150mg ovulation rate for the stair step and the traditional groups was 21.6 % and 14.7 % respectively. (P value 0.05) .The total ovulation rate was 64.6 % of women treated with the stair-step protocol, and this was higher than the ovulation rate of 40% with the traditional protocol (p value 0.05). 29 patients (38.7%) in traditional protocol where considered to be non-responder which is higher than that observed for the stair-step protocol 20% (P value 0.05) which is statistically significant as shown in table-4.

Mean endometrial thickness, number and size of follicle and pregnancy rate for both protocols after clomiphene citrate administration at time of ovulation

The mean endometrial thickness in the stair-step protocol was 1.01±0.169 which is significantly higher than that for the traditional protocol which was 0.832±0.153 (p-value 0.000). The highest number of follicle was found in the stair-step protocol 1.62± 0.63(p- value 0.043) and the largest size also found in the same protocol 20.69 (p-value 0.022) which are statistically significant. Regarding pregnancy rate in both protocols, 8 out of 65 patients (12.31%) became pregnant which is significantly higher than that for the traditional protocol which was 6 out of 75 patients (8%) (P-value0.000), as shown in table-5.

Endometrial thickness, number and size of the follicles, and ovulating dose for all pregnant women in both protocols

Table-6 shows the endometrial thickness, size of follicle, number of follicle, multiple pregnancy and dose of clomiphene citrate at which patients became pregnant for both protocols. About endometrial thickness 35.7% got pregnant at endometrial thickness measured between 7-9mm and the remaining 64.3% at 10-13mm. 78.6 % of those pregnant women have 2 follicles and 85.7% of all follicles their size measured between 20-23mm. About the ovulating dose 64.3% conceived at 100 mg clomiphene citrate and 35.7 % of them at 150mg. No one of our patients had multiple pregnancies, all of them had singleton pregnancy.

Discussion

The present study shows that in the stair-step protocol the rate of ovulation at 100mg and 150mg was 43% and 21.6% as compared to 25.3 % and 14.7% respectively for the traditional protocol at the same dose. The total ovulation rate for the stair step protocol was 80% which is higher than that for the traditional regimen which was 61.3%. Regarding time to achieve ovulation, for the traditional protocol it may require 42-70 days to achieve ovulation or to consider the patient non-responding to clomiphene citrate, while in the stair step regimen the time to achieve ovulation is between 21-28 days; which mean that the time required for stair step protocol is shorter than the traditional one by 21-42 day.

In our trial, the pregnancy rate for the stair-step protocol was 12.31%. When we compare this result with the traditional protocol, the pregnancy rate was 8%. Regarding pregnancy rate and clomiphene dose, we found that from all the pregnant women in both protocols, 68.3% became pregnant at 100mg and 36.3 % at 150mg. In those patients who became pregnant 64.3% of them get pregnancy with endometrial thickness of 10-13mm and the remaining 35.7% of them at endometrial thickness between 7-9mm , 85.7% conceived at follicular size between 20-23mm, 78.6% conceived with two follicles and 21.4 % with one follicle.

Strength and limitations of the study

1. It is not necessary to induce menses before increasing clomiphene doses in nonresponsive PCOS patients.
2. Selection bias: the method of selection was tossing a coin and therefore selection bias was excluded. Furthermore; the patients are consecutive series and this has left no possibility of selection bias.
3. Outcomes bias: The personal who assessed the results are the authors and not independent investigators. This may indicate a certain degree of bias; however authors tried very hard to be as honest as possible in reporting the results.
4. The groups of patient are not equal in number (one group 65, the other 75).

This was because of tossing the coin which left no option to the authors to interfere and make the groups equal. However the statistical analysis has shown no significant difference in the outcomes based on that factor.

