

## Effective of Sildenafil Citrate on Pregnancy Outcome in infertile women undergoing induction of ovulation by Letrozole and Clomiphene Citrate

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### ABSTRACT

**Background:** Endometrium consists of a simple columnar epithelium, forming numerous tubular glands, supported by a thick vascular stromal. Both glands and stromal undergo extensive changes during the menstrual cycle. The endometrium is functionally subdivided into two layers; stratum functionalis and stratum basalis.

**Aim of the work:** To assess the safety and efficacy of sildenafil on endometrium thickness, Number of mature follicle, Doppler indices of uterine artery and follow the effect on pregnancy outcome and rate of miscarriage.

**Patients and methods:** Randomized trial included 300 women recruited from outpatient obstetrics and gynecology clinic in Al -Azhar university Hospital, Assuit branch divided on three groups; Group (I): 100 patients (letrozole with sildenafil citrate). Group (II): 100 patients (letrozole with Placebo). Group (III): 100 patients (Clomiphene Citrate with Sildenafil Citrate).

**Results:** Our findings revealed that; In the current study, there was high significant difference between group I & group II and group II & III as regards endometrial thickness at HCG injection, all groups as regards number of follicle (18-22 mm) at HCG injection, Doppler indices at HCG injection (p 0.021 in p1 , p 0.239 p2), Pregnancy rate (p<0.001 in p1, p< 0.285 in p2), abortion rate I& group II (p<0.002 in p1 , p< 0.0280 in p2), all group in ovarian hyper stimulation syndrome rate (p<0.001HS). No significant difference between all groups as regard of congenital fetal malformation (p 0.773 NS).

**Conclusion:** Sildenafil citrate with induction of ovulation was significantly effective in increasing pregnancy rate and decreasing abortion rate.

**Keywords:** Sildenafil citrate; Pregnancy rate; Clomiphene citrate; Letrozole; Endometrial pattern; Abortion rate.

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### INTRODUCTION

Endometrial thickness of less than 14 mm is generally considered normal at any stage of the menstrual cycle.<sup>1</sup> During menstruation, the diameter of the endometrium in premenopausal women is between 2 and 4 mm.<sup>2</sup>

Sildenafil Citrate improve uterine blood flow and leads to estrogen-induce proliferation of the endometrium.<sup>2,3</sup> The powerful effects of sildenafil led researchers to evaluate sildenafil as a treatment in assisted reproduction where reduced uterine blood flow is thought to contribute to implantation failure.<sup>3</sup>

Uterine heart rate indices decreased in women receiving sildenafil while undergoing simultaneous in vitro fertilization, indicating decreased vascular

resistance in the uterus and increased uterine blood flow.<sup>4</sup>

Letrozole was introduced as reproduction therapy after the occurrence of many side effects of CC, the limited therapeutic success of CC and the complexity of gonadotropin treatment.<sup>5</sup> Letrozole suppresses estrogen production in the hypothalamic-pituitary axis, resulting in increased GnRH and FSH. Letrozole has a selective effect by blocking the peripheral conversion of androgens into estrogen and reducing the amount of estrogen, which creates a positive feedback in the pituitary gland, increases FSH and improves ovulation.<sup>6</sup>

Clomiphene competes and binds to estrogen receptors throughout the reproductive system, remaining bound for a long period of time, ultimately reducing receptor levels by interfering with receptor

recycling.<sup>7</sup> Decreased estrogen negative feedback leads to natural compensatory mechanisms that alter the pattern of GnRH secretion and stimulate overexpression of pituitary gonadotropin, which in turn fuels follicle growth in the ovaries.<sup>8</sup>

At the hypothalamic level, estrogen receptor depletion prevents accurate interpretation of circulating estrogen level.<sup>9</sup> Reduced estrogen negative feedback triggers normal compensatory mechanisms that alter the pattern of Gonadotropin-Releasing Hormone (GnRH) secretion and stimulate increased pituitary gonadotropin release, which in turn drives ovarian follicular development.<sup>10</sup>

This study purposes to assess the safety and efficacy of sildenafil on endometrium thickness, Number of mature follicle, Doppler indices of uterine artery and determine the effect on pregnancy outcome and rate of miscarriage.

**PATIENTS AND METHODS**

This study was conducted at the Department of Obstetrics & Gynecology in Assuit Al- Azhar University Hospital. Ethical committee approved the study protocol and an informed consent was obtained from every participant prior to commencing the study.

The study was conducted on 300 Patients were included in the study that performed in the department of obstetrics and gynecology of Al-Azhar University Hospital (Assuit) Egypt. Divided into 3 groups: Group I: included 100 patients (letrozole with sildenafil citrate). Group II: included 100 patients (letrozole with Placebo). Group III: included 100 patients (Clomiphene Citrate with Sildenafil Citrate).

