Oral sildenafil citrate: a potential approach for improvement of endometrial thickness and treatment of unexplained infertility in women

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Abstract. – **OBJECTIVE:** The study aimed to determine the impact of using sildenafil citrate as an adjuvant with clomiphene citrate (CC) in the treatment of women with unexplained infertility.

PATIENTS AND METHODS: 130 women with unexplained infertility were enrolled in a prospective randomized study. After dividing into two groups, all patients received CC 50 mg-BD from the 2nd to the 7th day of the cycle. Oral sildenafil citrate 20 mg was given BD to the study group from the end of menstruation till ovulation. A transvaginal ultrasound was carried out for all patients to assess ovulation, number of follicles, and endometrial thickness (ET). The beta-hCG blood test was used to determine pregnancy two weeks after ovulation followed by an ultrasound to confirm viability. Adverse effects were recorded and miscarriage, ectopic, and multi-fetal pregnancy were followed up for twelve weeks.

RESULTS: Median ET in the study group was 8 mm compared to 7 mm in the control group ($p<0.01$). The number of pregnancies increased in the study group but with no significant difference. The median ET was greater in the study group with an infertility duration lower than 2 years. Headache was the most significant adverse effect in the study group (9.2% *vs.* 1.5%, $p=0.052$).

CONCLUSIONS: Adding sildenafil citrate to CC is a good choice for overcoming the antiestrogenic action of CC and improving ET in women with unexplained infertility, especially in those with less than 2 years of infertility.

Key Words:
Unexplained infertility, Clomiphene citrate, Sildenafil citrate, Endometrial thickness, Ovulation.

Introduction

Infertility is a problem with multiple etiology. However, many women complain of unexplained infertility and their treatment represents a great dilemma[1,2]. Medications causing ovarian stimulation represent the most preferred way to treat such cases compared to invasive methods[2].

Clomiphene citrate (CC) is one of the non-invasive first-line treatments of infertility which gained great popularity due to its low price, tolerability, and safety profile[3,4]. Ovarian stimulation relies on its antiestrogenic impact to reduce estrogen-mediated feedback inhibition and promote luteinizing hormone (LH) and follicle stimulating hormone (FSH) production[5]. CC has a high rate of miscarriage and low pregnancy rate, despite its strong ovarian stimulation ratio[6]. This can be attributed to its antiestrogenic action on both the endometrium and cervical mucus[7].

The harmful effect of CC can be overcome by using phosphodiesterase (PDE) inhibitors[8]. Sildenafil citrate is a PDE5 inhibitor representing a wonderful precedent in this field[9-11]. It inhibits both cGMP-dependent protein kinases (PKGs) and phosphodiesterase-5 (PDE5) and increase nitric oxide (NO). Potentiation of nitric oxide effect by sildenafil on vascular smooth muscle can explain its action on the endometrium and fetal growth, as it relaxes the smooth muscles in blood vessels causing vasodilatation and thus increasing uterine blood flow. This can improve perfusion to the placenta and uterus in compromised pregnancies[12-14].

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The vasodilator effect of sildenafil can also enhance the development of the endometrium which is essential for implantation, placental exchange, and development of the fetus. The growth of the endometrium is mediated by hormones (estrogen and progesterone) and growth factors such as vascular endothelial growth factor (VEGF)[10,15]. It is necessary to transport some of these elements to the endometrium. These elements need enough uterine blood flow to reach the endometrium, especially its functional layer[16].

After menstruation, endometrial growth induced by estrogen is mostly influenced by the blood supply to the endometrial basal layer[17]. Consequently, sildenafil can increase the clinical and biochemical pregnancy rate in women having thin endometrium by improving endometrial thickness[18].

The purpose of this study was to determine if the combination of sildenafil and clomiphene citrate is superior to clomiphene citrate alone in improving endometrial thickness, ovulation, and rates of pregnancy in women with unexplained infertility.

Patients and Methods

Study Design

Between October 01, 2021, and April 30, 2022, a prospective randomized study was conducted at Beni Suef University Hospital, Beni-Suef, Egypt, in the gynecology outpatient clinic. One hundred and thirty women with unexplained infertility, ranging in age 18-40, participated in the study. Patients were randomly assigned to one of two groups. A computer-generated list randomly assigned patients to the sildenafil or control groups using sequential numbers. In addition, identical sealed envelopes were used, each including a paper with a printed code denoting the allocated group. The use of visually identical tablets for both the active tablet and the placebo tablet helped to guarantee double-blinding. The first group (n=65), which was the study group, received clomiphene citrate 50 mg (Tecnovula®, Techno Pharmaceuticals, 4th Industrial Zone, Borg El-Arab City, Alexandria, Egypt) orally twice daily from the 2nd to 7th day of the cycle and sildenafil (Respatio®, Pharma right group, Cairo, Egypt) 20 mg tablets from the end of menstruation till ovulation, twice daily. While the second group (n=65), which was the control group, received the same dose of clomiphene citrate 50 mg (Tecnovula®) as in the first group in addition to a placebo tablet.

