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The Impact of Usage of Sildenafil Citrate with Clomiphene Citrate on The Endometrial Thickness during Ovulation Induction

Heba Farag Salama[1], Said Abd Elatti Saleh[1], Sara Sami Mohamed Abo Shanab[2]*, Mohamed Zakaria Sayer Dayer[1]

[1]Department of Obstetrics and Gynecology, Faculty of Medicine, Menoufia University, Egypt

[2]Department of Obstetrics and Gynecology, Alshohadaa Central Hospital, Egypt

***Corresponding author:** Sara Sami Mohamed Abo Shanab, **Mobile:** (+20) 01001265131, **E-mail:** samisara9999@gmail.com

ABSTRACT

Background: Clomiphene citrate (CC)-induced ovarian stimulation has been linked to detrimental endometrial consequences that are anti-estrogenic.

Objectives: To assess the effects of sildenafil vaginal tablet combined to CC on the endometrial thickness (ET) during ovulation induction.

Patients and Methods: This randomized controlled study was conducted at Departments of Obstetrics and Gynecology of Menoufia University Hospital and Alshohadaa Central Hospital. A total of 124 women were recruited. The patients were blinded to group allocation. Group I: (Clomiphene citrate (CC) alone): received clomiphene citrate (50 mg oral tablet twice daily from day 3 to day 7 of the menstrual cycle). Group II: (Clomiphene and sildenafil): received clomiphene citrate (50 mg oral tablet twice daily from day 3 to day 7 of the menstrual cycle) plus vaginal sildenafil tablets (25 mg/12h daily from day 7 up to ovulation trigger)**.** Mean endometrial stripe thickness measured on day 13 of the cycle and pregnancy rates were the primary endpoints.

Results: There were no significant differences between both groups regarding demographic, clinical and basal hormonal profiles. The mean ET measured on day 13 of menstrual cycle was statistically significantly higher among patients received clomiphene and sildenafil than those received clomiphene alone (11.09± 3.83 mm versus 9.22± 3.90 mm, p value 0.021). Also, pregnancy rates were 32.3% and 48.4% in group 1 and 2 respectively with significant differences.

Conclusion: Incorporation of sildenafil to CC regimen of ovulation induction has a positive impact on endometrial stripe thickness and so pregnancy outcomes.

Keywords: Sildenafil, Clomiphene Citrate, ET, Ovulation Induction.

INTRODUCTION

The failure to conceive after 12 months of frequent, unprotected sexual activity is the hallmark of infertility. About 85% of infertile couples have a recognizable cause such as anovulation, male factor or tubal factor and the remainder 15% have unexplained infertility [(1)].

About 20% of the reasons of infertility are related to ovulatory dysfunction. Polycystic ovary syndrome (PCOS) accounts for around 85% of anovulatory infertility (classified by the WHO as type 2 anovulation) [(2)]. PCOS is the most often identified endocrine condition in reproductive-aged women (frequency of 5%-10%) and is the primary cause of anovulation [(3)]**.**

The standard treatment for anovulatory infertility is ovulation induction, and CC is the first-line therapeutic drug. It possesses the characteristics of an estrogen agonist and antagonist and is a selective non-steroidal estrogen receptor modulator. It binds to estrogen receptors, mostly in the hypothalamus, where it disrupts the negative feedback loop caused by rising estrogen levels. This leads to sustained FSH production, which promotes the maturation and expansion of follicles [(4)].

Starting on the third day of the cycle, clomiphene is administered for five days in a row. The patient is deemed CC-resistant if the ovulation fails despite taking 150 mg daily for at least six cycles in a row [(5)]. The antiestrogenic effects of clomiphene are seen in the cervix and endometrium [(6)]**.** Despite the fact that CC is simple to use and induces ovulation in the majority of patients (57-91%), the pregnancy rates (27–40%) are dismal. It is a result of CC's detrimental effects, which are most felt during stimulation on the endometrium and cervical mucus quality [(7)].

A healthy endometrial receptivity is necessary for a pregnancy to be successful. A particular phosphodiesterase type 5 inhibitor called sildenafil citrate increases the vasodilatory effects of NO on vascular smooth muscle by inhibiting the breakdown of cGMP, which may enhance uterine blood flow and promote endometrial growth [(8,9)]. Certain genes involved in the blastocyst implantation process such as vascular endothelial growth factor, p53 tumor suppressor and plasminogen activator inhibitor that induce the production of proteins needed for endometrial matrix digestion, cell growth regulation and angiogenesis. Sildenafil promotes angiogenesis via increasing p53 and VEGF expression [(2)]**.**

The purpose of this study was to assess the effects of sildenafil vaginal tablet combined to CC on the ET during ovulation induction.

