
Comparison between Clomiphene Citrate / Metformin Stair Step Protocol and Traditional Protocol in Treatment of Polycystic Ovary Syndrome

Waleed F. Ghareeb ¹, Nourhan E. Ahmad ², Elham H. Madny ³, Osama E. Ashour⁴
Department of obstetrics and gynecology, faculty of medicine, Suez Canal University Hospital, Ismailia, Egypt.
¹.Assistant professor of Obstetrics & Gynecology, Faculty of Medicine- Suez Canal University, Ismailia, Egypt.
².M.B.B.CH 2018, Suez Canal University, Resident of Obstetrics & Gynecology, Faculty of Medicine, Suez Canal University Hospitals, Ismailia, Egypt.
³.Professor of Obstetrics & Gynecology, Faculty of Medicine- Suez Canal University, Ismailia, Egypt.
⁴.Lecturer of Obstetrics & Gynecology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt.

Corresponding author:
Nourhan Ez Eldeen Ahmad,
Resident of Obstetrics & Gynecology, Faculty of Medicine-Suez Canal University, Ismailia, Egypt.
Address: Ismailia, Egypt
e-mail: noor.ez1995@gmail.com.
Phone: +201009590489

Abstract

Background: As Clomiphene citrate (CC) is widely used in treatment of infertility in polycystic ovarian syndrome (PCOS).

Aim: To evaluate rate of ovulation using CC “stair step protocol” compared to “traditional protocol”.

Methods: A randomized clinical trial conducted on 60 infertile PCO were randomly divided to 2 groups, group (A) “Stair Step protocol” in which CC was administrated on day 2 to day 6 of menses then transvaginal ultrasound (TVUS) was done, if follicular diameter less than 18 mm on day 14 increasing dose to 100 mg days for further 5 days, then TVUS on day 21, if no response, an increasing the dose to 150 mg daily. In group (B), “Traditional protocol” started with CC 50 mg on day 2 to day 6 of menses, if no response, in subsequent cycles the dose increased by 50 mg till dose 150 mg daily.

Metformin dose was 500mg daily then increased weekly by 500 mg till 1500 mg daily.

Results: the rate of ovulation and pregnancy were higher in the stair-step than the traditional group (76.7 vs. 63.3 %, respectively) (40 vs. 26.7 %, respectively). With significantly shorter duration of treatment was in stair-step than traditional protocol (21 ± 7.0 vs. 49 ± 24 days, respectively).

Conclusion: the stair step protocol has a higher efficacy than traditional protocol.

Keywords: Clomiphene citrate, metformin, ovulation, stair-step.

Synopsis: stair step protocol has higher ovulation and pregnancy rate and significant shorter duration of treatment making it superior on traditional protocol.

Introduction

PCOS is common endocrinal metabolic disorder affecting females at childbearing period ⁽¹⁾, with reported global prevalence varies between 3-10%⁽²⁾. Diagnosis of

PCOS is based on the Rotterdam criteria⁽³⁾ that requires 2 of the 3 diagnostic criteria, namely: - Oligo\anovulatory menstrual cycle, - Hyperandrogenic state that presented clinically or biochemically, and - Polycystic ovaries by high resolution transvaginal Ultrasound, in this setting follicle count per ovary should be ≥ 20 or ovarian volume ≥ 10 mL⁽⁴⁾. Treatment of infertility in women with PCOS includes non-pharmacological counseling about importance of life style modification and weight loss that would improve ovulation rates resulting in higher birth rates⁽⁵⁾. Pharmacological treatment for ovulation induction in PCOS includes CC and aromatase inhibitors are considered the first line, while gonadotropins are considered as second line, when the previous lines fail the next steps are laparoscopic ovarian drilling or assisted reproductive technologies⁽⁶⁾. CC acts as selective estrogen receptor modulator (SERM) that competes with estrogen ,consequently increasing levels of endogenous gonadotropins and the dominant follicle with highest number of FSH receptors is recruited, the antiestrogenic effect affects endometrium as well as cervical mucus thus potentially inhibits implantation⁽⁷⁾. According to the “traditional dosage protocol” an initial dose 50 mg daily is given for five days starting in days 2 to day 5 of menstrual cycle then TVUS is done to measure follicular diameter, if it is found less than 18 mm on cycle day 14, the dose increased by 50 mg in the subsequent cycles till reaching maximum dose 150 mg daily⁽⁷⁾. On the other hand, in “stair step protocol” CC is administrated after onset of menses for 5 days then TVUS is done to measure follicular diameter, if it is found less than 18 mm on cycle day 14, the dosage is increased to 100 mg daily for 5 days and re-evaluate by TVUS after one week of increasing dose, an increasing of in the same manner till reaching a maximum dose⁽⁸⁾. Previous studies showed that the ovulation rate was increased in the stair step group in comparison with traditional group⁽⁹⁾. This study aims to compare the efficacy of

