e-ISSN: 0975-5160, p-ISSN: 2820-2651

Available online on www.ijtpr.com

International Journal of Toxicological and Pharmacological Research 2023; 13(1); 09-14

Original Research Article

# An Observational Study on Assessment of Effect of Estradiol Valerate on Endometrial Thickness during Clomiphene Citrate Induced Ovulation

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**Received: 15-04-2022 / Revised: 18-05-2022 / Accepted: 01-06-2022**
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**Conflict of interest: Nil**

**Abstract**

**Background:** Clomiphene citrate is a first line therapy for ovulation induction in polycystic ovarian syndrome (PCOS) with anovulatory infertility. The drug binds and blocks estrogen receptors and is thought to have an anti-estrogenic effect on endometrial volume thus may have adverse effects on fertility despite good ovulation rates. Hence to counteract anti-estrogenic effects of clomiphene on endometrium, estrogen was added in the proliferative phase in clomiphene citrate induced ovulation and its effects on endometrial thickness and pregnancy rates was studied.

**Methods:** A hospital based observational prospective study was done at Medical College and Hospital, Kolkata in the department of Obstetrics and Gynaecology. Study period was 18 months and the sample size was 45.

**Results:** In our study we found that the endometrial thickness improved after adding estradiol valerate and among the study population 31.1% patients achieved pregnancy and among those who achieved pregnancy, frequency of live birth was more compared to abortion.

**Conclusions:** Adding estradiol valerate to clomiphene citrate induced ovulation produces a favorable endometrial response in infertile women with anovulatory cycle.

**Keywords:** Endometrial thickness, Estradiol, Comiphene citrate, ovulation induction



## Introduction

Clomiphene citrate (CC) is used for ovulation induction in polycystic ovarian syndrome (PCOS) with anovulatory infertility. It is a selective estrogen receptor modulator

(SERM), having both estrogen agonist and antagonist properties. Clomiphene citrate, a competitive antagonist of 17 β estradiol competes with endogenous estrogen for nuclear estrogen receptors at sites throughout the body. However, unlike Estrogen, clomiphene blinds to nuclear estrogen receptors for an extended interval of time and thereby depletes receptor concentrations by interfering with receptor cycling [1]. Reduced negative estrogen feedback triggers normal compensatory mechanisms that alter the pattern of gonadotropin-releasing hormone secretion and stimulate increased pituitary gonadotropin release which, in turn, drives ovarian follicular development [2] At the pituitary level, clomiphene might also increase the sensitivity of gonadotropin to GNRH [3]. In Anovulatory women with WHO Type II anovulation, clomiphene has been reported to induce ovulation in 60-85% of patients and achieve a pregnancy rate of 15-50% per women [4]. Anti-estrogenic effects of clomiphene on the endometrium are likely to be one of the causes of suboptimal pregnancy rates in spite of good ovulation rates. In addition to desirable central actions, clomiphene can exert less desirable anti-estrogenic effects at peripheral sites in the reproductive system. Estrogen plays a critical role in the formation of endometrium in natural cycles. Apart from its major role in the proliferative phase, it also primes the endometrium for the luteal phase by further proliferation of the basal cell layer and the induction of progesterone receptors [5] ensuring the capacity of the endometrium to become secretary. There are studies, that have shown, that adverse effects of CC on endometrium can be prevented by administering estrogen together with or after clomiphene [6]

Undoubtedly the endometrial thickness is one of the most important factors in infertility treatment. The pregnancy rate can be very low if the endometrial thickness is less-than 6-8mm.The reduction in endometrial thickness among treatment modalities containing clomiphene citrate is widely shown in the literature [7]. The use of drugs to improve endometrial thickness is of interest to researchers [8]. Endometrial thickness facilitates embryo transplantation therefore it reduces the risk of miscarriage and also increases pregnancy rate. Physicians have tried to adjust Clomiphene effects using ethinyl estradiol [9] (E2). Our aims and objectives of the study was to study the effects of estradiol valerate on endometrial thickness in clomiphene citrate induced ovulation and pregnancy rates as outcome.

## Materials and Methods

It is a hospital based observational prospective study which was conducted in the department of obstetrics and Gynaecology, Eden Hospital, Medical College, Kolkata. The study was conducted over 18 months i.e. from May 2020 to October 2021.Study population was derived from the patients presenting at Gynaecology OPD, MCH. These were the patients diagnosed with anovulatory infertility and has not responded to the initial treatment. The sample size was 45 which was calculated based on previous studies. The inclusion criteria were the patients with first treatment cycle, age between 25 to 30 years, infertility for at least 2 years duration and women with normal concentrations of prolactin, free thyroxine and thyroid stimulating hormone. Women whose partners have an abnormal semen analysis report – based on the World Health Organization criteria, women with uterine or tubal abnormalities on hysterosalpingography, women with body mass index > 30 kg/$m^2$ were excluded from the study.