PATIENTS AND METHODS

This is a randomized controlled study that was conducted at Departments of Obstetrics and Gynecology of Menoufia University Hospital and Alshohadaa Central Hospital. A total of 124 women were recruited.

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Sample size estimation:
Based on an analysis of earlier study by **Aboelroose *et al.*** [10] who found that adding sildenafil citrate improved ovulation and pregnancy rate from (40%) at the group received clomiphene alone to (65%) at the group received both clomiphene and sildenafil citrate, Sample size pro software version 6 and statistics were used to compute sample size; the lowest sample size estimated was 124, the study's power was 80%, and the confidence level was 95%.

Randomization and allocation:

Recruited patients were assigned to two groups using computer-generated random numbers with a 1:1 ratio. Patients were divided into two groups: Group I (clomiphene citrate alone) and Group II (clomiphene citrate with sildenafil), with 62 patients in each. Independent pharmacists distributed either (CC) alone or (CC) with sildenafil pills based on the computer-generated randomization list. The participants were not aware of their group allocation.

Women aged 18– 35 years, BMI < 35 kg/m^2, normal endometrial cavity and normal semen analysis were included.

Participants were excluded if: Age more than 35 years, BMI > 35 kg/m^2, presence of congenital uterine malformations, fibroids, adenomyosis, endometrial polyp (s), Asherman's syndrome, endocrine / thyroid disorders, tubal infertility detected by HSG, and contraindications to sildenafil use (cardiovascular, renal or hepatic disorders).

The eligible cases were subjected to complete history taking (age – parity – infertility duration – history of miscarriage), general examination, pelvic examination, hormonal profile (basal follicle stimulating hormone, luteinizing hormone, estradiol, prolactin, thyroid-stimulating hormone and midluteal serum progesterone), semen analysis and hysterosalpingography (to investigate tubal patency). Basal ultrasound examination was performed on cycle day 3 using 4-9 MHz endovaginal transducer (to exclude any uterine or adnexal pathology, to measure the basal endometrial stripe thickness and to assess antral follicle count (AFC)).

Ovulation induction:
Group I: (Clomiphene citrate alone): received clomiphene citrate (Clomid, 50 mg oral tablet) twice daily from day three to day seven of the menstrual cycle.

Group II: (Clomiphene and sildenafil): received clomiphene citrate (Clomid, 50 mg oral tablet) twice daily from day three to day seven of the menstrual cycle plus vaginal sildenafil tablets 25 mg/12h daily from day 7 up to ovulation trigger**.**

Follow up:
Folliculometry using transvaginal ultrasound was done on days 9, 11, and 13 until follicular size reached 18–25 mm. Then, 5000 IU of hCG (Human chorionic gonadotrophin) was administered intramuscularly to trigger ovulation if the follicular size was 18 mm – 20 mm.

Primary outcomes:
Mean endometrial stripe thickness measured on day 13, number of mature follicles, occurrence of ovarian hyperstimulation and +ve pregnancy test rate were the primary endpoints.

Secondary outcomes:
The participants were followed thereafter for eight weeks for miscarriages, ectopic and multiple gestations rates.

Ethical approval:
The study protocol was authorized by Menoufia University's Local Ethics Council of the College of Medicine. Prior to recruitment, and following an explanation of the study's objectives and protocols, all participants completed informed consent forms. Throughout the course of the investigation, the Helsinki Declaration was adhered to.

Statistical analysis:
Software called SPSS version 24.0 was used to tabulate and analyze data. Numbers and percentages were used to display categorical data. To assess categorical variables, the X^2-test was employed. The means ± SD were used to show the quantitative data. Two means of two independent groups were compared using the student "t" test. Significantly was defined as $P < 0.05$.

RESULTS
After 140 women underwent eligibility screening, 16 were deemed ineligible (10 individuals failed to satisfy the inclusion requirements, and 6 subjects declined to take part in the study), and 124 participants were included (62 women allocated per group), as shown in CONSORT flow chart **(Figure 1)**.

