

TO COMPARE THE COMBINED EFFECT OF ORAL SILDENAFIL AND ESTRADIOL VALERATE TO ESTRADIOL VALERATE ALONE IN PATIENTS WITH CLOMIPHENE INDUCED CYCLES IN INFERTILE WOMEN ON ENDOMETRIAL THICKNESS.

Gynecology

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ABSTRACT

Objective- Endometrial thickness <7 mm accepted as a reliable sign of suboptimal implantation potential. Clomiphene citrate (CC) has some negative effects on the endometrium. We evaluated the effect of concomitant administration of oral sildenafil and estradiol valerate on endometrial thickness

Aim- To compare the combined effect of oral sildenafil and estradiol valerate to estradiol valerate alone in patients with clomiphene induced cycles in infertile women on endometrial thickness.

Material And Method- This randomized study was conducted on infertile women attending infertility OPD in SN Medical College, Agra over a period of 2 years from January 2019 to December 2020. Infertile patients were randomly divided into two equal groups. In control group, 50 patients were given clomiphene citrate 50 mg daily from D2 to D6 along with estradiol valerate 2 mg thrice daily from 2nd day of the cycle till the day of trigger of ovulation. In study group, 50 patients were given clomiphene citrate 50 mg daily from D2 to D6 along with estradiol valerate 2mg 12 thrice daily plus oral sildenafil citrate 25mg every 8 h from 2nd day of the cycle till the day of hCG trigger.

Results- The results in this study confirm the superiority of using oral sildenafil with estradiol for improving endometrial thickness, vascularity and pregnancy rates.

Conclusion- Addition of oral sildenafil citrate to estradiol valerate in ovulation induction cycles has better results in improving endometrial thickness, endometrial blood flow and pregnancy outcome as compared to estradiol valerate alone in women with anovulatory infertility.

KEYWORDS

Sildenafil citrate, endometrial thickness, anovulation, infertility, estradiol valerate.

INTRODUCTION-

Female causes account for 30% of the total infertility cases[1] of these causes 5% are due to uterine factors. Endometrial thickness is one of the strongest predictors of implantation rate and ongoing success rates of pregnancy. Endometrial factors and implantation failure are the known causes of infertility. Endometrium less than 7mm is a reliable sign of suboptimal implantation potential. Endometrial growth is dependent on uterine blood flow and angiogenesis. Endometrium is a dynamic tissue that responds to changing hormonal signals throughout the cycle. Thin endometrium can be due to various reasons mainly surgical procedures such as curettage, inadequate blood flow, Mullerian anomalies, use of clomiphene citrate, radiotherapy, recurrent infections. Hence adjuvants causing vasodilation have been suggested to be beneficial in women with thin endometrium. Several agents such as aspirin, heparin, L-arginine, vitamin-E, pentoxifylline, tocopherol, estrogen, sildenafil citrate and intrauterine infusion of growth factors were tried to achieve endometrial growth. The presence of a receptive endometrium is a major factor in determining conception. A good blood supply is considered as an essential requirement for implantation.

Clomiphene, a selective estrogen receptor modulator (SERM), with estrogen agonist and antagonist characteristics was used to stimulate the ovulation for the patients who experienced ovulatory dysfunction and chronic oligo-anovulation. Clomiphene citrate representing a competitive antagonist of 17β estradiol competes with endogenous estrogen for nuclear estrogen receptors at different sites throughout the body. Although the ovulation rates are good, clomiphene has anti-estrogenic effects on the endometrium which in turn causes suboptimal pregnancy rate.

Sildenafil is considered a type 5-specific phosphodiesterase inhibitor; therefore, it increases the nitric oxide vasodilatory effects through the degradation prevention of cGMP. Vascular smooth muscle is relaxed during a cGMP-mediated pathway by Nitric oxide (NO) and NO synthase isoforms is found in the uterus. Sildenafil potentiates uterine blood flow and in conjunction with estrogen, it increases estrogen induced proliferation of the endometrium.

AIM AND OBJECTIVES-

To compare the effect of oral sildenafil and estradiol valerate to estradiol valerate alone in patients with clomiphene induced cycles in infertile women on endometrial thickness, blood flow and pregnancy rates.

MATERIAL AND METHODS- The study will be conducted in the department of Obstetrics and Gynaecology, S.N. Medical College, Agra during the study period of January 2019 to December 2020.