Study Population and Clinical Follow-Up

Personal information was collected from all studied patients including age and weight. Body mass index was calculated and one hysterosalpingography was carried out at the beginning of the study to confirm that all patients had patent tubes. On the third day of the menstrual cycle, serum levels of LH, FSH, and prolactin were assessed as baseline hormonal profiles.

Using transvaginal ultrasonography, folliculometry was performed. This was carried out on the third day of the cycle to rule out the potential for ovarian cysts. On days 9, 11, and 13, the endometrial thickness and number of follicles were measured until the follicles attained a diameter between 18 and 22 mm. The trial was double-blinded, the observer was the same fertility consultant, and finally, we took the mean of three measurements of endometrial thickness to exclude any bias. Human chorionic gonadotropin (hCG) with a dose of 5,000 IU was administered intravenously (IV) to both groups when follicles were 18 mm or greater to trigger ovulation. The median ET for comparing the two patient groups in the study was calculated using the hCG trigger day. The beta-hCG blood test was used to determine pregnancy two weeks following ovulation followed by an ultrasound to confirm clinical pregnancy. Clinical pregnancy is defined as a pregnancy diagnosed by ultrasonographic detection of one or more gestational sacs or definite clinical symptoms of pregnancy. It involves a clinically verified ectopic pregnancy in addition to an intrauterine pregnancy[19]. All patients were observed thoroughly for undesirable consequences. In addition, miscarriages, multifetal, and ectopic pregnancies were observed for a period of twelve weeks in each patient.

Inclusion Criteria

The study included women aged 18-40 with primary or secondary unexplained infertility, a regular menstrual cycle, patent tubes on hysterosalpingography, and partners with normal sperm analysis. No subjects had had fertility treatment in the six months prior to recruitment. Before starting the study, patients underwent ultrasound follow-ups to confirm unexplained infertility and eligibility. On day 3, an ultrasound measured the antral follicle count (AFC) and reviewed FSH and LH labs. Folliculometry was done on day 3 to rule out ovarian cysts, and on days 9, 11, and 13 to count follicles, endometrial thickness, and other uterine abnormalities.

Exclusion Criteria

Exclusion criteria included hypotension; renal and cardiovascular diseases; hepatic abnormalities; uncontrolled diabetic cases; adhesions in the pelvic region; high level of prolactin hormone; thyroid abnormalities; multiple fibroids in the uterus; patients taking nitrates; ovarian cysts; suspected adenomyosis and endometriosis, anovulatory infertility; and hormonal profile abnormalities. Prior to the study, patients were evaluated between day 16 and 22 from the previous cycle to exclude any local abnormalities in the uterus such as an endometrial polyp, myoma, cavity fluids, thin endometrium to ensure their eligibility as unexplained infertility patients.

Clinical Outcomes

The primary outcome of the study was the rate of pregnancy. While secondary outcomes included counts of mature follicles, endometrial thickness, and ovulation. In addition, drug side effects were also reported as one of the secondary outcomes.

Ethics Statement and Clinical Trial Registration

The Research Ethical Committee of Beni-Suef University, Faculty of Pharmacy, validated this study (REC-H-PhBSU-21015), which was registered at clinical trial.gov (NCT05846906). Participants gave written consent in advance and were told they could withdraw at any time during the study. The study followed the Helsinki Declaration and its subsequent revisions.

Sample Size Calculation

The sample size for the two-arm prospective randomized clinical study was determined using the proportion of pregnant women in the sildenafil/clomiphene group compared to the clomiphene group (65% *vs*. 40% respectively) from a prior study (P_1, P_2).

Using a statistical significance level (α) of 0.05 and power (1-β) of 0.8, the calculated sample size per group based on a 1:1 randomization ratio was 61 patients per study group using the below equation[20]:

$$n = (\frac{Z_{1-\frac{\alpha}{2}} \times \sqrt{P_1 \times (1 - P_1)} + Z_{1-\beta} \times \sqrt{P_2 \times (1 - P_2)}}{(P_1 - P_2)})^2$$

Statistical Analysis

Numbers and percentages were used to illustrate categorical data, and Chi-square and the Fisher exact test were used for making comparisons when appropriate. For normally distributed data, comparisons were made using the unpaired t-test on means and standard deviations for continuous variables.