Included criteria were; age 20-35 years old, patient hypothalamic pituitary ovarian axis, patients having ovulatory infertility, no medical disorders as DM, chronic HTN, cardiac, renal or blood disorders, women with documented ovulation, having patent tubes and Normal uterine cavity, conceive with sexual intercourse, normal hormonal profile and not using hormonal contraception. Exclusion criteria were; age less than 20- or more than 35 years old, male factor of infertility, congenital uterine anomaly, tubal factor that relates to infertility, history of medical disorders, resistance to clomiphene, pelvic adhesion, any contraindication to sildenafil citrate, letrozole or clomiphene citrate.

Clinical examination were general, abdominal, and pelvic, vaginal and cervical, ultrasound (Abdominal and Vaginal) and Doppler Indices. Laboratory investigations were complete blood count, Hormonal profile (F.S.H, L.H, DHEA, serum testosterone, TSH and PRL), progesterone level at med luteal phase (>5 ng/ml), Liver function tests, Random blood sugar,

kidney function profile, Semen analysis (satisfaction) and B HCG Subunit and titre.

In patients undergoing induction of ovulation, oral sildenafil citrate may improve endometrial thickness, number and diameter of follicles, uterine artery blood flow and pregnancy rate. Group (I); which will include of 100 infertile women will receive: Aromatase inhibitor (letrozole 2.5 mg) 1 tablet twice daily for induction of ovulation for five-days, beginning from the 2<sup>nd</sup> day of cycle, and Sildenafil citrate: 1 tablet three times per day from with the start of Letrzole therapy until the day of HCG administration. Group (II); which will include of 100 infertile women will receive: Aromatase inhibitor (letrozole 2.5 mg) 1 tablet twice daily for induction of ovulation for five-days, starting from the 2<sup>nd</sup> day of cycle and Placebo tablets: 1 tablet three times per day from with the start of Letrzole therapy until the day of HCG administration. Group (III); which will include of 100 infertile women will receive: Clomiphene citrate (clomide 50 mg) 2-tablet once daily for induction of ovulation for five-days, starting from the 2<sup>nd</sup> day of cycle and Sildenafil citrate: 1 tablet three times per day from with the start of Clomiphene Citrate therapy until the day of HCG administration.

Each patient will receive one treatment regimen for 3 consecutive cycles unless ovulation or pregnancy occurs. The development of the ovarian follicles were monitored by Tran's vaginal ultrasound measurement of the mean follicular diameter every 3 days during the follicular phase starting from day 10 until ovulation occur (18-20 mm) also the endometrial thickness and pattern were monitored by the trans-vaginal ultrasound. Doppler flow parameters of uterine vessels were measured on the third day of the cycle and on the day of HCG administration using colored Doppler US for comparison. The study was discontinued with positive pregnancy test or after three successive treatment cycles. Serum B-HCG were done to detect pregnancy occurrence.

Statistical data analysis was performed using SPSS as follows: quantitative variables were described as the mean, standard deviation and range were described, qualitative variables and percentages were described, and the non-comparative t-test was used for the comparison used. Two groups as follows: Consider a qualitative variable in the parametric data (SD < 50% mean), Chi2 test was used for statistical analysis of categorical data, P value >0.05 insignificant, P value <0.05 significant and P value <0.01 highly significant. The results were tabulated and statistically analyzed.

**RESULTS**

	Group I letrozole + sildenafil (n=100)		Group II letrozole only (n=100)		Group III clomiphene citrate+ sildenafil (n=100)		P. value	P1	P2	P3
	No.	%	No.	%	No.	%				
<b>Endometrial thickness at HCG inj.( 8-12mm)</b>										
<b>Unhealthy</b>	9	9.00	33	33.00	14	14.00	<0.001** HS	<0.001** HS	0.37	0.003** HS
<b>Healthy (triple line)</b>	91	91.00	67	67.00	86	86.00			5	

**Table 1:** comparison between three groups in endometrial thickness at HCG injection

P. value: Comparison between all groups calculated by Chi-square test for categorical data. (Significant difference). P1: (High significant difference). P2: (Not significant difference). P3: (High significant difference).

