

Traditional Clomiphene Citrate with Phytoestrogen Versus Traditional Clomiphene Citrate Versus Stair Step Protocol in Patients with Polycystic Ovaries: A Randomized Clinical Trial

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ABSTRACT

Introduction: Polycystic ovary syndrome (PCOS) ranks among the most prevalent endocrine disease affecting women of reproductive age. Multiple therapeutic approaches have been suggested for the management of PCOS. Anovulation is responsible for approximately 40% of female infertility cases, and a significant portion of individuals dealing with infertility are also diagnosed with polycystic ovary syndrome.

Patients and methods: This was a prospective multi-center randomized clinical trial conducted from April 2022 till March 2023 involving one hundred fifty female patients with PCOS categorized into three categories, category I (traditional protocol) involved 50 subjects who received traditional clomiphene citrate (CC) 100 mg for 5 days, and category II stair-step protocol (SS) involved 50 subjects who received clomiphene citrate 50 mg for 5 days followed by 100 mg for another 5 days and phytoestrogen given for the whole 10 days. In addition, group III clomiphene + phytoestrogen (CCP) included 50 patients who received 100 mg of clomiphene citrate for 5 days and phytoestrogen for 10 days. **Results:** There was significantly thinner endometrium in the CC group as compared to the other two groups; the greatest endometrial thickness was in the SS protocol group, followed by the CCP group; a higher pregnancy rate and ovulation rate were in the SS protocol group, followed by the CCP group; and the lowest pregnancy rate and ovulation rate were in the CC group.

Conclusions: Clomiphene, when used as an SS regimen, improves pregnancy rates without any side effects compared to traditional regimens. Adding phytoestrogens to CC is associated with improving endometrial thickness and pregnancy rates.

Keywords: Polycystic ovary syndrome; Stair-step protocol; Phytoestrogen.

INTRODUCTION

PCOS is the most prevalent endocrine disorder among women aged 18 to 44, affecting approximately 6% to 20% of this demographic⁽¹⁾. To diagnose PCOS, two out of the following three criteria are required: amenorrhea or oligomenorrhea, signs of hyperandrogenism, or the presence of PCOS characteristics on ultrasound scans⁽²⁾.

Clomiphene citrate is used to stimulate ovulation by blocking estrogen receptors in the hypothalamus and negating the inhibitory effect of estradiol, resulting in increased secretion of endogenous FSH⁽³⁾.

Combining phytoestrogen with clomiphene citrate in ovulation induction for females with PCOS aims at counteracting the antiestrogenic effect of clomiphene citrate on the endometrium, which may cause endometrial hyperplasia and subsequently increase endometrial thickness⁽⁴⁾.

Utilizing a stair-step protocol with a combination of clomiphene citrate and phytoestrogen in women with PCOS has shown greater effectiveness in terms of promoting ovulation and improving pregnancy rates compared to the traditional protocol⁽⁵⁾.

Aim: to treat infertile women with polycystic ovary syndrome by improving endometrial thickness, pregnancy rate, and fertility rate.

PATIENTS AND METHODS

This study is a multi-center randomized clinical trial, conducted on female patients with PCOS.

This research enrolled 150 female patients with PCOS who were divided into three groups; Group I involved 50 patients who received clomiphene citrate 100 mg for 5 days, and Group II (Stair-step protocol) involved 50 patients who received clomiphene citrate 50 mg for 5 days followed by 100 mg for another 5 days and phytoestrogen given for the whole 10 days. In addition, group III (clomiphene + phytoestrogen) included 50 patients who received 100 mg of clomiphene citrate for 5 days and phytoestrogen for 10 days. The study setting was on October 6 University Hospital and Al Kasr Alainy University Hospital from the period of April 2022 till March 2023.