clomiphene citrate by stair step protocol with traditional protocol in combination with metformin in induction of ovulation of PCOS patients.

Aim of the work: To evaluate the rate of ovulation using Clomiphene Citrate (CC) and Metformin “stair step protocol” versus “traditional protocol” in PCO and determine the uterine side effect & systemic side effects of cumulative doses in one cycle.

Patients and Methods

Study design: Randomized clinical trial (RCT)

Study setting: The study was carried out at obstetrics and Gynecology department outpatient clinic in Suez Canal University Hospital from the start of August 2021 to July 2022.

Study population and sample: A 60 participants diagnosed as PCO based on “Rotterdam 2003 criteria” after approval by local ethics committee and filling an informed written consent to participate in the study after receiving full information about the study.

Inclusion criteria:

1. Patients ages between 20 -35 years.
2. Patients diagnosed as PCOS based on “Rotterdam 2003 criteria”.
3. Tubal patency confirmed by either hysterosalpingogram or during diagnostic laparoscope.
4. Normal husband semen analysis according to WHO criteria (2010).

Exclusion criteria:

- Patient ages < 20 years or > 35 years.
- Patients who do not meet criteria for PCOS.
- Other causes of infertility as tubal factor or male abnormalities.
- Presence of endocrinological disease as

hypo, hyperthyroidism, hyperprolactinemia or other pelvic causes of infertility.

- Pregnancy or receiving other ovulation induction medications.

Randomization and Allocation: A probability simple random sampling was done by computer generated program. -

Allocation: two groups with 1:1 ratio.

- **Group (A):** stair step protocol (n=30).
- **Group (B):** traditional protocol (n=30).

Procedure

All selected patients are subjected to:

1. complete history.
2. full general and local examination.
3. laboratory tests: liver, renal function tests, TSH, prolactin, FSH, LH and husband semen analysis
4. TVUS: 7.5 MHz vaginal probe of ultrasound (Models DC 60, MINDRAY, CHINA) to identify ovarian cysts before starting treatment.

Ovulation induction groups:

Group (A): 50 mg of CC was administered daily on cycle day 2 for consecutive 5 days then TVUS is done on day 9 to measure follicular mean diameter. If less than 18 mm on day 14, the dose is increased immediately to 100 mg daily for consecutive 5 days and re-evaluated by TVUS on day 21, if follicular mean diameter less than 18 mm, the dose increased immediately to 150 mg for consecutive 5 days and U/S is performed 1 week after the second U/S, as shown in figure (1).

Group (B): 50 mg of clomiphene citrate was administered daily on cycle day 2 for consecutive 5 days then transvaginal ultrasound is done on day 9 and day after day to measure follicular mean diameter. If less than 18 mm on cycle day 14, the dose increased to 100 mg in the next cycle in the same manner till reaching 150 mg of CC. When the follicular mean diameter

was 18 mm or more, HCG was administered. **Metformin:** For both protocols, the starting dose was 500 mg once daily then increased weekly by 500 mg till reaching maximum dose of 1500 mg daily.

The measures of primary outcome through:

- Rate of ovulation: TVUS folliculometry assessing number and mean diameter of growing follicles during the given cycle.
- Detection of the ovulation by transvaginal ultrasound through:
- Sudden decrease in follicular size with irregularity of margins.
- appearance of intra follicular echoes.
- Follicle suddenly becomes more echogenic.
- Free fluid in pouch of Douglas.