	Group I letrazole + sildenafil (n=100)	Group II letrazole only (n=100)	Group III clomiphene+ sildenafil (n=100)	P. value	P1	P2	P3
<b>Number of follicle(18-22mm) at HCG injection</b>							
Immature follicle	(9%)9%	(29%)29%	(15%)15%	<0.001** HS	**0.001HS	<0.001** HS	**0.005HS
One follicle	(90%)90%	(66%)66%	(64%)64%				
Two follicle	(1%)1%	(5%)5%	(14%)14%				
Three follicle or more	0	0	(7%)7%				

**Table 2:** comparison between three groups in number of follicle18-22mm at HCG injection

P. value: Comparison between all groups calculated by Chi-square test. (High significant difference). P1: (High significant difference). P2: (High significant difference). P3: (High significant difference).

	Group I letrazole + sildenafil (n=100)	Group II letrazole only (n=100)	Group III clomiphene + sildenafil (n=100)	P. value	P1	P2	P3
	No. %	No. %	No. %				
<b>Doppler indices at HCG inj</b>							
Normal	93 93.0	81 81.0	87 87.0	0.041* S	*0.021 S	0.239	0.335
Abnormal	7 7.0	19 19.0	13 13.0				

**Table 3:** comparison between three groups in Doppler indices at HCG injection.

P. value: Comparison between all groups calculated by Chi-square test for categorical data. (Significant difference). P1: (Significant difference). P2: (Not significant difference). P3: (Not significant difference).

	Group I letrazole + sildenafil (n=100)	Group II letrazole only (n=100)	Group III clomiphene + sildenafil (n=100)	P. value	P1	P2	P3
	No %	No %	No %				
<b>Pregnancy</b>							
+ve	73 73.0	49 49.0	65 65.0	<0.001*	<0.001**	0.285	*0.032 HS
-ve	27 27.0	51 51.0	35 35.0	* HS	HS		

**Table 4:** comparison between three groups in pregnancy rate.

P. value: Comparison between all groups calculated by Chi-square test for categorical data. (Not significant difference). P1: (High significant difference). P2: (Not significant difference). P3: (High significant difference).

	Group I letrazole + sildenafil (n=100)	Group II letrazole only (n=100)	Group III clomiphene+ sildenafil (n=100)	P. value	P1	P2	P3
	No. %	No. %	No. %				
<b>Abortion</b>							
Yes	9 9.0	27 27.0	22 22.0	0.004 HS**	0.002**	*0.020 S	0.511
No	91 91.0	73 73.0	78 78.0				

**Table 5:** comparison between three groups in Abortion rate.

P. value: Comparison between all groups calculated by Chi-square test for categorical data. (Not significant difference). P1: (High significant difference). P2: (Significant difference). P3: (Not significant difference).

	Group I letrazole + sildenafil (n=100)	Group II letrazole only (n=100)	Group III clomiphene + sildenafil (n=100)	P. value
	No. %	No. %	No. %	
<b>OHSS</b>				
No	100 100.0	0 0.0	97 97.0	<0.001** HS
Yes	0 0.0	0 0.0	3 3.0	

**Table 6:** comparison between three groups in ovarian hyper stimulation syndrome rate.

P. value: Comparison between all groups calculated by Kruskal Wallis test. (High significant difference).

	Group I letrozole + sildenafile (n=100)		Group II letrozole only (n=100)		Group III clomid + sildenafile (n=100)		P. value
	No.	%	No.	%	No.	%	
<b>CFMF</b>							
<b>Anencephaly</b>	0	0.0	0	0.0	1	1.0	0.773 NS
<b>Blighted ovum</b>	2	2.0	1	1.0	2	2.0	
<b>Partial mole</b>	0	0.0	0	0.0	2	2.0	
<b>Complete Vesicular mole</b>	0	0.0	1	1.0	1	1.0	
<b>No</b>	97	97.0	98	98.0	94	94.0	

**Table 7:** comparison between three groups in CFMF rate.

P. value: Comparison between all groups calculated by Chi-square test for categorical data. (Not significant difference). CFMF=congenital fetal malformation

## DISCUSSION

Infertility affects up to one in seven couples all over the world. Ovarian stimulation protocols make numerous oocytes available per cycle and it has shown that a large variety exists within a single cohort.<sup>11,12</sup>

In this research, the effect of oral sildenafil citrate with (letrozole or clomiphene citrate) on different endometrial parameters were studied, in addition to vaginal ultrasonography and color Doppler to examine the endometrium and uterine vasculature in infertile patients.

According to previous study Hale al.<sup>3</sup> the sonographer features of the endometrium (thickness and pattern) on the day of HCG-administration, no differences between pregnant and non-pregnant patient, however; pregnancy rate declined beyond two limits of endometrial thickness.

In the present work, the mean endometrial thickness for patients receiving letrozole and sildenafil citrate (group I), on HCG was (P <0.001), while for (group II) patients receiving letrozol and placebo the mean endometrial thickness, on HCG-administration, was (P 0.375). However; (group III) patients receiving clomiphene citrate and sildenafil citrate the mean, on HCG-administration, was (P 0.003).

