The Egyptian Journal of Hospital Medicine (October 2021) Vol. 85 (1), Page 2804-2808

Role of Adding Phytoestrogen to Clomiphene Citrate in Improving Endometrial Thickness in Woman with Unexplained Infertility

Ali El-Shabrawy Ali, Sherin Attia Shazley, Waleed Abd - Allah Abd Al-Salam, Marwa Yahia Ramadan Metwali*

Department of Obstetrics & Gynecology, Faculty of Medicine – Zagazig University, Egypt.

***Corresponding author:** Marwa Yahia Ramadan, **Mobile:** (+20) 01091677724, **E-Mail:** marwayahia120@gmail.com

ABSTRACT

Background: Unexplained infertility is failure to conceive after 1 year in couples with normal semen samples and no abnormality found during an infertility work-up. Clomiphene citrate (CC) induces ovulation.

Objective: The aim was to evaluate the effect of follicular phase oral phytoestrogens (PES) in patients with unexplained infertility, managed by clomiphene ovulation induction, triggered by HCG injection and timed intercourse, in terms of pregnancy rate, follicular maturation and endometrial thickness.

Patients and Methods: This study included 56 infertile patients seeking for pregnancy. Clomiphene was given orally at a dose of one tablet twice per day (tablet 50 mg), from day 3 to day 7 for all patients (group I and group II), while phytoestrogens were given at a dose of two tablets one shot per day (tablet 20 mg), from day 1 to day 12 only for group I, followed by HCG injection on attaining mature follicle(s) by trans-vaginal US scan with diameter ≥ 18 mm in both group. The study was conducted in Department of Obstetrics and Gynecology, Zagazig University Hospitals.

Results: The number of days till HCG injection were significantly lower in group I (13.8 ± 1.8 days) than in group II (14.9 ± 2.1 days), which indicates a better ovulation. As for the endometrial thickness, it was significantly higher in group I than in group II. The number of ovulatory follicle(s) as single, two or three showed no significant difference between the studied groups.

Conclusion: Adding PES to CC induced cycles, improved the endometrial thickness, promoted follicular maturation in a shorter time and also, improved pregnancy rates.

Keywords: Phytoestrogen, Clomiphene citrate, Endometrial thickness, Infertility.

INTRODUCTION

Unexplained infertility is defined as failure to conceive after 1 year in couples with normal semen samples and no abnormality found during an infertility work-up [(1)].

Clomiphene citrate (CC) is most commonly used as a first-line treatment of unexplained infertility [(2)]. It is an estrogen analogue that was first used in induction of ovulation in 1961, and clinically approved in the United States in 1967. Clomiphene exerts both estrogen agonist effects and antagonist effects [(3)]. Also, CC is successful in ovulation induction in 80% of cases, however the cumulative pregnancy rates reaches 30-40% only after its use for a few cycles. The discrepancy between ovulation and cumulative pregnancy rates is due to the anti-estrogenic effects of CC on both endometrial lining and the quality of cervical mucus [(3)].

Phytoestrogens (PES) are natural compounds with estrogenic activity that occur in many plants and fungi. The effects of different PES on estrogen-sensitive genes, their uterotropic activity and their selective affinity to estrogenic receptor subtypes, have been investigated and characterized in numerous studies. The affinity of PES for β estrogen receptors is 20–30 folds higher than that for alpha-estrogen receptor [(4)].

In general, phytoestrogens such as soy isoflavones have been thought to have predominant affinity to estrogen receptor beta (ER β) [(5)].

The study aimed to evaluate the effect of follicular phase oral phytoestrogens in patients with unexplained infertility, managed by clomiphene citrate ovulation induction, triggered by HCG injection and timed intercourse, in terms of endometrial thickness, follicular maturation and pregnancy rate.