Inclusion criteria

This study included women who met the following criteria: aged between 20 and 35 years, diagnosed with either primary or secondary infertility based on the Rotterdam criteria from 2003 for PCOS, experienced infertility for a duration of 1 to 5 years, had partners with normal semen analysis, exhibited normal uterine conditions as confirmed by transvaginal ultrasound, and had normal hysterosalpingography (HSG) results. Additionally, participants were free from

medical disorders such as renal, hepatic, and cardiac conditions, and there were no other identified factors contributing to infertility, such as tubal pathology or male factor infertility.

Exclusion criteria

The study excluded individuals with other potential infertility causes, such as tubal pathology, endocrine disorders, previous gynecological operations, and females aged over 35 years. Randomization was done by a computer-generated table of random numbers in three groups of drugs:

Group I: Traditional protocol group ⁽⁶⁾ (n=50):

In this particular category, clomiphene citrate was provided at a daily dosage of 100 mg for 5 days, and this treatment commenced after the onset of a progestin-induced menstrual cycle. The response of follicles in the ovaries was closely monitored using transvaginal ultrasonography, with the monitoring beginning on day 8 of the menstrual cycle. When the mean diameter of the leading follicle reached 17 mm, human chorionic gonadotropin (HCG) was administered. If no follicular response was observed by cycle day 20, the cycle was terminated. Patients who did not respond to this dosage of clomiphene citrate were considered to have experienced treatment failure.

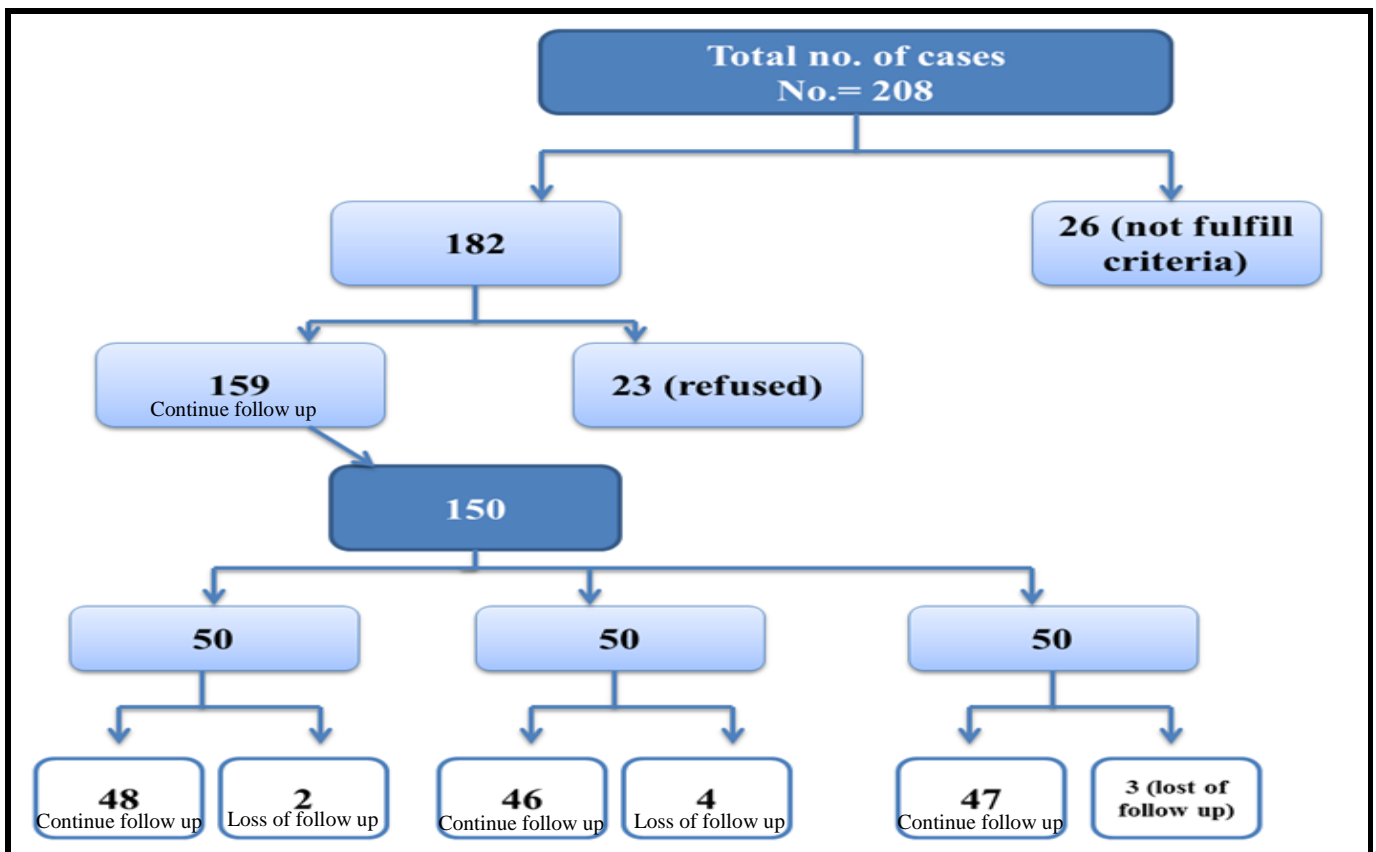
Group II: Stair-step protocol group ⁽⁶⁾ (n=50):