The measures of secondary outcome through:

Uterine side effect of CC through:

- Uterine artery doppler US pulsatility and resistivity index between 8:00 am-12:00 pm
- Endometrial thickness by TVUS as an indirect marker for endometrial receptivity

Systemic side effect of CC: was evaluated by questionnaire that investigates hot flushes, mood changes, nausea, vomiting, breast tenderness and headache.

Statistical Analysis:

- The collected data was computerized and statistically analyzed using SPSS program (Statistical Package for Social Science) version 26.
- Data was tested for normal distribution using the Shapiro Walk test.
- Qualitative data was represented as frequencies and relative percentages.
- Chi square test (χ^2) and Fisher exact was used to calculate difference between qualitative variables as indicated.
- Quantitative data was expressed as mean and standard deviation.

- Student t test and Mann Whitney test were used to calculate difference between quantitative variables in two groups for parametric and non-parametric variables.
- Level of P-value < 0.05 indicates significant while, $P \geq 0.05$ indicates non-significant difference.

Results

from the beginnings of August 2021 to the end of July 2022, RCT study conducted on 60 infertile PCO patients after exclusion of 18 patients who dropped out during study, then were randomly allocated into Group (A) "stairstep protocol" & Group(B) "traditional protocol". there were no statistically significant differences between the study group means regarding: the age, BMI, parity, residency & occupation, as shown in table (1).

There was a higher ovulation rate in the group (A) than group B (76.7% V.S 63.3%), although not statistically significant. When comparing ovulation rates by dose, there was higher ovulation rate (43.3%) on dose 100 mg at group (A) compared to higher ovulation rate (33.3%) on 150 mg in group B. regarding clinical pregnancy rate, group (A) was significant higher clinical pregnancy rate compared to group B. When comparing pregnancy rate by dose, there was higher pregnancy rate on dose 100 mg at group (A) while the rate was higher on dose 150 mg in group B. That was statistically significant. Overall, the treatment duration was statistically significantly shorter in group (A) (21 ± 7 days) compared to group B (49 ± 24 days), as shown in table (2).

There was no statistically significant difference for systemic side effects of CC between the two studied groups, as shown in figure (2). While local side effects, the endometrial thickness was less in group (A) than group (B) that was statistically significant. Regarding mean uterine artery PI and RI, the mean for each was significantly higher in group (A), as shown in table (3).

Discussion

In the current study, CC was administrated with metformin using stair step protocol compared with traditional protocol to assess the ovulation rate in both protocol and to compare the systemic and local side effect of both protocols.

A meta-analysis had suggested that about 52% ovulate in response to treatment with clomiphene citrate 50 mg, the dose dependent ovulation rate was higher " 64%" at 100 mg in stairstep protocol compared to "22%" for traditional protocol, with shorter duration of treatment by 32-53 days in comparison to traditional protocol⁽¹⁰⁾. on these grounds, the use of stairstep protocol in ovulation induction shows higher ovulation rate with shorter duration of treatment and this effect enhanced with combination with metformin in order to improve ovulation and pregnancy rate. This is why the study was selected to compare the efficacy of use of clomiphene citrate combined with metformin by stairstep protocol with traditional protocol regarding ovulation, pregnancy rate and duration of treatment for both protocols.

In the current study results have revealed that There was a higher ovulation rate of (76.7%) in the stair-step protocol group when compared to (63.3%) in the traditional protocol group, however it did not reach statistical significance. When comparing ovulation rates by dose, there was higher ovulation rate 43.3% on dose 100 mg and 23.3% at dose 150 mg observed at stair step protocol compared with 20% ovulation on 100 mg and 33.3% ovulation on 150 mg in traditional protocol. These results were in coincidence with Ali et al., 2018 study that found that the ovulation rate was higher in stair step group (80%) in comparison to traditional group (63.3%) but not statistically significance⁽⁹⁾. In addition Deveci et al., 2014 study found the higher ovulation rate in stair step group 43.3% versus 33.3% in traditional group was not statistically significance⁽⁸⁾. However, Kader et al., 2021 study stated that the ovulation