Shapiro-WIlk tests were used to determine the normality of data. Median, range, and Mann-Whitney U tests were used to present continuous data with non-normal distribution. Each studied group was further divided based on infertility duration (≤ 2 years *vs*. > 2 years) and compared using the Kruskal-Wallis' test.

The Mann-Whitney test and Bonferroni correction were used for post-hoc pairwise statistical analysis. Lambda association measured the connection between two nominal variables

The Mann-Whitney test was used for post-hoc pairwise statistical analysis, and the findings were adjusted using the Bonferroni method. The results were reported in terms of lambda values, which measure a proportionate reduction in error (PRE), and lambda is used to quantify the degree of association between two nominal variables. Non-normally distributed numerical variables were correlated using the Spearman correlation, with rho (ρ) values indicating the direction and strength of the association. All tests were 2-sided, and results with a *p*-value of 0.05 or lower were considered significant. SPSS 26 (IBM Corp., Armonk, NY, USA) was used for all statistical analyses.

Results

Baseline Clinical Characteristics

Figure 1 shows the flowchart of all subjects. 156 patients were examined for eligibility, and 26 were excluded, since 12 of them refused to participate and 14 did not meet the inclusion criteria. The remaining 130 patients were enrolled and randomly assigned to two groups based on treatment beginning (study *vs*. control group), with a median age of 32 years for both groups; however, minimum and maximum ages varied between 23 years and 40 years in the study group, compared to 22 years and 39 years in the control group, respectively. Median body mass index also was the same in the two groups (29 Kg/m^2) although in the study group it had a greater range. The age (*p*=0.46) and BMI distribution (*p*=0.76) did not show significant difference between both groups as shown in Table I.
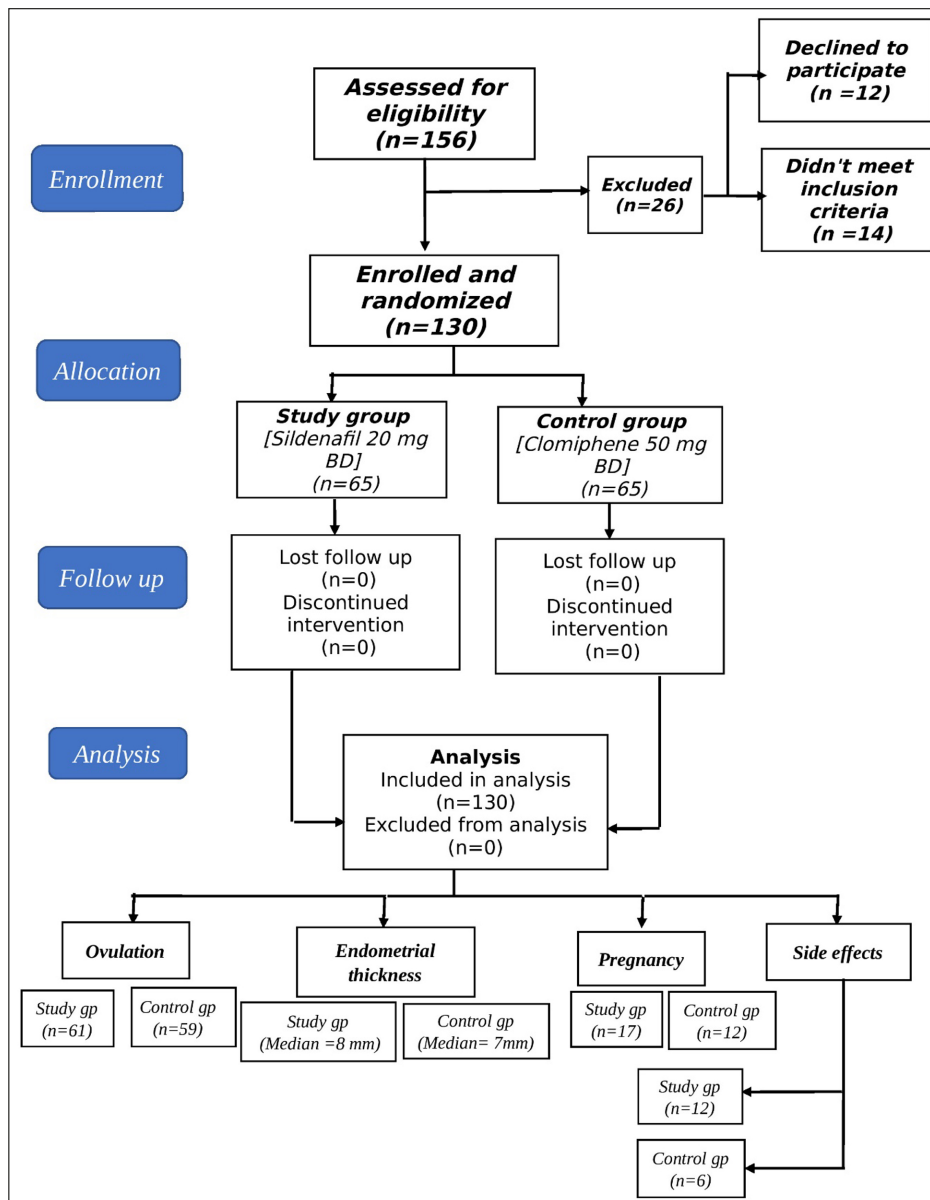
Figure 1. Flowchart of studied patients.