The study population was previously administered with Clomiphene citrate 100mg/d from $3^{th}$ to $7^{th}$ day of menstruation, in spite of the follicles greater than 18mm after administration of Clomiphene citrate their endometrial thickness was less than 7mm as diagnosed by transvaginal sonography. The frequency of transvaginal sonography was

9MHz.These patients received 100 mg Clomiphene citrate from day 3 to day 7 of menstruation and 4 mg estradiol valerate after the 8th day of menstruation until 14th day. In these patients endometrial thickness was measured by transvaginal sonography on the 14th day of menstruation and then timed intercourse was recommended. The SPSS 25.0 software was used for statistical analysis. Parametric data were expressed as mean and standard deviation. Differences between groups were compared by Student's/test for independent samples for continuous values and $x^2$ test or Fisher's exact test for categorical variables.This work has been done after ethical clearance from the institution.

## Results

45 patients with anovulatory infertility were included in the study. The results are depicted as tables whereby Table 1 shows the distribution of the basic parameters of the patients and Table 2 shows the association between those parameters and pregnancy.

**Table 1: Basic parameters of the patients**

| Parameter | No: of patients | Percentage |
|---|---|---|
| Age in years | | |
| 21-25 | 6 | 13.3% |
| 26-30 | 39 | 86.7% |
| Distribution of Infertility | | |
| Primary | 32 | 71.1% |
| Secondary | 13 | 28.9% |
| Dominant Follicle size before Ovulation | | |
| 18 mm | 11 | 24.4% |
| 19 mm | 9 | 20.0% |
| 20 mm | 11 | 24.4% |
| 21 mm | 9 | 20.0% |
| 22 mm | 5 | 11.1% |
| Distribution of Achieved Pregnancy | | |
| No | 31 | 68.9% |
| Yes | 14 | 31.1% |
| Outcome of Achieved Pregnancy | | |
| Abortion | 5 | 35.7% |
| Live Birth | 9 | 64.3% |

In our study, the mean Baseline endometrial thickness (mean±s.d.) of patients was 6.4933±0.5404 and the mean Endometrial thickness after induction (mean±s.d.) of patients was 10.3689± .7999. There is a statistically significant increase in endometrial thickness with p value 0.0106. Hence addition of estradiol valerate has resulted in the increase of endometrial thickness. 6 (13.3%) patients were 21-25 years old and 39 (86.7%) patients were 26-30 years old. The mean Age (mean±s.d.) of patients was 27.3111± 1.6627. 32 (71.1%) patients had Primary Infertility and 13 (28.9%) patients had Secondary infertility. After induction the dominant follicle size was measured on day-14. 11 (24.4%) patients had Dominant follicle of 18 mm ,9 (20.0%) patients had Dominant follicle of 19 mm , 11 (24.4%) patients were having Dominant follicle of 20 mm, 9 (20.0%) patients were having Dominant follicle of 21 mm and 5 (11.1%) patients were having Dominant follicle of 22 mm.

Among 45 patients 14(31.1%) achieved pregnancy. Among those who achieved

pregnancy 5(35.7%) patients had abortion and 9(64.3%) had live birth.

**Among patients who achieved pregnancy**:

3 (21.4%) patients were 21-25 years old and 11 (78.6%) patients were 26-30 years old. 11 (78.6%) patients had Primary Infertility and 3 (21.4%) patients had Secondary Infertility. 7 (50.0%) patients were having Dominant follicle size of 20 mm on day-14, 3 (21.4%) patients had a size of 21 mm, and 4 (28.6%) patients had a size of 22 mm. Among patients who achieved pregnancy, 5 (35.7%) patients had abortion and 9 (64.3%) patients had live birth. The mean Age (mean± s.d.) of patients was 26.8571± 1.6575. The mean BMI (mean± s.d.) of patients was 25.5714± 2.3073. The mean Duration of infertility (mean± s.d.) was 5.9677± 2.0080. The mean Baseline endometrial thickness (mean± s.d.) was 6.5032± 5630.The mean endometrial thickness after induction (mean± s.d.) of patients was 10.3806± .8348.

**Among patients who did not achieve pregnancy:**

3 (9.7%) patients were 21-25 years old and 28 (90.3%) patients were 26-30 years old .21 (67.7%) patients had Primary Infertility and 10 (32.3%) patients had Secondary Infertility .11 (35.5%) patients were having Dominant follicle size of 18 mm on day-14, 9 (29.0%) patients had a size of 19 mm , 4 (12.9%) patients had a size of 20 mm, 6 (19.4%) patients had a size of 21 mm and 1 (3.2%) patient had a size of 22 mm on day-14.The mean Age (mean± s.d.) of patients was 27.5161± 1.6507 .The mean BMI (mean± s.d.) of patients was 25.9258± 3.2389. The mean Duration of infertility (mean± s.d.) of patients was 6.1429± 1.9158. The mean baseline endometrial thickness (mean± s.d.) of patients was 6.4714± .5060 The mean endometrial thickness after induction (mean± s.d.) of patients was 10.3429± .7460.