PATIENTS AND METHODS

This was a controlled clinical trial conducted on fifty six (56) unexplained infertile patients seeking for pregnancy to evaluate the effect of follicular phase oral phytoestrogens in patients with unexplained infertility, managed by clomiphene citrate. Ovulation induction was triggered by HCG injection and timed intercourse. Fifty six (56) unexplained infertile women seeking for pregnancy were divided into two groups: The 1st group (study group) included twenty eight (28) women receiving CC in the form of clomid 50 mg tablet (Sanofi Aventis, France) at a dose of 100 mg/day in two divided doses, starting from day 3 to day 7 of the cycle plus phytoestrogens (black cohosh/cimicifugae racemosa) in the form of Klimadynon uno tablet 20 mg (Bionorica, Germany) at a dose of 2 tablet/day in single dose 40 mg. Choriomon 5000 I.U. ampoule (Ibsa, Switzerland) at a dose of two ampoules i.m. one shot for attaining mature follicle(s) by trans-vaginal US scan with diameter ≥ 18 mm for triggering ovulation.

Received: 10 /5 /2021
Accepted:6 /7 /2021

The 2nd group (control group) included twenty eight (28) women receiving clomiphene citrate only at a dose of 100 mg/day, starting from day 3 to day 7 of the cycle, followed by HCG injection (Choriomon 5000 I.U. ampoule, Ibsa, Switzerland) at a dose of two ampoules i.m. one shot for attaining mature follicle (s) by trans-vaginal US scan with diameter ≥ 18 mm for triggering ovulation.

The idea of giving phytoestrogens in this study before starting clomiphene was to block estrogen receptors before the clomiphene occupies them [(6)]. Phytoestrogens, by binding to estrogen receptors, have either an estrogenic agonist activity, the magnitude of which is far less than estradiol, or a less antagonist effect than clomiphene [(5)]. In order to explain this raised pregnancy rate as well as other results of other studies, the questions should be raised about whether this adjuvant treatment of follicular phase supplementation with phytoestrogens has a direct estrogenic effect or whether it merely reverts the anti-estrogenic effect of clomiphene [(7)].

Inclusion criteria: Patient aged 18-35years old with at least one year of infertility, primary and secondary infertility, duration of infertility from 1 to 5 years, normal husband semen analysis, normal uterus by trans-vaginal ultrasound, and normal hysterosalpingogram (HSG).

Exclusion criteria: Age > 35 years or <18 year, patient whose husband has a male factor of infertility, organic pelvic disease, previous pelvic surgery, abnormality detected by HSG as blocked tubes and hepatic, renal, diabetic, thyroid or cardiovascular disorders.

All patients were subjected to:

A) History taking: Full history taking including period and duration of infertility.

B) Full physical examination: Complete general, abdominal and local examination

C) Full consent (verbal and written consent): Patients were informed about the dose, side effects and complications of the drugs used.

D) Trans-vaginal ultrasound: It was performed after bladder emptying using 2D endovaginal prope ultrasound machine with frequency 7 MHz (SIMENS ACUSON X 300 Medical Systems, Korea) for both groups for follicular monitoring from day 7 and day 9 and individualized according to patients' response. Assessment of endometrial thickness, number of follicles and their size. for attaining mature follicle (s) with diameter ≥ 18 mm, HCG injection (Choriomon, Ibsa, Switzerland, 10000 IU, i.m.) was given.

- Endometrial thickness was measured by placing electronic calipers on the outer walls of the endometrium at the widest diameter as seen in the longitudinal axis (sagittal plane) of the uterine body at right angle to the cavity. The highest value of the thickness in the sagittal plane was recorded. An endometrial thickness of minimum 6 mm is required on day of HCG injection, but 8mm to 10 mm is optimum.
- Ultrasound follicular monitoring at this study began at day 7, the follicle diameter was measured when the follicle was seen as a rounded structure, and at least three measurements must be taken perpendicular to each other and take a mean measurement as a follicular diameter. Further monitoring of follicle number and size is done according to patient's response till detecting mature follicle(s). A mature follicle is characterized by being ≥ 18 mm, has thin walls, regular round shape and no echogenicity in the lumen.
- All ultrasound scans were done by the same investigator to avoid interobserver variations.

E) Timed intercourse is advised for 36-40 hours after HCG administration.