Table I. Age and Body Mass Index comparison among Sildenafil/clomiphene (study) and Clomiphene (control) groups.

	Study Group (n=65)	Control Group (n=65)	Test Statistic	*p*-value
Age & BMI				
Median age in yrs. (Range)	32 (17)	32 (17)	*U*= 1955	0.46
Minimum age in years (Maximum)	23 (40)	22 (39)		
Age Categories				
Age from 18 to 24 no. (%)	6 (9.2%)	4 (6.2%)		
Age from 25 to 29 no. (%)	19 (29.2%)	17 (26.2%)		
Age from 30 to 34 no. (%)	24 (36.9%)	29 (44.6%)	χ^2=1.02	0.79
Age above 35 no. (%)	16 (24.6%)	15 (23.1%)		
Body Mass Index (BMI)				
Median BMI in Kg/m^2 (Range)	29 (10)	29 (4)	U= 1938.5	0.41
Minimum BMI in Kg/m^2 (Maximum)	22 (32)	27 (31)		

In the study group, 46.2% of included patients were defined as primary infertile compared to 35.4% in the control group. The median duration of infertility was 2 years in both groups; however, patients in the study group with infertility >2 years constituted 41.5% of included patients compared to 27.7% in the control group. Neither the median number of years of infertility nor the proportion of infertility lasting longer than two years showed a statistically significant difference between the two groups. There was no significant statistical difference between both groups in basal FSH or LH levels, as shown in Table II.

Study Outcomes

The follicles numbers, ovulation percentages, and pregnancy rates indicated no statistically significant difference between the two groups. On the other hand, the median of the endometrium thickness was 8 mm in the study group compared to a median of 7 mm in the control group, showing a statistically significant difference ($p<0.01$, Table III). Endometrial thickness was also measured in each group sub-population based on infertility duration (Table IV). Median endometrial thickness showed both greater median (8.25 mm) and range (8 mm) in the study group with duration of infertility lower than two years compared to other treatment sub-groups. Post-hoc comparison was done between each pair of subgroups as illustrated in Table V and Figure 2.

When comparing each sub-group's median endometrial thickness, there were statistically significant differences between all sub-groups except intra-treatment arm sub-groups (i.e., sub-groups of the control group showed no statistical difference between patients with infertility greater than or less than two years, sub-groups of the study group showed no statistical difference as well).

Table II. Type and duration of infertility, and basal hormonal levels among Sildenafil/clomiphene (study) and Clomiphene (control) groups.

	Study Group (n=65)	Control Group (n=65)	Test Statistic	p-value
Type of infertility				
Primary infertility no. (%)	30 (46.2%)	23 (35.4%)	$\chi^2 = 1.56$	0.21
Secondary infertility no. (%)	35 (53.8%)	42 (64.6%)		
Duration of infertility				
Median duration of infertility in years (Range)	2 (7)	2 (8)	$U = 1804$	0.14
Infertility duration > 2 years no. (%)	27 (41.5%)	18 (27.7%)	$\chi^2 = 1.56$	0.97
Basal Hormonal Levels				
Median Basal FSH (Range)	7 (8)	8 (9)	$U = 1890.5$	0.29
Median Basal LH (Range)	5.5 (7)	6 (8)	$U = 1858$	0.24