Clomiphene citrate was initially received at a daily dosage of 50 mg for 5 days after the onset of either

a spontaneous or progestin-induced menstrual cycle. The response of ovarian follicles was closely monitored using transvaginal ultrasonography (TVS), with the monitoring commencing on day 8 of the menstrual cycle. If the mean diameter of the follicles was found to be below 11 mm on cycle day 14, the dosage was increased to 100 mg/day for 5 days. Then, on cycle day 19, re-evaluations were conducted using TVS. When the mean diameter of the leading follicle reached 17 mm, human chorionic gonadotropin (HCG) was administered. If no follicular response was observed by cycle day 23, the cycle was canceled.

Group III: (Clomiphene+ Phytoestrogen) group ⁽⁶⁾ (n = 50):

In this specific group, the treatment involved administering clomiphene citrate at a daily dosage of 100 mg for 5 days following the initiation of a progestin-induced menstrual cycle. The response of ovarian follicles was monitored using transvaginal ultrasonography, starting from day 8. When the mean diameter of the leading follicle reached 17 mm, human chorionic gonadotropin (HCG) was administered. If there was no observable follicular response by cycle day 20, the cycle was terminated. Additionally, patients in this group began taking phytoestrogen on the third day of their menstrual cycle, and this continued for 10 days at a daily dosage of 1500 mg. This was done to mitigate the potential adverse effects of clomiphene citrate on the endometrium.



The subjects in the study underwent the following processes:

History Assessment:

- **Personal history:** Gathering information about the patient's background, medical history, and relevant details.
- **Present history:** Exploring the current health status and issues the patient is experiencing.
- **Contraceptive history:** Understanding the patient's history of contraceptive use.
- **Physical examination:** A thorough examination of the patient's physical condition.

Sample size evaluation:

The sample size was based on the primary outcome, which was the improvement of endometrial thickness. The study was designed with a statistical power of 80%, which means it had an 80% chance of detecting an effect if it truly existed. The alpha error (α), which represents the significance level, was set at 0.05, and the beta error (β), which complements the power, was set at 0.2. These parameters help ensure that the study is adequately powered to determine meaningful impacts while reducing the risk of types I and II errors in statistical hypothesis testing.

Ethical Approval:

Institutional approval was obtained from The Research Ethics Committee of October 6 University No. PMC-Me-2201004. Every patient signed an informed consent form. The attending obstetrician who recruited patients was responsible for obtaining informed consent. This research has been performed in accordance with The Declaration of Helsinki for researching involving humans.