Type of study : Meta Analysis

Total no. of women assessed for eligibility (n=135)
Infertile women excluded (n=35): Reason -

- Did not meet inclusion criteria (n=25)
- Refused to participate (n=10)

Final Randomized infertile women (n=100)

Allocated to group A (n=50)	Allocated to group B (n=50)
Lost of follow up (n=0)	Lost of follow up (n=0)
Analyzed (n=50)	Analyzed (n=50)

Fig. 1: Algorithm of patient's distribution

Selection of Cases: A total of 100 women in the outpatient and inpatient setup of the Department of Obstetrics and Gynaecology, S.N. Medical College, Agra were included in the study, fulfilling the inclusion criteria and exclusion criteria.(Fig.1)

Study population- 100 infertile women with thin endometrium were randomly divided into two groups.

Group A comprised of 50 women in whom clomiphene citrate 50 mg daily from D2 to D6 along with estradiol valerate 2 mg thrice daily from D2.

Group B of 50 women in whom clomiphene citrate 50 mg daily from

D2 to D6, estradiol valerate 2 mg thrice daily from D2 and sildenafil citrate 25 mg orally thrice daily from D7 to the day of ovulation trigger were given. Trans-vaginal ultrasound was done on alternate days from D8 of cycle for monitoring of follicular development and endometrial characters. HCG trigger was given once the dominant follicle reached >18 mm. Total three cycles of ovulation were studied.

Selected patients were subjected to-

Detailed clinical history, detailed general, systemic and local examination, routine blood investigation, urine microscopy, transvaginal ultrasound.

INCLUSION CRITERIA:

Women less than 35 years of age; with primary or secondary infertility; with patent fallopian tubes; without any organic pelvic pathology; with endometrium thickness less than 7 mm on the day of ovulation or Day 14 in previous clomiphene induced cycle.

EXCLUSION CRITERIA:

Congenital uterine anomalies, acquired deformities of uterus [Asherman's Syndrome], tubal factors of infertility, sensitivity to sildenafil citrate, contraindications for estrogen treatment [history of stroke, deep vein thrombosis, benign liver disease], moderate and severe male factor infertility.

After taking clearance from ethical committee and informed consent from the patient, 100 infertile women were taken up for study. Detailed history, general and local examination was done. Basic investigations along with hormonal profile [FSH, LH, Prolactin, AMH] on Day2 of cycle, TSH were done. Transvaginal Ultrasound of uterus and adnexa for any pelvic pathology, endometrial thickness on Day2 and Day14 was done. Those patients whose endometrium was less than 7mm on Day14 were taken up for study in next cycle. Semen analysis was done to rule out male factor.

Zone of vascular penetration into subendometrial and endometrial regions have been defined as:

Zone 1: A 2 mm thick area surrounding the hyperechoic outer layer of endometrium

Zone 2: Hyperechoic outer layer of endometrium

Zone 3: Hypoechoic inner layer of endometrium

Zone 4: Endometrial cavity.

Patients were randomly divided into 2 groups, each consisting of 50 patients. A maximum of 3 cycles of ovulation induction were continued and followed up till 7 weeks of gestation

Group A: 50 patients--Ovulation induction with Tab. Clomiphene Citrate 50mg daily from D2 to D6, along with Tab. Estradiol valerate 2mg thrice daily from D2 to D16

Group B: 50 patients--Ovulation induction with Tab. Clomiphene Citrate 50mg daily from D2 to D6, along with Tab. Estradiol valerate 2mg thrice daily from D2 to D16 and Tab. Sildenafil citrate 25mg 8th hourly vaginally from D7 till HCG trigger. In both the groups Ultrasound monitoring (Fig.2, 3,4) was done on alternate days from D8 onwards till HCG trigger and ovulation to check endometrial thickness, endometrial blood flow and follicular size. HCG trigger was given once the follicle reached more than 18mm. IUI was done in few patients if associated with mild male factor infertility.

OBSERVATIONS AND RESULTS-

Table 1 Patient characteristics among the studied groups

Variables	Group A (N=50) [CC+estradiol]	Group B (N=50) [CC+estradiol+sildenafil]
Median Age in years	30.20	30.4
Primary infertility	42[84%]	44[88%]
Secondary infertility	8[16%]	6[12%]
Mean BMI [Kg/m ²]	28.3	27.9
Median duration of infertility	4.26 years	4.08 years
FSH (mIU/ml)	6.8±1.5	7.1±1.3
LH (mIU/ml)	5.6±1.6	5.8±1.5
Prolactin (ng/ml)	12.6±5.0	12.3±4.8
E2(pg/ml)	54±9.8	57.1±10.5
TSH(iu/ml)	1.82±0.34	1.85±0.45

Values are mean ± standard deviation (SD)

In Group A the Median age was 30.20 years, primary infertility in 21[84%] women, secondary infertility in 4[16%] women, Mean BMI was 28.3 and median duration of infertility was 4.26 years. In Group B the Median age was 30.4 years, primary infertility in 22 [88%] women, secondary infertility in 3[12%] women, Mean BMI was 27.9 and Median duration of infertility was 4.08 years. The difference between both the groups is insignificant as regards to age, BMI, duration of infertility and type of infertility.