Table III. Outcomes of the study

	Study Group (n=65)	Control Group (n=65)	Test Statistic	p-value
Endometrial Thickness and number of follicles				
Median no. of follicles (Range)	1 (2)	1 (2)	$U = 2112.5$	1
Median endometrial Thickness (Range)	8 (8)	7 (6)	$U = 916.5$	< 0.01
Ovulation				
Positive no. (%)	61 (93.8%)	59 (90.8%)	$\chi^2 = 0.43$	0.51
Negative no. (%)	4 (6.2%)	6 (9.2%)		
Chemical pregnancy				
Positive no. (%)	9 (13.8%)	7 (10.8%)	$\chi^2 = 0.29$	0.59
Negative no. (%)	56 (86.2%)	58 (89.2%)		
Clinical pregnancy				
Positive no. (%)	8 (12.3%)	5 (7.7%)	$\chi^2 = 0.77$	0.38
Negative no. (%)	57 (87.7%)	60 (92.3%)		

Table IV. The thickness of endometrium between among sub-populations.

Treatment Group	Sub-Group	No. of patients (%)	Endometrial thickness in mm		Test Statistic	
			Median	Range		p-value
Sildenafil/ Clomiphene Group	Infertility ≤ 2 Years	38 (58.5%)	8.25	8	33.1[a]	< 0.01
	Infertility > 2 Years	27 (41.5%)	8	3		
Clomiphene group	Infertility ≤ 2 Years	47 (72.3%)	7	6		
	Infertility > 2 Years	18 (27.7%)	7	3		

[a]Test Statistic is adjusted for ties.

Table V. The thickness of endometrium post-hoc analysis among treatment subgroups.

Sub-group 1	Sub-group 2	Standard test statistic	Adjusted significance[†]
Clomiphene with infertility duration > 2 Yrs	Sildenafil/Clomiphene with infertility duration ≤ 2 Yrs	4.07	< 0.01
Clomiphene With infertility Duration > 2 Yrs	Sildenafil/Clomiphene with infertility duration > 2 Yrs	4.10	< 0.01
Clomiphene with infertility duration ≤ 2 Yrs	Sildenafil/Clomiphene with infertility duration ≤ 2 Yrs	4.02	< 0.01
Clomiphene with infertility duration ≤ 2 Yrs	Sildenafil/Clomiphene with infertility duration > 2 Yrs	3.97	< 0.01
Clomiphene with infertility duration > 2 Yrs	Clomiphene with infertility duration ≤ 2 Yrs	1.04	1.00
Sildenafil/Clomiphene with infertility duration > 2 Yrs	Sildenafil/Clomiphene with infertility duration ≤ 2 Yrs	-0.32	1.00

[†]Significance values have been adjusted by the Bonferroni correction for multiple tests.

Median endometrial thickness was statistically significantly greater in sub-groups of the study group compared to the sub-groups of the control group. Different sizes of follicles, endometrial thickness, and pregnancy in both groups can be recognized in Figure 3.

Clinical and chemical pregnancy in the different sub-groups was statistically similar in both the study and control groups. Two patients in each study group experienced miscarriage, while only one patient developed multi-fetal pregnancy in the sildenafil/clomiphene treatment group, and no cases were reported with ectopic pregnancy in both groups.

Six patients in the study group (9.2%) experienced headache compared to one in the control group with statistical significance (χ^2= 3.78, p=0.052). Regarding other adverse effects in the study and control group, flushing (4 *vs.* 1), blurred vision (1 *vs.* 1), GIT upset (1 *vs.* 1) were reported in both groups respectively. When comparing all side effects distribution, there was no statistically significant difference between the two groups.

Associations

The association between nominal variables was evaluated using the lambda test. No statistically significant correlations were found between the treatment group and ovulation, different types of pregnancy, or even the adverse events. Duration of infertility was negatively associated with the thickness of endometrium in the control group with a statistically significant difference [$\rho(63)$=-0.25, p=0.04], as shown in Figure 4. Other studied correlations in both treatment and control groups are tabulated in Table VI and show no statistically significant associations.