Association of Age in group with Achieved pregnancy was not statistically significant (p=0.2830) and association of Primary or Secondary Infertility with Achieved pregnancy was also not statistically significant (p=0.4580) but association of Dominant follicle size on day-14 with Achieved pregnancy was statistically significant (p=0.0005). Distribution of mean Age in patients who achieved pregnancy was also not statistically significant (p=0.2223), even distribution of mean BMI with Achieved pregnancy was also not statistically significant (p=0.7144). Distribution of mean Duration of infertility with Achieved pregnancy was also not statistically significant (p=0.7850) The distribution of mean baseline endometrial thickness with Achieved pregnancy was not statistically significant (p=0.8574) and the distribution of mean Endometrial thickness after induction with Achieved pregnancy was also not statistically significant (p=0.8853).

**Table 2: Association between Parameters and Pregnancy**

| Parameter | Achieved Pregnancy | |
|---|---|---|
| | Yes | **No** |
| Age (in Years) | | |
| 21-25 | 3 | 3 |
| 26-30 | 11 | 28 |
| Type of Infertility | | |
| Primary | 11 | 21 |
| Secondary | 3 | 10 |
| DF size before Ovulation | | |
| 18 mm | 0 | 11 |
| 19 mm | 0 | 9 |

| | | |
|---|---|---|
| 20 mm | 7 | 4 |
| 21 mm | 3 | 6 |
| 22 mm | 4 | 1 |
| Mean Endometrial Thickness (Mean±SD) | | |
| Baseline ET | 6.5032 ± 0.5630 | 6.4714 ± 0.5060 |
| ET after induction | 10.3806 ± 0.8348 | 10.3429 ± 0.7460 |

## Discussion

In our study we found that the endometrial thickness improved after adding estradiol valerate and 31.1% patients had achieved pregnancy. Among those who achieved pregnancy the frequency of live birth was more than abortion.

Favaedi M *et al* [10] studied the effectiveness of clomiphene citrate used alone and in combination with ethinyl estradiol for the induction of ovulation in patients undergoing IUI. There was a statistically significant difference in endometrial thickness between the two groups. Our study showed similar results but the difference was not statistically significant. Another study conducted by Satirapod C *et al* [11] also showed the positive effects of adding estradiol valerate (EV) on the thickness of clomiphene citrate (CC) stimulated endometrium. Gupta S *et al* [12] or modulator, has been used to induce ovulation in patients suffering from chronic oligo-anovulation and ovulatory dysfunction with ovulation rates of 60-85% and pregnancy rates of 15-50% per woman. Supplementation with estradiol valerate or gonadotropins in CC stimulated IUI cycles did not have any positive effect on the endometrium on the day of trigger. The study failed to show a statistically significant difference in pregnancy rates, however abortion rates decreased marginally in estradiol or gonadotropin supplemented groups. Our study showed an increase in endometrial thickness but the difference was not statistically significant, it also showed that 14 (31.1%) patients achieved pregnancy and among them 5 (35.7%) patients had abortion and 9 (64.3%) patients had live birth. Dhama V *et al* [13] found that the endometrium plays an important role in infertility. The growth of endometrium depends on serum estradiol level and blood flow to the uterus. The purpose of this study was to evaluate the role of endometrial thickness and its outcome in natural and stimulated cycles in infertile women. In our study we also found that there is a positive effect of estrogen on endometrium. Swasti KS *et al* [14] compared the effectiveness of clomiphene citrate used alone and in combination with ethinyl estradiol on endometrial receptivity in infertile women with polycystic ovaries (PCO). Color Doppler ultrasonographic evaluation of endometrial thickness (ET) and pulsatility index (PI) was done for 27 infertile women with polycystic ovaries and found a positive effect of estradiol on endometrial thickness. Similarly, Atashpour S *et al* [15] found that polycystic ovarian syndrome (PCOS) is the most common endocrine disorder in women which affects fertility. Clomiphene citrate is used as first-line treatment for this disorder, which is associated with some complications and therapeutic resistance. Our study also showed that the limitations of clomiphene citrate can be overcome by adding estradiol valerate.

So, we conclude that there is an increase in endometrial thickness after adding estradiol valerate which was statistically significant and the pregnancy rate also improved though it was not statistically significant.

Limitations of our study was that the sample size was very small and it was not a multicentric study. So, a larger multicentric study is required in this area to show a significant increase in pregnancy ratesS and that is the implications for future research.

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