Table 2 1st cycle of Ovulation Induction. USG monitoring on the day of ovulation trigger

	Group A [N=50] [CC+estradiol]	Group B [N=50] [CC+estradiol+sildenafil]
No of follicles more than 18 mm diameter	1.28	1.36
Endometrial thickness	8.6±1.4 mm	10.2±1.8 mm
Endometrial pattern		
Trilaminar	20 [40%]	46 [92%]
Bilaminar	30 [60%]	4 [8%]
Endometrial blood flow		
Zone 1	2 [4%]	Nil
Zone 2	4[8%]	2[4%]
Zone 3	26[52%]	8[16%]
Zone 4	18[36%]	40[80%]
Clinical pregnancy	4[8%]	6[12%]

Average number of dominant follicles in Group A were 1.28, endometrial thickness 8.6±1.4 mm, trilaminar endometrial pattern in 20[40%] women, zone 4 endometrium in 18[36%] women where as in Group B average number of dominant follicles were 1.36, endometrial thickness 10.2±1.8 mm, trilaminar endometrium in 46[92%] women, zone 4 endometrium in 40[80%] women. Four women from group A and six women from group B achieved clinical pregnancy.

Table 3 2nd cycle of Ovulation Induction.USG Monitoring on the day of Ovulation trigger

	Group A [N=46] [CC+estradiol]	Group B [N=42] [CC+estradiol+sildenafil]
No of follicles more than 18 mm diameter	1.20	1.32
Endometrial thickness	8.4±1.6 mm	10.4±1.6 mm
Endometrial pattern		
Trilaminar	20 [43.47%]	40 [95.23%]
Bilaminar	26 [56.52%]	2[4.76%]
Endometrial blood flow		
Zone 1	2 [4.34%]	Nil
Zone 2	6[13.04%]	2[4.76%]
Zone 3	28[60.86%]	8[19.04%]
Zone 4	10 [21.73%]	32[76.19%]
Clinical pregnancy	6[13.04%]	10[23.80%]

Total number of patients in Group A were 23 women and 21 patients in Group B. One patient didn't come for follow up. In Group A average number of dominant follicles were 1.20, endometrial thickness was 8.4±1.6 mm, trilaminar endometrial pattern was noted in 20[43.47%] women, zone 4 endometrial blood flow was noted in 10[21.73%] women. In Group B average number of dominant follicles were 1.32, average endometrial thickness was 10.4±1.6mm, trilaminar endometrial pattern was seen in 40 [95.23%] women, zone 4 endometrial blood flow was observed in 32 [76.19%] women. Six out of 46 women in group A and 10 out of 42 women in group B achieved clinical pregnancy.

Table 4 3rd cycle of Ovulation Induction.USG Monitoring on the day of Ovulation trigger

	Group A [N=38] [CC+estradiol]	Group B [N=32] [CC+estradiol+sildenafil]
No of follicles more than 18 mm diameter	1.24	1.26
Endometrial thickness	8.6±1.2 mm	10±1.4 mm
Endometrial pattern		
Trilaminar	14[36.84%]	30 [93.75%]
Bilaminar	24 [63.15%]	2[6.25%]
Endometrial blood flow		

Zone 1	2 [5.26%]	Nil
Zone 2	6[15.78%]	2[6.25%]
Zone 3	22[57.89%]	4[12.5%]
Zone 4	8[21.05%]	26[81.25%]
Clinical pregnancy	8[21.05%]	10[31.25%]

Total number of patients in Group A were 38 patients and 32 patients in Group B. One patient didn't come for follow up In Group A. In Group A average number of dominant follicles were 1.24, average endometrial thickness was 8.6 ± 1.2 mm, trilaminar endometrial pattern was noted in 14[36.84%] patients, zone 4 endometrial blood flow was noted in 8[21.05%] and 8 out of the 38 women achieved clinical pregnancy. In Group B average number of dominant follicles were 1.26, average endometrial thickness was 10 ± 1.4 mm, trilaminar endometrial pattern was seen in 30[93.75%] patients, zone 4 endometrial blood flow was observed in 26[81.25%] and 10 out of 32 women achieved clinical pregnancy.