Discussion

The use of CC for ovulation induction is associated with high ovulation rates (70 to 80%) and low pregnancy rates (10 to 20% per cycle). However, low endometrial development during induction cycles may be attributable to its anti-estrogenic effect. Diverse methods for decreasing
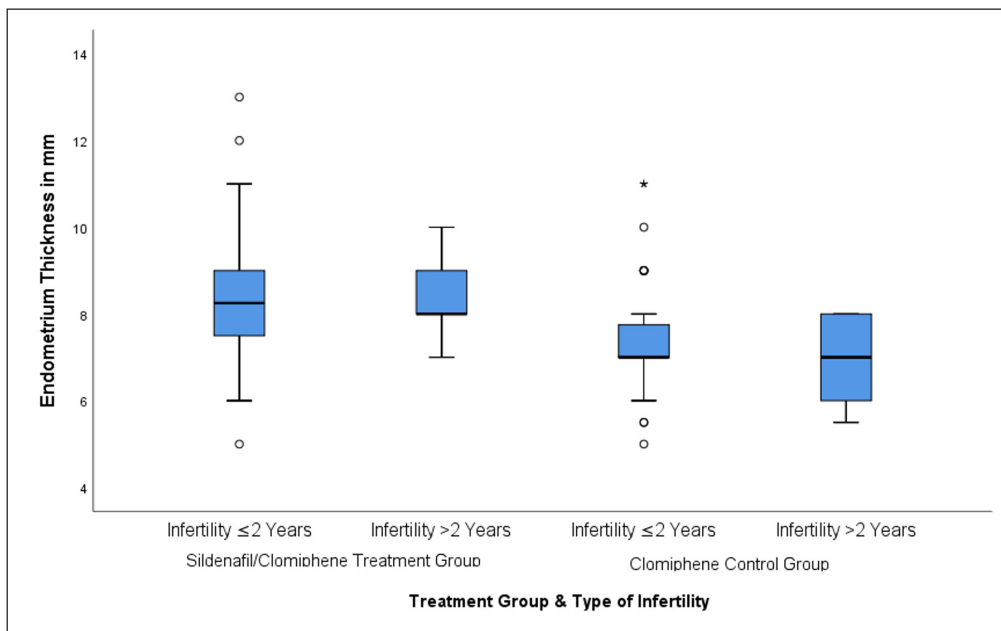
Figure 2. Endometrial thickness distribution among different treatment sub-groups.

the estrogenic antagonistic effects of CC have been investigated, with variable results[21-23]. By combining sildenafil with clomiphene citrate in the present study, we aimed to improve rates of pregnancy and endometrial thickness in females with unexplained infertility.
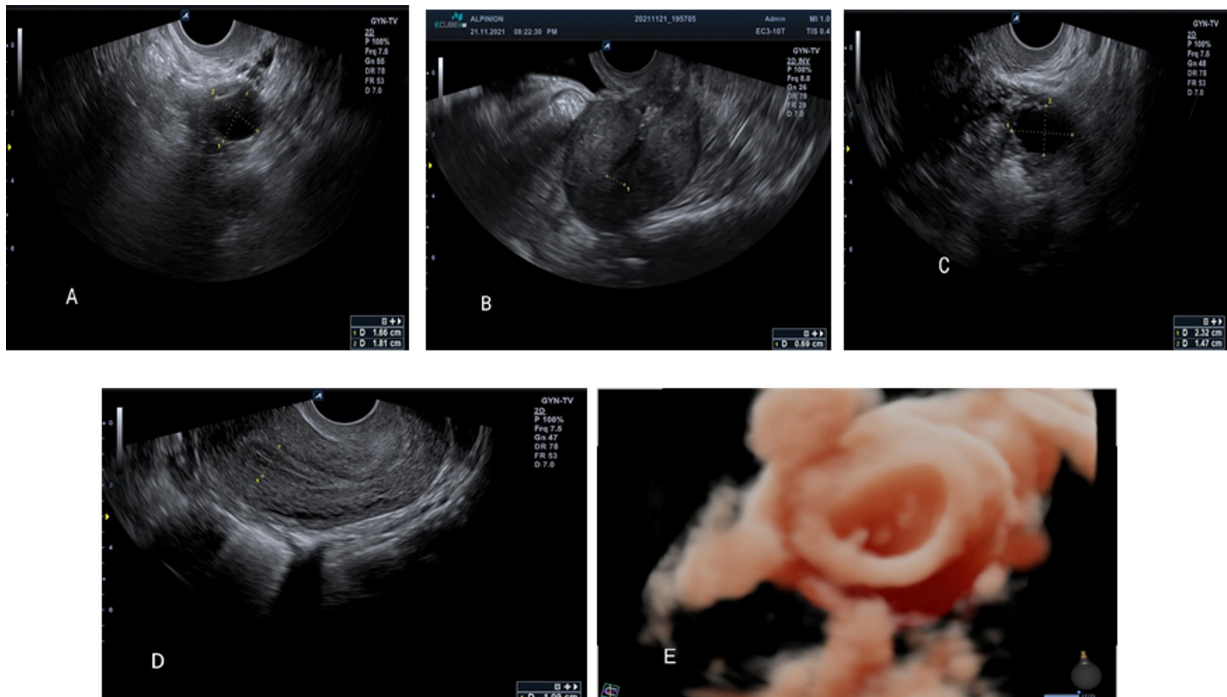