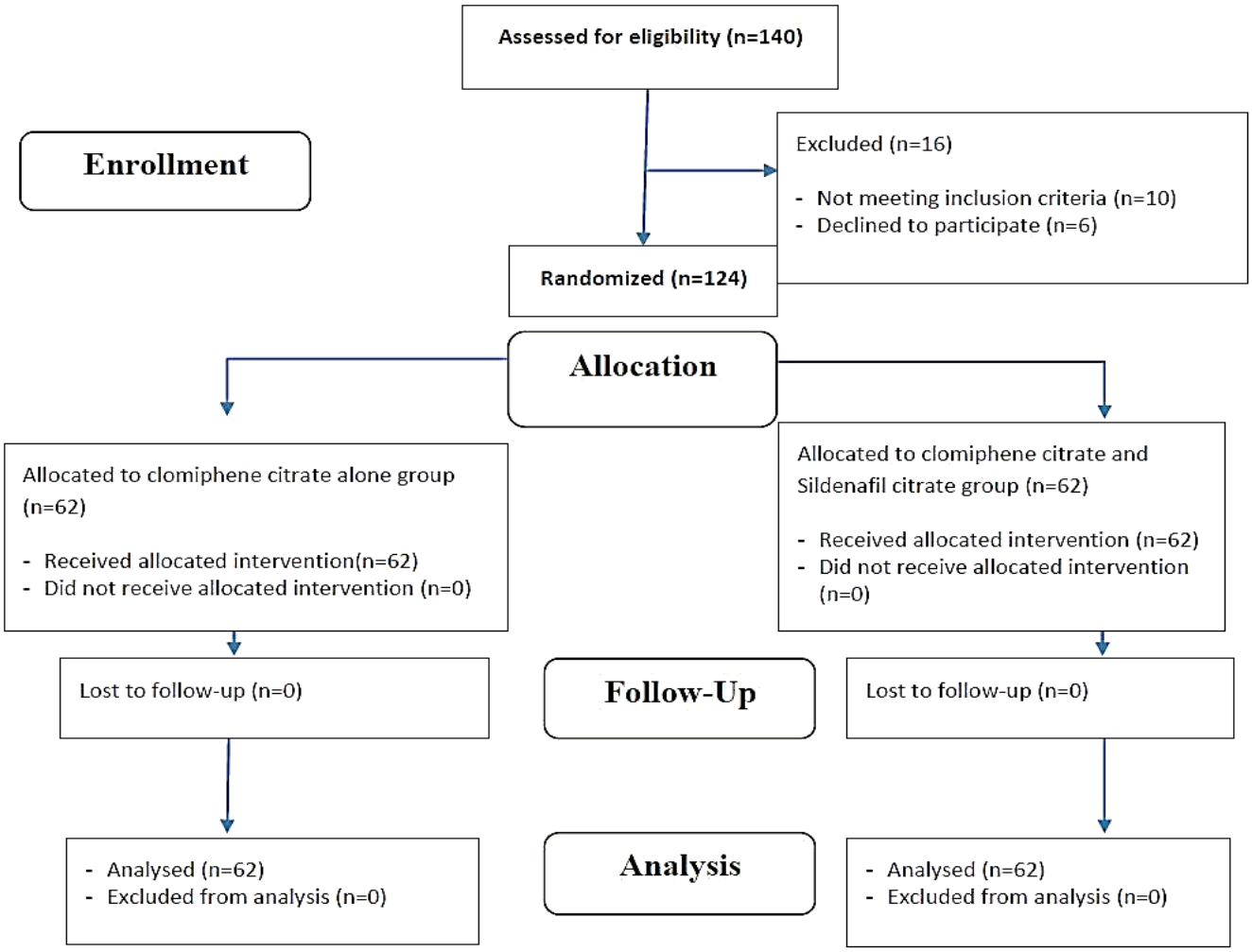

Figure (1): The CONSORT flow chart.

Baseline demographic and clinical data (age, BMI, parity and infertility type) were matched between groups **(Table 1)**.

Table (1): Baseline demographic and clinical data of study population

		Group I	Group II	t. test	P. value
Age (years)	Mean ± SD	27.61 ± 4.062	27.58± 4.44	0.042	0.966
BMI	Mean ± SD	29.95± 2.87	29.69± 2.51	0.537	0.592
Parity	Nulligrada 1 2 3	26(41.9%) 26(41.9%) 9(14.5%) 1(1.6%)	23(37.1%) 22(35.5%) 15(24.2%) 2(3.2%)	2.350	0.503
Infertility type	primary secondary	26(41.9%) 36 (58.1%)	25(40.3%) 37(59.7%)	0.033	0.855

There were no detected statistically significant differences between both groups concerning basal hormonal profile **(Table 2)**.