rate was statistically significant higher in stairstep group (71.43%) compared with traditional group (55.68%) with higher dose dependent ovulation rate at dose 150 mg in stairstep group 34.52% compared to 20.45% in traditional group⁽¹¹⁾. High ovulation rate is thought to be the result of a cumulative effect of multiple doses. As Clomiphene citrate formed of two geometric isomers mixed in 3:2 ratio, Enclomiphene and Zuclomiphene, respectively. Enclomiphene is more potent with short half-life about 5–7 days and is primarily responsible for ovulation induction. When patients take their next dose, active isomers are still circulating in the system, making the total circulating concentration higher than in traditional protocols. In current study, there was statistically significant difference between the two groups regarding clinical pregnancy rate that was about 40% in stair step group compared to 26% in traditional group. That was high at dose 100 mg in stair step group (23.33%) but was high at dose 150 mg in traditional group (13.33%) Which is consistent with previous studies. In line with our results, Kader et al., 2021 study showed higher pregnancy rate difference that reach significance statistically between both groups, that was 45.24% in stairstep, while 30.68% for traditional group⁽¹¹⁾. These results disagreed with result obtained by Jones et al., 2018 study that revealed there was no difference in pregnancy rates per ovulatory cycle in the stair-step (16.3%) and traditional groups (18.1%)⁽¹²⁾. This difference in pregnancy rate between our study and his study could be explained that in Jones retrospective study depended on historical results not comparison of study and control groups, also administration of metformin play important role in improving ovulation and pregnancy rate as demonstrated in previous studies⁽¹³⁾. although the ovulation rate increased significantly, the pregnancy rates remain low. the low pregnancy rates explained by many reports through antiestrogenic effect of clomiphene and its metabolites on cervical mucus, endometrial, and oocytes.

Further, decrease in uterine vascularity in periovulatory period and endometrial thinning may disturb implantation and cause increase in pregnancy loss⁽¹³⁾. The results of our work revealed that there was no statistically significant difference between the two studied groups regarding the distribution of systematic side effects of the used drugs including hot flushes, mood changes, nausea, vomiting, breast tenderness and headache. These results are similar to that of studies conducted by Ali et al., 2018 and Deveci et al., 2014 studies^(8, 9).

Since CC causes a central misperception of low estrogen levels, natural vasomotor symptoms may be observed. Nausea and vomiting are the most side effects related to CC (60%). Mood changes and transient hot flushes occur in 56.7% of women. Headache was reported in 50% and breast tenderness in 43.3% of cases. Visual disturbances were the least side effects (36.7%). In the current study, the rate of systemic side effects was higher in the stair-step protocol but did not reach statistical significance. The major anti-estrogenic effect of CC interferes with the estrogen proliferation of endometrium. Endometrial receptivity is evaluated indirectly by measuring the endometrial. That was reported that the endometrial thickness should be at least 6 mm for implantation.

In the current study, there was statistically significant difference between both groups regarding endometrial thickness, RI and PI. These results showed disagreement with those of Eran Horowitz and Ariel Weissman 2020 and Deveci et al., 2014 studies which showed no statistically significant difference between the two groups regarding endometrial thickness and uterine artery Doppler ultrasound^(7, 10). On the other hand, there was partial agreement between our results and those of Ali et al., 2018 study which showed the difference between both groups was statistically significant regarding endometrial thickness and RI but not the PI.

that may explain decreased pregnancy rate in comparison to ovulation rate in each group ⁽⁹⁾.

The strengths and limitations of the current study:

The main strengths of this study include prospective study between study group and control group with strict selection of our samples and evaluation of different clinical outcomes and assessment of the ultrasound images in ovulation induction by clomiphene citrate in both groups. While the main limitation of this study was the small sample size of the study. Therefore, the presented study is accepted as a pilot study. A multicenter study is needed to provide required sample size for power. Also, this protocol requires multiple visits in a shorter period of time and more ultrasound monitoring.

Recommendations for future studies:

Further studies are needed to assess the efficacy of stair step protocol using letrozole and gonadotropins in ovulation induction.