F) Further follow-up: Follow-up for occurrence of pregnancy was done, as serum βeta-subunit human chorionic gonadotropin (β –HCG) concentration was determined 14 days after HCG injection. Pregnancy was defined as an increase in the serum β-HCG concentration on two determinations at least 2 days apart. Biochemical pregnancy was defined as falling β-HCG concentration on serial determination. Clinical pregnancy was defined as positive gestational sac, with positive fetal heart beat, viewed by trans-vaginal ultrasound at 6 weeks of gestation (really for TVS to detect gestational sac and heart beats this is associated with β-HCG concentration more than 1500 mIU/mL, which coincides with 6 weeks of gestation). Early miscarriages were defined as biochemical pregnancies and/or cases with positive β-HCG testing who aborted spontaneously before reaching the stage of clinical pregnancy.

Ethical approval:

An approval of the study was obtained from Zagazig University academic and ethical committee. Every patient signed an informed written consent for acceptance of the operation. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Outcome of primary and secondary group included days until HCG injection, endometrial thickness (mm), number of mature pre-ovulatory follicles, and pregnancy outcome.

Clinical Check-up Schedule: Inclusion and follow up visits were done according to the following schedule:

- 1st session: inclusion and exclusion criteria, examination and informing patient about time to start taking drugs.
- Follow up sessions: from day 7 and then individualized for clinical checkup and transvaginal US assessment of follicles and endometrial thickness.
- Latter session: to assess occurrence of pregnancy.

Statistical analysis

Analysis of data was carried out using statistical package of social science (SPSS version 20). Description of quantitative variables was given as mean, and standard deviation (SD). Chi square test (χ^2 –test) was used to compare qualitative variables between groups. The t-test was used to compare quantitative variables in parametric data. The Z-test was used for proportions. P-value ≤ 0.05 was considered significant and P ≤ 0.01 were considered highly significant.

RESULTS

Table (1) showed that there was no statistically significant difference between the two studied groups regarding age and BMI**.**

Table (2) showed that there was no statistically significant difference between the two studied groups regarding type of infertility, and duration of infertility.

Endometrial thickness on 7th, 9th days and at day of HCG injection was statistically significantly thicker in the 1st group (4.89 ± 1.2, 7.78 ± 2.4 &10.1 ± 2.9 mm respectively) than in the 2nd group (3.75 ± 1.1, 5.14 ± 1.3 & 7.57 ± 1.4 mm respectively). However the days till HCG injection were significantly lower in the 1st group (13.8 ± 1.8 days where oral phytoestrogen was added to CC) than in the 2nd group (14.9 ± 2.1 days where CC was used only) as shown in table (3).

Table (4) and figure (2) showed that there was no statistically significant difference between the two studied groups in number of pre-ovulatory follicles (≥ 18 mm). There were three pre-ovulatory follicles ≥ 18 mm (17.9%) in the oral phytoestrogen plus CC group, while in clomiphene citrate group, only 7.1% had three pre-ovulatory follicles ≥ 18 mm.

there was statistically significant difference between the two studied groups in total pregnancies with 42.85% in the oral phytoestrogen plus CC group (39.3% had clinical pregnancies at 6 weeks and 3.55% had biochemical pregnancies. While in CC only group, 35.7% had pregnancies (21.4% had clinical pregnancies at 6 weeks and 14.3% had biochemical pregnancies) as shown in table (5).

Table (1): Basic data of the studied groups (NO=56)

Variable	1st group (28)	2nd group (28)	t-test	p-value
Age (years)				
• mean ± SD • range • median	24.5 ± 4.3 (18-34) 24	24.3 ± 4.6 (18 -35) 23	0.2	0.8
BMI (kg/M2)				
• mean ± SD • range • median	31.9 ± 4.2 (20-37) 30.7	30.6 ± 4.6 (20 -35) 29.5	1.2	0.4

Table (2): Distribution of studied patients according to types and duration of infertility (NO=56):

Variable	1st group		2nd group		p-value
	No. (28)	%	No. (28)	%	
Type of Infertility: -Primary -Secondary	 12 16	 42.9% 57.1%	 15 13	 53.6% 46.4%	0.4
Duration of Infertility (years): Mean ± SD	4.6 ± 1.1		3.9 ± 1.03		0.2