Relation with other literature

Comparable results have been found in the study by Hurst *et al* [12] who studied anovulatory or oligoovulatory women with PCOS who failed to respond to 50 mg clomiphene for 5 days and were subsequently treated with the stair-step protocol from 2000 to 2007. They found a significantly higher ovulation rate of 64% with the stair-step protocol at a clomiphene dose of 100 mg compared with the expected ovulation rate of 22% with this dose in a traditional regimen. 74% of women treated with the stair-step protocol ovulated on 100 or 150 mg of clomiphene, and this was higher than the expected ovulation rate of 35.5% expected with a traditional protocol. They also found that the time to ovulation was shorter with the stair-step protocol (21-28 days) as compared with a traditional progestin withdrawal regimen (55-88 days).

Farhi J. *et al* in 2010 [14] concluded that time can be saved and the process made more efficient, without affecting the outcome of treatment, by starting CC at a time unrelated to the onset of bleeding, on condition that no dominant follicle is present and ovulation has not occurred. CC treatment was started regardless of the time since their menstrual bleeding (day 7-29 of cycle). This allows time saving in the initiation of treatment for newly diagnosed patients and justifies a stair-step protocol allowing a step-up in dose after a lack of response to CC, without having to induce a menstrual period [14].

A higher pregnancy rate was achieved by Hurst *et al* in 2009 [12] with pregnancy rate for the stair step protocol of 13% and for traditional protocol of 15%. This difference in pregnancy rate between our study and his study could be explained that in Hurst et al the total number of patients enrolled in the stair step protocol was 31 patients while in our study the total number was 65 patients, and about traditional protocol Hurst et al depended on historical result from published data and not from a controlled study group to compare the result of both protocols as compared to our study.

Regarding pregnancy rate and clomiphene dose, the results was higher than that reported by Dicky *et al* in 1997 [15] who found that 14.7% of patients conceived at clomiphene citrate dose of more than 100mg and this is probably because of small number of patients who conceived in our study as compared to their study. A previous study done by Dickey *et al* in 1992 [16] found that the dose of clomiphene was positively related to pregnancy rates and birth rates, but not to multiple birth rates, abortion or ectopic pregnancies. Doses more than 100 mg/day were responsible for 16% of births and 11% of multiple births.

Richter KS *et al* in 2007 [17] who reported a direct relationship between clinical pregnancy and live birth or ongoing pregnancy rates with increasing endometrial thickness. In Esmailzadeh study in 2007 [18], endometrial thickness on the day of HCG administration was significantly greater in cycles where pregnancy was achieved (10.1±3 *vs*. 7.7 ±3.5), they found that endometrial thickness was positively associated with pregnancy outcome. However other study contradicts our finding, Ziba Zahiri *et al* in 2007 [19] found that there is no significant relation between endometrial thickness and pregnancy rate.

Farhi j. *et al* in 2010 [20] who found that pregnancy rate increase with increasing follicular size, although an earlier study done by Ghosh C. *et al* in 2003 [21] found that women with follicular diameter of 20mm and more are less likely to become pregnant as compared to women of follicular diameter of 15-19mm.He conclude that women with the largest follicular diameter of ≥20 mm in a cycle appeared to have a 40% less chance of conceiving as compared to women with the largest follicular diameter between 15 and 19.99 mm. Possible explanation for this differences is that in Ghosh C. *et al* in their study sample a large proportion of women had advanced stage endometriosis, and they did not exclude women with history of other chronic diseases or prior surgery.

MME Van Rumste *et al* in 2008 [22], Karuppaswamy J *et al* in 2009 [23] and Dickey *et al* in 1992 [16] who found that multifollicular growth associated with increasing pregnancy outcome in couples with controlled ovarian hyperstimulation, but doesn't go with Sonika and Mittal in 2004 [24] who demonstrated no relation between

We **concluded** that the clomiphene stair step protocol decreases the time to ovulation and is shown to improve ovulation rates in clomiphene-resistant women as pregnancies occur in less time compared with a traditional protocol. We recommend further study with larger number of patients with a wide spectrum of infertility problems to put more emphasis on pregnancy rate and to report on any difference in adverse effects between the two regimes.

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Competing interests

The authors declare that there is no conflict of interest.

Author Contributions

The author wrote, read and approved the final manuscript.

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