Table (2): Basal hormonal profile of study population

		Group I	Group II	t. test	P. value
FSH (IU/ml)	Mean ± SD	6.14± 1.47	6.35± 1.50	-0.415	0.679
LH (IU/ml)	Mean ± SD	7.46± 1.84	7.86± 1.94	-0.560	0.577
Prolactin (ng/ml)	Mean ± SD	17.71± 4.31	17.63± 4.31	0.084	0.933
TSH (ulU/ml)	Mean ± SD	2.37± 0.58	2.59± 0.64	-1.671	0.097

There were no statistically significant differences between groups regarding antral follicle count and basal ET measured by transvaginal ultrasound on the 3rd day of menstrual cycle **(Table 3)**.

Table (3): Basal transvaginal ultrasound on day 3 of the menstrual cycle

		Group I	Group II	t. test	P. value
Antral follicle count	Mean ± SD	9.77± 3.55	10.01± 3.12	-0.403	0.688
Endometrial thickness (mm)	Mean ± SD	2.95± 1.03	3.87± .999	0.952	0.019

Mean endometrial stripe thickness on day 13 of menstrual cycle was statistically significantly higher among patients received clomiphene and sildenafil than those received clomiphene alone. There were no observed statistically significant differences between both groups regarding number of mature follicles on day 13 ultrasound examination (**Table 4)**.

Table (4): Transvaginal ultrasound on day 13 of the menstrual cycle

		Group I	Group II	X^2	P. value
Endometrial thickness (mm)	Mean ± SD	9.22± 3.90	11.09± 3.83	2.53	0.021*
Number of mature follicles	Mean ± SD	1.61± 1.45	1.82± 1.36	0.751	0.269

*: Significant

Pregnancy rates were statistically significantly higher among patients received clomiphene and sildenafil than those received clomiphene alone (**Table 5)**.

Table (5): Pregnancy test results of both groups

	Group I	Group II	X^2	P. value
Pregnancy test				
Negative	42(67.7%)	32(51.6%)	0.852	0. 011*
Positive	20(32.3%)	30(48.4%)		

*: Significant

There were no detected statistically significant differences between both groups regarding occurrence of ovarian hyperstimulation, spontaneous miscarriages, ectopic or multiple pregnancy **(Table 6)**.

Table (6): Comparison between Group I and Group II regarding occurrence of ovarian hyperstimulation, spontaneous miscarriages, and ectopic/multiple pregnancy

	Group I	Group II	X^2	P. value
Ovarian hyperstimulation				
No	58(93.5%)	57(91.9%)	0.120	0.729
Yes	4(6.5%)	5(8.1%)		
Spontaneous miscarriages	**(N=20)**	**(N=30)**		
No	17(85%)	26(86.7%)	0.028	0.868
Yes	3(15%)	4(13.3%)		
Ectopic pregnancy	**(N=20)**	**(N=30)**		
No	19(95%)	29(96.7%)	0.087	0.768
Yes	1(5%)	1(3.3%)		
Multiple pregnancy	**(N=20)**	**(N=30)**		
No	16(80%)	25(83.3%)	0.090	0.764
Yes	4(20%)	5(16.7%)		

DISCUSSION

We investigated the impact of adding sildenafil vaginal tablet combined to CC on the ET during ovulation induction. Our data confirmed that sildenafil had a positive impact on endometrial stripe thickness and pregnancy rates.

In current study, there were no significant differences between both groups regarding age, BMI, parity, infertility type and basal hormonal profile. This was concordant with **Abdel Hamid *et al.*** [7] who compared the effects of adding vaginal sildenafil to a clomiphene regimen on endometrial stripe thickness and pregnancy rates in PCOS patients (group 1 received clomiphene, and group 2 received clomiphene citrate and Sildenafil). The demographic data revealed no significant differences between the two groups in terms of age, parity, BMI, type, and duration of infertility. Regarding the hormonal profiles of the study groups. Both groups had comparable TSH, LH, FSH, and prolactin blood levels, with no statistically significant differences.

The current study reported that mean endometrial stripe thickness on day 13 of menstrual cycle was statistically significantly higher among patients received clomiphene and sildenafil (11.09± 3.83mm) than those received clomiphene alone (9.22± 3.90) (P value=0.019). This was in line with **Abdel Hamid *et al.*** [7] who showed that endometrium was significantly thicker in sildenafil+ clomiphene group than controls (13.4±1.814 mm versus 8.52±2.081 mm). Other studies have shown that combining sildenafil with clomiphene during ovulation induction results in improved ET [11,12].