Table 5 Summary of 3 cycles of Ovulation Induction

	Group A [N=134] [CC+estradiol]	Group B [N=124] [CC+estradiol +sildenafil]
No of follicles more than 18mm diameter	1.24	1.31
Endometrial thickness	8.53 ± 1.4 mm	10.2 ± 1.6 mm
Endometrial pattern		
Trilaminar	54 [40.29%]	116 [93.54%]
Bilaminar	80 [59.70%]	8[6.45%]
Endometrial blood flow		
Zone 1	6 [4.47%]	Nil
Zone 2	16[11.94%]	6[4.83%]
Zone 3	76[56.71%]	20[16.12%]
Zone 4	36[26.86%]	98[79.03%]
Clinical cycle pregnancy	18[13.45%]	26[20.96%]
Total pregnancies in 25 women, (%)	18(36%)	26 (52%)

Out of 134 ovulation induced cycles in Group A average number of dominant follicles were 1.24, endometrial thickness was 8.53 ± 1.4 mm, trilaminar endometrial pattern was seen in 54[40.29%] women, zone 4 endometrium in 36[26.86%] women and pregnancy per cycle was 13.45%. In Group B out of 124 ovulation induced cycles average number of follicles were 1.31, endometrial thickness was 10.2 ± 1.6 mm, trilaminar endometrial pattern was seen in 116[93.54%] women, zone 4 endometrium in 98[79.03%] women and pregnancy per cycle was 20.96%.

DISCUSSION

Endometrium is a key factor for implantation. Clomiphene citrate is the first line of treatment for induction of ovulation. Despite good results reported with clomiphene for induction of ovulation a large proportion of females do not achieve pregnancy and the cause may be attributed to failure of implantation. Although most of the studies have compared estradiol or sildenafil alone with patients previous cycle as control, this study was done to compare the addition of sildenafil citrate on an already established benefit of using estradiol valerate.

We report statistically insignificant increase in mean number of follicles greater than 18mm on the day of the HCG trigger. Ahmed et al.,(11) also reported mean increase in the number of follicles with sildenafil was insignificant.

In our study we found significant increase in the endometrial thickness to mean of 10.2mm with a standard deviation of 1.6mm in Group B compared to 8.53mm with a standard deviation of 1.4mm in Group A which is in accordance with Takasaki et al., (a prospective study of 61 patients with thin endometrium there was a significant improvement in endometrial thickness)(5) which was also observed with Jerzal et al.,(7) Dalia Ibrahim et al.,(12) studies. Endometrial triple line [trilaminar pattern] in Group B was [93.54%] compared to 40.29% in Group A which was also seen in Firouzabadi et al., (a study of endometrium thickness and triple line pattern with significantly higher with sildenafil and estradiol compared to estradiol alone)(6).

In the present study there was endometrial blood flow zone 4 in 79.03% of women in Group B compared to 26.86% in Group A which is statistically significant. According to Mangal et al.,(8) there was a

significant increase in endometrial thickness, endometrial pattern, sub endometrial blood flow in patients using sildenafil with estradiol valerate. Mishra et al.,(10) reported statistically significant improvement in endometrial vascularity. In present clinical study clinical pregnancy rate per cycle was 20.96% in Group B where as 13.45% in Group A. Eighteen out of 50 women (36%) in group A and 26 out of 50 (52%) in group B had confirmed clinical pregnancy in the three months study period.

These significant results are due to direct effect of sildenafil on endometrial vascularity. In a prospective study, Wang et al.,(13) studied the endometrial thickness, echo pattern and blood flow on transvaginal sonography in 182 women 8 hours prior to HCG injection, they observed a higher clinical pregnancy rate and implantation in women with detectable blood flow. In Firouzabadi et al.,(6) study clinical pregnancy rates were higher but not significant.

The results in this study confirm the superiority of using oral sildenafil with estradiol for improving endometrial thickness, vascularity and pregnancy rates.



FIG. 2 Endometrium

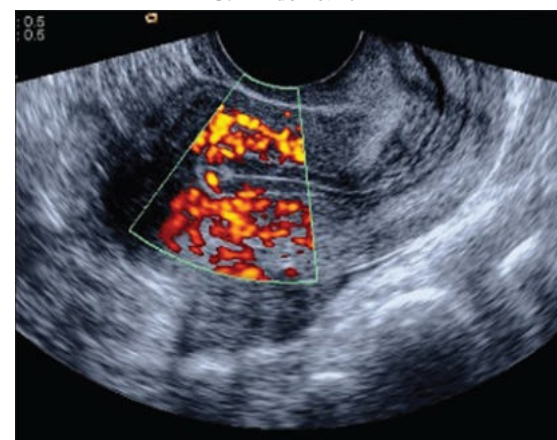


Fig. 3 Endometrial thickness in color doppler

CONCLUSION

Addition of oral sildenafil citrate to estradiol valerate in ovulation induction cycles has better results in improving endometrial thickness, endometrial blood flow and pregnancy outcome as compared to estradiol valerate alone in women with anovulatory infertility (undergoing induction of ovulation by clomiphene citrate).

Conflict of Interest: Nil

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