Figure 3. A, Transvaginal ultrasound shows right ovary with dominant follicle = 17.5 mm in the study group, **B**, Transvaginal ultrasound shows thin endometrium = 6.9 mm in the control group, **C**, Transvaginal ultrasound shows left ovary with dominant follicle = 19 mm, **D**, Transvaginal ultrasound shows trilaminar endometrium with thickness =10.9 mm in the study group, **E**, HD live shows gestational sac of 8 weeks pregnancy with fetal pole and yolk sac inside.
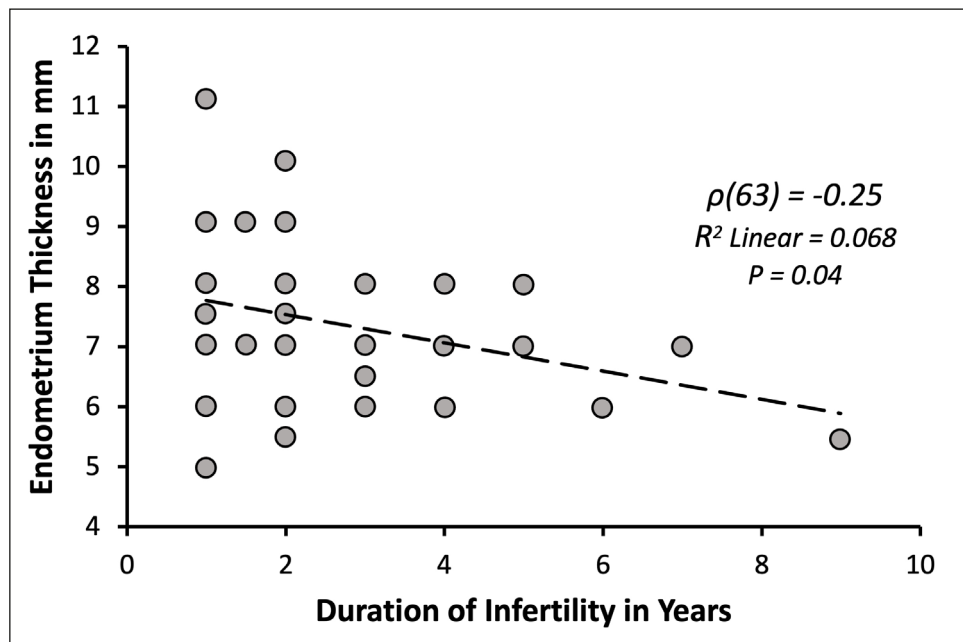
Figure 4. Correlation between the thickness of endometrium and the infertility duration in the control group.

Table VI. Correlation between the thickness of endometrium and number of follicles with different variables in both study and control groups.

	Endometrium thickness				No. of follicles			
	Study group		Control group		Study group		Control group	
	p	p-value	p	p-value	p	p-value	p	p-value
Age	-0.04	0.73	0.09	0.4	-0.23	0.06	-0.07	0.57
BMI	-0.004	0.9	0.18	0.14	0.01	0.95	0.009	0.98
Duration of infertility in Years	-0.09	0.45	-0.25	0.04*	0.03	0.75	-0.03	0.7
Basal FSH Level	-0.07	0.53	0.01	0.9	0.06	0.6	-0.05	0.66
Basal LH	-0.058	0.64	0.07	0.57	0.05	0.63	-0.08	0.47

*: Correlation is significant at the 0.05 level (2-tailed).

In this study, the median thickness of endometrium in the study group was significantly greater than the control group's ($p<0.01$). According to a prior study[24], the use of sildenafil citrate suppositories for unexplained infertility treatment in women was associated with an improvement in endometrial growth. This study explained these findings as a result of an increase in uterine spiral artery (SA) blood flow due to a decrease in spiral artery (SA) resistance index (RI) and, consequently, an increase in endometrial growth[24]. This effect is attributed to the vasodilator action of sildenafil as a PDE5 inhibitor, which is needed for supplying the endometrium with sufficient blood flow required for transporting many factors that are essential for endometrial growth as estrogen, progesterone, and VEGF. This may increase the likelihood of a successful pregnancy[10,15,25,26].

Although there was a significant difference in the thickness of endometrium between the two groups, the rise in clinical and chemical pregnancy detected in the study group did not reach the level of statistical significance when compared to the control group. Fetih et al[27] supported such findings, indicating a significant rise in the thickness of endometrium and increase in uterine blood flow after using sildenafil vaginal gel with CC in women with failed CC response. Although higher doses of sildenafil were utilized in that study than in the present one (50 mg vaginal gel

twice daily), the increase in pregnancy rate was also insufficient to draw statistically significant conclusions. This means that large doses may be required to make a substantial increase in the rate of pregnancy when sildenafil is used locally.