Our findings proved that the administration of sildenafil citrate had a reversing power on the unfavorable changes in endometrial thickness due to Letrozol or CC induction with high significant difference in comparison between group I and II and Not significantly difference in comparison between group I and III and high significant difference in comparison between group II and III.

These results agree with Gonen and Casper<sup>5</sup> found that a significant reduction in endometrial thickness occurs in CC stimulated cycles.

Our findings revealed that; mature follicles was highly significant in women of (all group): in (group I) mature follicle 91%: immature follicle 9%, (group II) mature follicle 71%: immature follicle 29%, (group III) mature follicle 85%: immature follicle 15% (p<0.001).

Also our findings comparing between both groups to Doppler indices at the day of HCG administration shows that (group 1); 93% normal -7% abnormal (letrozol with sildenafil) has significant deference with (group II) ;81 % normal- 19% abnormal (letrozol only without use sildenafil); but with no significant deference between (group1) and (group 111):87% normal-13% abnormal. And no significant deference between (group11) and (group 111). (P = < 0.041); indicating better blood flow to the uterine artery in (group 1).

Consistent with previous studies, Jokubkiene al.<sup>1</sup> concluded that there was no concept of a cycle in which the pulmonary artery flowing showed an undetermined rate of fluctuation of diastolic flow.

Kansouh and El-Naggaer<sup>4</sup> reported vaginal administration of Viagra to 105 women with recurrent IVF deficiency due to persistent endometrium.

According to previous research, Haritha and Rajagopalan<sup>2</sup> Sildenafil Citrate improves myometric endothelial function of the blood vessels and can be a potential therapeutic strategy to correct intestinal bleeding.

In addition to Fisher al,<sup>6</sup> more than 500 women have been treated since treatment was introduced, and many babies need IVF treatments after relaxed.

Previous studies between Clomidium and Letrozol and their results showed that the mean cell-maturity number on the day of HCG administration was statistically higher in the CC group than in the letrozoli group (6.8 + 0.3 vs. 4.4 + 0, 4) as mentioned by Ernesto.<sup>7</sup>

Giorgetti al,<sup>9</sup> the mean number of dominant cells at 18 mm was not statistically different in the letrozolo and CC groups (letrozole 1.86 0.06 vs. CC 1.92 0.17, P = 0.126).

Furthermore; Branigan and Estes<sup>10</sup> the incidence of ovulation in gonadotropin stimulation was 95% as 39 cases ovulated in 41 cases, and concluded that the incidence of OHSS was 2.5% in HMG stimulation group per cycle.

In this study, pregnancy higher with the concomitant use of sildenafil citrate rather than placebo with the

use of induction of ovulation by letrozole or clomiphene citrate.

In addition to Balen and Jacob<sup>11</sup> described that earlier administration of clomiphene citrate resulted in marked reduction of endometrial thickness.

In contrary, Alatas and Yagci<sup>12</sup> no decrease in endometrial thickness or affection of the implantation window in clomiphene citrate treated cycles.

Thin endometrium during the periovulatory phase as measured by transvaginal ultrasound has been reported to be associated with clinical pregnancy rates as thin endometrium, usually less than 7 mm, is associated with implant defects.<sup>13</sup>

Get on; Badawy al.<sup>14</sup> If the endometrium is less than 5 mm thick then no pregnancy will take place, but if the endometrium is larger than 7.5 mm then no pregnancy will take place.

One final point that all researchers agree on about endometrial thickness is that the endometrium is thick enough (6-8mm) to allow for embryonic implantation.

Previous studies have already shown that implantation and pregnancy rates are significantly lower and that the number of abortions in patients with an endometrial thickness greater than 14 mm on the day of HCG administration may increase.<sup>15</sup>

But Alcazar<sup>16</sup> also found an endometrial thickness of 5 mm that was achieved with large clinical implant sizes and for pregnant women.

We also found a significant difference in abortion rate between group and group, a significant difference in abortion rate between group and group, and no significant difference in abortion rate between group and group (P-value 0.004).

In this study; detect that 3% of ovulated cases suffered from OHSS only in group III (P<0.001)

In this study we also detect congenital featal malformation in group I was (3%), group II was (2%) while in group III was (6%).

### CONCLUSION

Highest clinical pregnancy rate is derived from letrozole with sildenafil citrate in comparison with clomiphene citrate with sildenafil citrate. But in the opposite side there was unfavorable pregnancy outcome in letrozole with placebo due to bad quality of ovum and endometrium pattern. Furthermore researches needed for achieving the best pregnancy outcome on infertility cases.

Conflict of interest : none

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