Also, our results were concordant with **Fahmy *et al.*** [12] who confirmed that sildenafil when applied as an adjuvant to CC during ovulation induction had improved the endometrial blood flow and ET**.** Moreover, **Mohammed *et al.*** [2] documented that mean endometrial stripe thickness among PCOS population was significantly thicker among the group receiving sildenafil citrate as an adjunct vs the group receiving clomiphene alone for the first three months and following medication switch. According to a study on the effects of sildenafil citrate therapy in infertile women with thin endometriums, the medication is useful in increasing the chances of clinical, chemical, and ET pregnancy in these women [8].

Furthermore, **Aboelroose *et al.*** [10] also illustrated that the addition of oral sildenafil citrate to the CC regimen in cases with unexplained infertility had increased ovulation rates and endometrial stripe thickness. **Abdel Hamid *et al.*** [13] reported that, adding sildenafil avoids the finding of thin refractory (< 7 mm) endometrium and only three patients (5.6%) had thin endometrium in sildenafil group compared to 14 out of 54 patients (25.9%) in clomiphene alone group and this difference was statistically significant. **Malinova *et al.*** [14] found that vaginal sildenafil might be utilized as an adjuvant to improve endometrial receptivity and fetomaternal immunotolerance, and that it was linked to enhanced endometrial blood flow and ET.

Mohamed [15] also investigated the usage of sildenafil citrate as an adjunct to letrozole in cases with PCOS and observed that thickness of endometrium among sildenafil/letrozole group were significantly higher compared to letrozole only group (12.7mm vs. 9.8mm, respectively).

However, when sildenafil is added to ovarian stimulation regimens, some investigators have documented a non-significant rise in ET [16].

In the current setting, there were no statistically significant differences between both groups concerning the count of mature follicles on day 13 or occurrence of ovarian hyperstimulation. In line with our findings, **Abdel Hamid *et al.*** [7] reported a comparable count of dominant (>18 mm) follicles in two study groups. Thus, it appears that sildenafil has no effect on follicular development or numbers. Contrary to our observations, **Fahmy and his colleagues**[12] reported that sildenafil citrate when combined to CC during ovulation induction had improved the follicular number.

We also documented that pregnancy rates were significantly higher among sildenafil/clomiphene group. This was consistent with **Aboelroose *et al.*** *'s* [10] study, the effects of sildenafil added to ovarian stimulation in individuals with infertility that cannot be explained were examined. The results showed that the sildenafil group had much greater conception rates. In addition, a research that assessed the advantages of administering sildenafil citrate to women undergoing assisted reproduction found that clinical pregnancy rates were considerably greater in women who got both sildenafil citrate and CC in combination than in those who received CC alone. Furthermore, compared to women who got estradiol valerate alone, those who received a combination of sildenafil citrate and estradiol valerate had a considerably higher rate of clinical pregnancy [17].

Also, **Aboelroose *et al.*** [10] reported a considerable rise in pregnancy rates among sildenafil patients. Moreover, **Li *et al.*** [8] concluded that sildenafil citrate had a positive impact in improving pregnancy outcome. **Ashoush and Abdelshafy** [18] observed that using SC as an adjuvant to CC for ovulation induction in PCOS women with CC failure was linked with significantly increased clinical pregnancy rates, ET, and improved subendometrial blood flow indices.

Frattarelli *et al.* [19] showed that whereas adjuvant sildenafil increases pregnancy rates, it has no effect on ET. Also**, Abbas *et al.*** [20] reported that positive pregnancy test rate was 39.8% in clomiphene/sildenafil group compared to just 30.1% in clomiphene group and when women that did not achieve pregnancy in clomiphene only group (about 145 candidates) underwent cross over to clomiphene and sildenafil combined regimen and followed up for a further three months, positive pregnancy outcome was increased to 38%.

In disagreement to our results, **Abdel Hamid *et al.*** [7] recorded a pregnancy rate of 27.6% in the group using sildenafil (8 patients) and 48.3% in the group taking clomiphene alone (14 patients). This non-significant differences regarding pregnancy rates observed in their trial could be explained by a small sample size included. Consequently, higher pregnancy rates might be demonstrated via a longer research with more participants.

In the current study, rates of miscarriages, ectopic and multiple pregnancy were comparable between both groups. This slightly agrees with **Sarhan *et al.*** [21] whose objective was to investigate the possible effects of combining sildenafil citrate with CC among women presented with unexplained infertility. They reported that sildenafil has no effect on decreasing the rates of miscarriages when compared to CC alone. Also, one case of multi-fetal pregnancy has been detected in the sildenafil group. Consequently, further settings with a longer period of follow-up are necessary for such cases.

CONCLUSION

Our findings suggested that incorporation of sildenafil to CC regimen of ovulation induction has a positive impact on endometrial stripe thickness and so pregnancy outcomes.

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