Statistical analysis

The collected data were entered into a computer system for further analysis. IBM SPSS 23.0 for Windows, developed by SPSS Inc. in Chicago, IL, USA, was employed for statistical analysis. Qualitative data were expressed in terms of counts and percentages (N. %) and were compared by Chi-square test. For quantitative data, the normality of the distribution was assessed using the Shapiro-Wilk test and they were presented as mean \pm standard deviation (SD) and range and compared by one-way ANOVA test. $P \leq 0.05$ was considered significant.

RESULTS

No significant variations were noted between the studied groups concerning demographic data like age, gender, or other demographic characteristics. There were no remarkable variances between the studied groups regarding BMI or infertility duration.

A statistically significant difference was found among the studied groups regarding the number of follicles (as depicted in table 1).

Table 1: Follicles number among studied groups:

Follicles number	Group I (n=50)	Group II (n=50)	Group III (n=50)	P-Value*
Follicles number mean \pm SD range	1.1 \pm 0.2 (0-3)	2 \pm 0.41 (1-5)	1.4 \pm 0.42 (0-4)	0.03

*Chi-square test.

There was a considerable difference between the studied groups as regards follicle diameter at the 9th, 11th, 13th, and 15th-day measurements by ultrasound, as Group II (stair-step protocol group) showed the highest diameter of follicles (Table 2).

Table 2: Ultrasound folliculometry among studied groups:

Follicle diameter (mm)	Group I (n=50)	Group II (n=50)	Group III (n=50)	P-Value*
At 9 th day mean \pm SD	15.2 \pm 1.09	15.5 \pm 0.9	16.4 \pm 0.3	<0.001
At 11 th day mean \pm SD	16.2 \pm 1.01	17.9 \pm 1.07	17.3 \pm 1.1	<0.001
At 13 th day mean \pm SD	17.9 \pm 1.03	19.3 \pm 2.5	18.8 \pm 1.2	<0.001
At 15 th day mean \pm SD	18.3 \pm 1.2	21.5 \pm 0.5	19.1 \pm 1.8	<0.001
Increase in follicle diameter	3.1 \pm 0.2	5.8 \pm 0.5	2.7 \pm 0.25	<0.001

*ANOVA test.

Group II (stair-step group) had the greatest endometrial thickness, followed by Group III (Clomiphene citrate + phytoestrogen group) then Group I (Clomiphene citrate group), which had the lowest endometrial thickness, and this variation among the groups was highly statistically significant (Table3).

Table 3: Endometrial thickness among studied groups

Endometrial thickness (mm)	Group I (n=50)	Group II (n=50)	Group III (n=50)	P-Value*
At 9 th day mean±SD	6.5±0.98	6.9±0.85	6.6±0.75	0.06
At 11 th day mean±SD	7±0.9	7.5±0.77	7.2±0.71	0.008
At 13 th day mean±SD	7.5±0.89	9.4±1.72	7.6±0.72	<0.001
At 15 th day mean±SD	7.8±0.6	10.1±0.5	8.2±0.5	<0.001
Increase in endometrial thickness mean±SD	1.2±0.16	3.2±0.16	1.6±0.13	<0.001

*ANOVA test.

Stair-step protocol Group (Group II) had the highest pregnancy rate, with 30% of the patient’s achieving pregnancy. The difference in pregnancy rates among the three groups was significant. Also, there was a highly significant difference between the studied groups in ovulation rate as Group II showed the highest rate in comparison to the other two groups (Table 4).