Table (3): Mean and standard deviation of days till HCG injection, endometrial thickness on 7th & 9th days and at day of HCG injection between the two studied groups (NO=56)

Variable	1st group (28) mean ± SD range	2nd group (28) mean ± SD range	t-test	p-value
Days till HCG injection				
• mean ± SD • median	13.8 ± 1.8 13	14.9 ± 2.1 14	5.9	**0.001****
Endomertrial thickness on 7th day				
• mean ± SD • median	4.89 ± 1.2 5	3.75 ± 1.1 4	3.5	**0.001****
Endomertrial thickness on 9th day				
• mean ± SD • median	7.78 ± 2.4 8	5.14 ± 1.3 5	5.1	**0.001****
Endometrial thickness at day of HCG injection (ovulation day)				
• mean ± SD • median	10.1 ± 2.9 10	7.57 ± 1.4 7	5.8	**0.001****

* Statistically significant difference (P ≤ 0.05)

Table (4): Number and percentage of mature pre-ovulatory follicles (≥ 18 mm) between the two studied groups (NO=56)

Variable	1st group		2nd group		χ^2	p-value
	No. (28)	%	No. (28)	%		
Anovulation	2	7.1	5	17.9	4.5	0.06
Single preovulatory follicles ≥18mm	11	39.3	13	46.4		
Two preovulatory follicles ≥18mm	10	35.7	8	28.6		
Three preovulatory follicles ≥18mm	5	17.9	2	7.1		

Table (5): Number and percentage of the pregnancy outcome between the two studied groups (No=56)

Variable	1st group		2nd group		χ^2	p-value
	No. (28)	%	No. (28)	%		
Clinical pregnancies at 6 weeks NO.=(16)	11	39.3	6	21.4	4.6	**0.03***
Biochemical pregnancies NO.=(2)	1	3.55	4	14.3		
Total pregnancies No.=(22)	12	42.85	10	35.7		

* Statistically significant difference (P ≤ 0.05)

DISCUSSION

The current controlled clinical trial study on patients undergoing IVF showed that both groups were matched with no statistically significant difference between them in age and BMI. This is the same as in a study conducted by **Unfer *et al.*** [6] whose study included 134 women divided into two groups; 1st group consisted of 65 women who CC Plus phytoestrogens and 2nd group consisted of 69 women who received CC only with no statistically significant difference between both groups in age.

Concerning primary or secondary type of infertility among the two studied groups, the current study showed that there was no statistically significant difference between the two studied groups in type of infertility with 42.9% of the oral phytoestrogen plus CC group had primary infertility type, while 53.6% of CC group only had primary infertility type that was the same as a study conducted by **Shahin *et al.*** [7] where 28.3% of the phytoestrogen plus CC group had primary infertility type while 25.4% of CC group only had

primary infertility type with no statistically significant difference between the two groups.

Regarding duration of infertility between the two studied groups, the present study showed that there was no statistically significant difference in duration of infertility between patients in both groups, which is in agreement with **Unfer *et al.*** [6] who reported no statistically significant difference in duration of infertility between the studied groups. Additionally, **Shahin *et al.*** [7] found the same data where the difference in duration of infertility between their studied groups wasn't statistically significant.

The present study showed that there was highly statistically significant difference between 1st and 2nd group patients in endometrial thickness at day of HCG injection which was higher in the first group (10.1 ± 2.9 mm) than in the second group (7.5 ± 1.2 mm). This is in agreement with **Unfer *et al.*** [6] who found a statistically significant difference between the 2 groups regarding endometrial thickness. Also, **Shahin *et al.*** [7] showed highly statistically significant increase in endometrial thickness in CC plus phytoestrogen group than in CC only (8.9 ± 1.4 mm versus 7.5 ± 1.3 mm respectively, P < 0.001). Additionally, **Kamel** [8] showed that the women in the second group (phytoestrogen group) had a significant thicker endometrium (p = 0.0004). Moreover, **Shahin and Mohamed** [9] also had higher endometrial thickness in CC plus phytoestrogen group than in CC only (12.5 ± 1.9 VS 8.5 ± 1.9 p < 0.001 mm).