In agreement with the present study, Aboelroose et al[1] also illustrated that the addition of oral sildenafil citrate to the CC regimen increased ovulation success and endometrial thickness in women with unexplained infertility. Interestingly, Aboelroose et al[1] reported a significant increase in the pregnancy rate among sildenafil patients. This can be explained by the period of follow-up and dosage of medication. The patients in the present study were followed up for only one cycle compared to 3 cycles in the other study, as women who had not conceived in the first cycle, were recruited for a second and third cycle (up to three cycles) of treatment using the same regimen. Regarding treatment dosage, higher doses of sildenafil citrate were used in the other study (25 mg *vs.* 20 mg) compared to ours.

The infertility duration should be taken into account during the unexplained infertility treatment. Median endometrial thickness showed both greater median (8.25 mm) and range (8 mm) in the study group with a duration of infertility lower than two years compared to other treatment sub-groups. This indicates that the optimal effect of sildenafil on the thickness of endometrium can be attained in women with unexplained infertility for a short duration (lower than 2 years). This finding has not yet been included in previous studies on sildenafil and is considered as a precedent for this current study. Also, the infertility duration was taken as the standard for subgroup analysis, as several other studies[28-31] in litearture demonstrated that it adversely affected pregnancy rates.

Moreover, the duration of infertility was negatively associated with endometrial thickness in the control group, showing a statistically significant difference (*p*=0.04). This suggests that, with a prolonged duration of infertility, CC will have little influence on endometrial thickness and the consequent pregnancy rate.

Sildenafil has been found to have no effect on decreasing the number of miscarriages compared to CC alone. Also, one case of multi-fetal pregnancy has been reported in the study group. Consequently, further studies with a longer duration of follow-up for such cases are required[32].

A more significant proportion of women in the study group experienced side effects than in the control group [12 (18.46%) *vs.* 6 (9.23%)]

but with no significant difference. Six patients in the study group (9.2%) experienced headache which was regarded as a statistically significant difference compared to the clomiphene group (χ^2= 3.78, *p*=0.052). A small number of patients experienced flushing, blurred vision, and GIT upset in the two groups. All reported side effects were described as mild effects, and no serious ones were recognized. In line with our findings, other previous studies[33,34] indicated that the most common sildenafil's side effects were dyspepsia, headache, nausea, and blurred vision. Basson et al[35] illustrated that severity of side effects on using sildenafil ranged from mild to moderate ones and were considered to be dose dependent.

Furthermore, as indicated by Legro et al[36], CC contributed to some of the identified side effects in the current investigation, including nausea, vomiting, abdominal distension, headache and altered eyesight. On the other hand, the significant difference reported between the two groups regarding headache can be attributed to the transient induction of elevated levels of glutamate by sildenafil in the brainstem. Consequently, the excitability of the neurons in the brainstem is transiently increased[37]. Also, sildenafil, as a vasoactive substance, is known to induce headache in healthy ones and attacks in migraine patients. Sildenafil's vasoactive effects are predominantly mediated by the activation of cyclic guanosine monophosphate (cGMP)[38].

Conclusions

Based on our findings, oral sildenafil citrate, as an adjuvant drug with CC, can improve endometrial thickness in women with unexplained infertility, especially when the infertility duration is lower than two years. Headache is the most frequently observed side effect of sildenafil treatment, but with no severe complaints. Further studies on a large number of subjects with more extended follow-up periods are required to estimate the effect of sildenafil on pregnancy, miscarriage, and ectopic and multi-fetal pregnancies.

Conflict of Interest
The authors declare no conflict of interest.

Authors' Contributions

Conceptualization, methodology, resources, visualization, supervision software, validation, formal analysis, investigation, writing-original draft preparation: Rania M. Sarhan, Sara Abdallah Mohamed Salem, Marian S. Boshra; data curation, writing-review and editing: Mohammed H. Elkomy, Rafaa Mohammed Albuhayran, R.R.S. Hussein; funding acquisition: Mohammed H. Elkomy, Rafaa Mohammed Albuhayran. All authors have read and agreed to the published version of the manuscript.

Data Availability

The data presented in this study are available on request from the corresponding author.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

Ethics Approval

The Research Ethical Committee of Beni-Suef University, Faculty of Pharmacy approved this study (REC-H-PhB-SU-21015).

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