Table 4: Outcomes among the groups

Outcomes	Group I (n=50)	Group II (n=50)	Group III (n=50)	P-Value*
Clinical Pregnancy				
No (N. %)	43 (86%)	35 (70%)	40 (80%)	0.14
Yes (N. %)	7 (14%)	15 (30%)	10 (20%)	
Multiple pregnancies				
Singleton (N. %)	46 (92%)	44 (88%)	47 (94%)	0.6
Multiple (N. %)	4 (8%)	6 (12%)	3 (6%)	
Ovarian hyper-stimulation				-
No (N. %)	50 (100%)	50 (100%)	50 (100%)	
Yes (N. %)	0 (0%)	0 (0%)	0 (0%)	
Ovulation rate				
No (N. %)	35(70%)	17 (34%)	31 (62%)	<0.001
Yes (N. %)	15 (30%)	33 (66%)	19 (38%)	

*Chi-square test

DISCUSSION

Polycystic ovary syndrome (PCOS) stands out as the primary cause of ovulatory dysfunction in women of reproductive age dealing with infertility⁽⁷⁾. Clomiphene citrate (CC) is recognized for its role as a

partially selective estrogen receptor modulator. It primarily influences the hypothalamus by altering the frequency of gonadotropin-releasing hormone (GnRH) pulses, consequently enhancing the release of follicle-stimulating hormone (FSH) from the pituitary gland. This, in turn, results in an ovulation rate ranging from 70% to 85% in each cycle⁽⁸⁾.

However, the application of CC for ovulation induction is associated with a common issue: endometrial thinning. To address this concern, phytoestrogens are introduced alongside CC to enhance endometrial thickness and subsequently boost pregnancy rates⁽⁹⁾.

Our results reveal that a notable difference exists in endometrial thickness between the clomiphene citrate group and the other two intervention groups. The stair-step protocol group exhibited the most substantial endometrial thickness, followed by the (CC + phytoestrogen) group. Furthermore, the stair-step protocol group displayed higher rates of pregnancy and ovulation, with the (CC + phytoestrogen) group following closely behind. On the other hand, the group utilizing only clomiphene citrate exhibited the lowest pregnancy and ovulation rates. In summary, our findings emphasize the significant impact of the chosen treatment approach on endometrial thickness, pregnancy, and ovulation rates in women with PCOS-related infertility. The stair-step protocol, particularly when combined with phytoestrogens, appears to offer a more favorable outcome regarding endometrial thickness and reproductive success compared to using clomiphene citrate alone. These results underscore the potential benefits of customized treatment regimens in managing infertility in PCOS patients.

In our study, a highly significant difference was observed among the studied groups in terms of ovulation rate (P < 0.001). The stair-step protocol (SSP) group displayed the highest ovulation rate at 66%, whereas the rates for the clomiphene citrate + phytoestrogen (CCP) and clomiphene citrate (CC) groups were 38% and 30%, respectively. These findings align with those of **Horowitz and Weissman** who also noted an increased ovulation rate with SSP, particularly at higher doses when compared to CC⁽¹⁰⁾.

However, our results differ from those of **Ali et al.**⁽¹¹⁾, who reported a higher rate of ovulation in the CC group, with 80% of patients experiencing an increase in ovulation rate, whereas the SSP group had a rate of 63.3%. This discrepancy could be attributed to Ali's study having a smaller sample size and potentially different characteristics, including a longer mean duration of infertility. In our study, the mean duration of infertility in the traditional group was 3.36 years, and in the stair-step protocol group, it was 3.38 years. Both studies found insignificant differences between the studied groups concerning BMI and age (p-values of 0.09 and 0.09, respectively). As a result, our study also revealed a statistically significant difference (P = 0.03) among the studied groups in terms of the number of