Conclusion

The current study outcomes suggest that stair step regimen combined with metformin improves the ovulation rate and pregnancy rate with shorter course of treatment of infertility in PCOS.

Ethical approval

This study was approved by the research Ethical committee, faculty of medicine, Suez Canal university.

Competing of interest

There is no competing of interest to declare.

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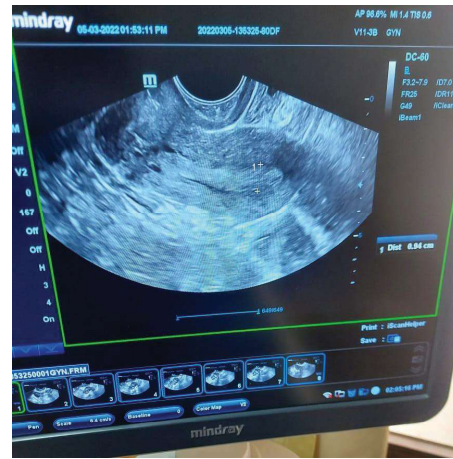
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Figure (1): these images demonstrating the ovarian morphology” left image” and endometrial thickness “RT image”

Before starting ovulation induction using stair step protocol



(a) PCOM



(b) Endometrial thickness was 9 mm

After ovulation induction using stair step protocol on dose 100 mg of CC



(c) Dominant follicle measuring 19*21 mm



(d) Endometrial thickness was 7 mm

Table (1): Basic characteristics of the two studied groups.

Variable		Group A n= 30	Group B n= 30	P value
Age (years)	Mean ± SD	26.9± 4.5	27.8± 4.9	0.495
	Median (Range)	26.5 (21, 35)	29 (20, 35)	
BMI	Mean ± SD	25.8± 2.5	27.0± 2.6	0.066
	Median (Range)	25 (22, 31)	27 (20,30)	
Parity	Mean ± SD	1.0± 0.9	1.0± 0.9	0.910
	Median (Range)	1 (0, 3)	1 (0, 3)	
Residency	Rural, n (%)	13 (43.3)	16 (53.3)	0.606
	Urban, n (%)	17 (56.7)	14 (46.7)	
Occupation	Employed, n (%)	11 (36.7)	10 (33.3)	>0.999
	Unemployed, n (%)	19 (63.3)	20 (66.7)	

Table (2): Ovulation rate and pregnancy rate among the two studied groups.

Variable		Group A n= 30	Group B n= 30	P value
Ovulation rate	Yes, n (%)	23 (76.7)	19 (63.3)	0.260
	No, n (%)	7 (23.3)	11 (36.6)	
Dose dependent ovulation rate	50 mg	3 (10)	3 (10)	
	100 mg	13 (43.3)	6 (20)	
	150 mg	7 (23.3)	10 (33.3)	
Clinical Pregnancy rate \ cycle	Yes, n (%)	12 (40.0)	8 (26.7)	0.017*
	50 mg	2 (6.67)	1 (3.33)	
	100 mg	7 (23.33)	3 (10)	
	150 mg	3 (10)	4 (13.33)	
	No, n (%)	18 (60.0)	22 (73.3)	
Duration of treatment (days)	Mean ± SD	21± 7	49± 24	<0.001*
	Median (Range)	21 (14, 28)	49 (21, 73)	

Table (3): Endometrial thickness and uterine artery doppler US among the two studied groups.

Variable		Group A n= 30	Group B n= 30	P value
Endometrial thickness	Mean ± SD	7.4± 1.1	8.8± 1.8	0.001*
	Median (Range)	7.7 (6.2, 9.2)	8.5 (6.3, 11.9)	
PI	Mean ± SD	1.1± 0.19	0.9± 0.31	<0.001*
	Median (Range)	1.2 (0.98, 1.58)	1.41 (0.85, 1.84)	
RI	Mean ± SD	0.66± 0.19	0.57± 0.11	0.020*
	Median (Range)	0.71 (0.21, 0.9)	0.58 (0.40, 0.78)	

Figure (2): Side effects of used drugs among the two studied groups.