Concerning the number of pre-ovulatory follicles, our study showed that there was no statistically significant difference between the two studied groups in number of pre-ovulatory follicles (≥ 18 mm). This is in concordance with **Shahin *et al.*** [7] whose results showed that there was no significant difference between number of patients having single (9 versus 10), two (28 versus 26) or three or more (23 versus 24) pre-ovulatory follicles reaching more than 17 mm in diameter in both groups. Also our study is in agreement with **Elkhateeb** [10] where higher but not statistically significant difference in number of pre-ovulatory follicles (≥ 17 mm) was present (3.7 ± 0.5 VS 3.2 ± 0.8) in the clomiphene citrate with phytoestrogen group compared to clomiphene citrate only respectively.

The present study showed that there was statistically significant difference between the two studied groups in total pregnancies with 42.85% of the phytoestrogen plus CC group had pregnancies, while in CC group only, 35.7% had pregnancies. This is consistent with **Unfer *et al.*** [6] who found the same results. Also, similar to **Shahin *et al.*** [7] where the clinical pregnancy rate was significantly higher in CC in combination with PE compared to CC alone (36.7% versus 13.6%, P < 0.01, respectively).

CONCLUSION

Phytoestrogens administration has the ability to decrease the adverse effects of CC on endometrial thickness. Adding phytoestrogens to CC as an alternative to estradiol in women with unexplained infertility increased number of dominant follicles and improved endometrial thickness and pattern with improvement of both ovulation and pregnancy rates.

REFERENCES

1. **Nardo L, Chouliaras S (2015):** Definitions and Epidemiology of Unexplained Female Infertility. In: Unexplained Infertility: Springer, Pp: 21-25. https://link.springer.com/book/10.1007/978-1-4939-2140-9
2. **Palomba S, Falbo A, Giallauria F *et al.* (2010):** Six weeks of structured exercise training and hypocaloric diet increases the probability of ovulation after clomiphene citrate in overweight and obese patients with polycystic ovary syndrome: a randomized controlled trial. Hum Reprod., 25 (11): 2783-91.
3. **Maged A, Deeb W (2015):** Phytoestrogens as an alternative to estradiol in reversing the antiestrogenic effect of clomid on endometrium in ovulation induction in cases of PCO. Uterus and Ovary, 1: 909-14.
4. **Shahin Y, Alaa M, Omar M (2009):** Supplementation of clomiphene citrate cycles with Cimicifuga racemosa or ethinyl oestradiol–a randomized trial. Reproductive Biomedicine Online, 19: 501-507.
5. **Casper R (2004):** Phytoestrogens, clomiphene, and the uterus. Journal of the Society for Gynecologic Investigations, 11: 261–262.
6. **Unfer V, Casini M, Costabile L (2004):** High dose of phytoestrogens can reverse the antiestrogenic effects of clomiphene citrate on the endometrium in patients undergoing intrauterine insemination: a randomized trial. Journal of the Society of Gynecological Investigations, 11: 323–328.
7. **Shahin A, Ismail A, Zahran K (2008):** Adding phytoestrogens to clomiphene induction in unexplained infertility-a randomized trial. Reproductive Bio Medicine, 16: 580–588.
8. **Kamel H (2013):** Role of phyto-oestrogens in ovulation induction in women with polycystic ovarian syndrome. Eur J Obstet Gynecol Reprod Biol., 168 (1): 60-63
9. **Shahin A, Mohammed S (2014):** Adding the phytoestrogen Cimicifugae Racemosae to clomiphene induction cycles with timed intercourse in polycystic ovary syndrome improves cycle outcomes and pregnancy rates - a randomized trial. Gynecological Endocrinology: the Official Journal of the International Society of Gynecological Endocrinology, 30: 505-510.
10. **Elkhateeb R (2017):** Peri-ovulatory Phytoestrogen Improves Pregnancy Rate in Women with Polycystic Ovarian Syndrome Receiving Ovulation Induction with Clomiphene Citrate. Androl Gynecol Curr Res., 5: 4-8.