follicles. The SSP group had the highest number of follicles with a mean of 2 ± 1.41 follicles and a range of 1 to 5 follicles. These findings are consistent with previous studies by **Jones et al.**⁽⁴⁾ and **Agrawal et al.**⁽¹³⁾. **Jones et al.** reported a significant increase in the number of follicles in the SSP group, with mean values of 2 in SSP and 1 in CC⁽⁴⁾. **Agrawal et al.** found the highest number of follicles and the largest follicle size in the SSP group⁽¹²⁾. However, our results do not align with those of **Pourhoseini et al.**, who found no significant differences between the two groups in terms of the number of medium, large, or total follicles (p-values of 0.288 for all)⁽¹³⁾. This discrepancy may be due to the smaller sample sizes in previous studies. Additionally, a significant variance was observed between the studied groups regarding follicle diameter on the 9th, 11th, 13th, and 15th-day measurements by ultrasound ($P < 0.05$). The Stair-Step group exhibited the highest follicle diameter at the 15th-day measurement, with a mean of 21.9 ± 0.5 , while the clomiphene citrate (CC) group had the lowest follicle diameter at 18.3 ± 1.2 . These findings are consistent with the findings of **Oglak et al.**, who reported a significantly larger follicle size in the SSP group (20.69 ± 2.112)⁽¹⁴⁾.

CC has been associated with the issue of endometrial thinning, which is why adding phytoestrogens to CC has been explored to improve endometrial thickness⁽⁴⁾. Our study demonstrates that the SSP group had the most substantial endometrial thickness, followed by the clomiphene citrate + phytoestrogen (CCP) group, and the lowest endometrial thickness was observed in the clomiphene citrate (CC) group. This variation between the groups was highly significant ($P < 0.05$). Our findings are consistent with those of **Ali et al.**⁽¹¹⁾ and **Pourhoseini et al.**⁽¹³⁾. **Ali et al.** reported a mean endometrial thickness of 7.41 in the CC group and 8.42 in the SSP group, with significantly better endometrial thickness in the SSP group (p-value 0.004)⁽¹¹⁾. **Pourhoseini et al.** found no significant variance between the two groups receiving clomiphene alone or a combination of clomiphene and phytoestrogen ($P = 0.281$)⁽¹³⁾. However, our results differ from studies by **Deveci et al.**⁽⁶⁾ and **Shahin**⁽¹⁵⁾. **Deveci et al.** found no significant difference in endometrial thickness between the SSP and CCP groups (8.3 ± 2.1 vs. 9.3 ± 2.4 mm) on the day of triggering (p-value 0.24)⁽⁶⁾. **Shahin** suggested that endometrial thickness in the range of 5.5–8.25 mm and a triple-line pattern are highly predictive of pregnancy⁽¹⁵⁾. These discrepancies could be due to the smaller sample sizes in their studies.

Regarding chemical pregnancy rates, we found a significant increase in the SSP group, with 30% of patients achieving chemical pregnancy, compared to 14% in the CC group and 20% in the CCP group. These findings align with several studies, including **Deveci et al.**⁽⁶⁾ and **Ali et al.**⁽¹¹⁾, who also reported significantly

higher pregnancy rates in the SSP group compared to CCP and CC (46.7%, 30%, 6.7%, respectively).

In conclusion, our study suggests that combining clomiphene with phytoestrogens, particularly using the stair-step protocol, can enhance endometrial thickness and increase pregnancy rates in comparison to clomiphene citrate alone. However, differences in study outcomes among different research might be attributed to variations in sample size and other factors.

Limitations of the study

Short duration, a multicenter study is needed to confirm or refute our results with more patients with different geno- and phenotype, and more cases needed to be recruited to reach a final opinion about the addition of phytoestrogen to clomiphene citrate.

CONCLUSION

From our study, we concluded that clomiphene when being used as a stair-step regimen improves pregnancy rate without any side effects compared to the traditional regimen. Ovulation induction is a solution for the large percentage of women with infertility due to PCOS and should be practiced at the general OB/GYN clinic, especially on low social economics, whatever is the period of infertility. Adding phytoestrogens to CC is associated with improving endometrial thickness and pregnancy rates as it antagonizes the antiestrogenic effect of CC on the endometrium. Pregnancy rates and endometrial thickness were higher in the SS protocol group than the CC+ phytoestrogens group, which is higher than the CC group.

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- **Conflicts of interest: No conflicts of interest are existed in this study.**
- **Trial registration number: PACTR202204757658869.**

REFERENCES

1. **Echiburú B, Crisosto N, Maliqueo M et al. (2016):** Metabolic profile in women with polycystic ovary syndrome across adult life. DOI: [10.1016/j.metabol.2016.01.006](https://doi.org/10.1016/j.metabol.2016.01.006)
2. **Fausser B, Tarlatzis B, Rebar R et al. (2012):** Consensus on women's health aspects of polycystic ovary syndrome: the Amsterdam ESHRFJ/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. DOI: [10.1016/j.fertnstert.2011.09.024](https://doi.org/10.1016/j.fertnstert.2011.09.024)
3. **Espinola M, Laganà A, Bilotta G et al. (2012):** Current issues in ovulation induction. DOI: [10.12659/AJCR.932722](https://doi.org/10.12659/AJCR.932722)
4. **Jones T, Ho J, Marc J et al. (2011):** Clomiphene stair-step protocol for ovulation induction in women with polycystic ovarian syndrome. MSc. Degree in Obstetrics and Gynecology, Faculty of Medicine, Ain Shams University, DOI: [10.1097/AOG.0000000000002418](https://doi.org/10.1097/AOG.0000000000002418)

5. **Tempfer C, Georg F, Heinze G *et al.* (2009):** effects of phytoestrogens: A meta-analysis of randomized trials. DOI: **10.1016/j.amjmed.2009.04.018**
6. **Deveci C, Demir B, Sengul O *et al.* (2015):** Clomiphene citrate 'stair-step' protocol vs. traditional protocol in patients with polycystic ovary syndrome: a randomized controlled trial. DOI: **10.1007/s00404-014-3398-y**
7. **Borghet M, Wyns C (2018):** Fertility and infertility: Definition and epidemiology. DOI: **10.1016/j.clinbiochem.2018.03.012**
8. **Anwar A, Torky H, Dief O *et al.* (2013):** The effect of clomiphene citrate versus tamoxifen versus letrozole on endometrial thickness and blood flow in ovulation induction in women with polycystic ovaries. DOI: **10.5530/ami.2016.2.19**
9. **Wang L, Wen X, Lv S *et al.* (2019):** Comparison of endometrial receptivity of clomiphene citrate versus letrozole in women with polycystic ovary syndrome: a randomized controlled study. DOI: **10.1080/09513590.2019.1612358**
10. **Horowitz E, Weissman A (2020):** The stair-step approach in the treatment of anovulatory PCOS patients. DOI: **10.1177/263349412090881**
11. **Ali A, Khalaf S, Farahat M *et al.* (2016):** Clomiphene citrate and metformin stair step protocol vs. traditional protocol in patients with polycystic ovary syndrome, MSc Degree in Obstetrics and Gynecology, Faculty of Medicine, Menoufia University, DOI: **10.21608/EJHM.2018.9511**
12. **Agrawal K, Gainder S, Dhaliwal L *et al.* (2017):** Ovulation Induction Using Clomiphene Citrate Using Stair – Step Regimen versus Traditional Regimen in Polycystic Ovary Syndrome Women – A Randomized Control Trial. DOI: **10.4103/hrs.JHRS_15_17**
13. **Pourhoseini S, Mahmoudinia M, Najafi M *et al.* (2022):** The effect of phytoestrogens (Cimicifuga racemose) in combination with clomiphene in ovulation induction in women with polycystic ovarian syndrome: a clinical trial study. DOI: **10.22038/AJP.2021.47998.2595**
14. **Sakar M, Oğlak S (2021):** Comparison of the efficacy of letrozole stair-step protocol with clomiphene citrate stair-step protocol in the management of clomiphene citrate-resistant polycystic ovary syndrome patients. DOI: **10.1111/jog.14936**
15. **Shahin A (2008):** Endometrial sonographic characters predicting pregnancy following recurrent clomiphene induction in unexplained infertility. DOI: **10.1016/s1472-6483(10)60407-5.**