

## Research Article

# Effect of Sildenafil Citrate on Endometrial Thickness and Pregnancy Rate in Frozen-Thawed Embryo Transfer Cycles

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Received: January 06, 2020; Accepted: January 29, 2020; Published: February 05, 2020

## Abstract

**Background:** Successful implantation requires good embryo quality, appropriately timed and arranged endometrial receptivity, and efficient matching between the embryo and the receptive endometrium.

**Objective:** The aim of work was to assess the effect of sildenafil citrate on endometrial thickness and pregnancy rate of frozen-thawed embryo transfer cycles.

**Subjective:** The study was carried out on 40 patients with history of poor endometrial response. Patients were randomly allocated into two equal groups. Group A received estradiol valerat 2mg/tab, three tablets from the first to the 12<sup>th</sup> day of the menstrual. Group B treated by the same protocol plus sildenafil citrate tablets (50mg) daily from the first day of the cycle until the day of starting progesterone to be stopped 3 days before embryo transfer.

**Results:** The results of this study showed insignificant difference between the two studied groups regarding endometrial thickness, number of embryo, quality of embryo and pregnancy rate. Conclusion: There was a clinical increase in pregnancy rate in study group more than the control group, but this increasing not significant.

**Keywords:** Sildenafil Citrate; Endometrial Thickness; Pregnancy Rate; Embryo Transfer Cycles

## Introduction

Despite the improvement in ovarian stimulation protocols and subsequent access to a number of dominant follicles, mature oocytes, and embryos for transfer, *In Vitro* Fertilization (IVF) has successfully reached a plateau. Therefore, more attention should be focused on implantation and endometrial receptivity. Implantation remains a major limiting step of Assisted Reproductive Technology (ART) and uterine receptivity is essential for successful [1] arranged endometrial receptivity, and efficient crosstalk between the embryo and the receptive endometrium. It is thought that the impairment of any one of these factors or [2] during the window. Implantation window is a period during which the endometrium is optimally receptive to implanting blastocyst within the cycle days 20 and 24. It is Endometrial receptivity during the [4,5] implantation in all species. Successful implantation requires good embryo quality, appropriately timed and biological processes may result in implantation failure. The endometrium is normally a non-receptive environment for an embryo, except [3] implantation window depends on the following factors as Endometrial thickness, Endometrial echogenic pattern, Endometrial and sub endometrial blood flows. During implantation window, the endometrial epithelium encompasses four cell types [6]: microvilli-rich cells, pinopode cells, vesiculated cells, and ciliated cells. Treatment options for improving the implantation: (a) blastocyst transfer, (b) assisted hatching, (c) co-culture, (d) preimplantation genetic screening, (e) hysteroscopy, (f) sildenafil

citrate, (g) salpingectomy for tubal disease, (h) oocyte donation, (i) transfer of six or more embryos, (j) intratubal embryo transfer, (k) natural-cycle IVF, (l) antiphospholipid antibodies (APA) testing and a vascular connection to the mother.

Estrogen induced endometrial proliferation is in large part dependent upon blood flow treatment, (m) allogenic lymphocyte therapy, and (n) IV immunoglobulin therapy [7,8]. Guanyl Monophosphate (cGMP) mediated pathway. Nitric oxide synthase isoforms have been identified in the vascular muscles of both human endometrium and myometrium. Phosphodiesterase (PDE) is a family of isoenzymes that hydrolyze cyclic nucleotides, such as cGMP. The inhibitors of specific Phosphodiesterase (PDE) subtypes have been identified with an ability to augment the effects of cyclic nucleotides on target tissues as the [8] Phosphodiesterase type 5 (PDE5) that prevents the breakdown of cGMP and potentiates the to the basal endometrium. Nitric Oxide (NO) leads to relaxation of vascular smooth muscles through a cyclic endometrium.

Sildenafil citrate is a potent and selective inhibitor of cGMP specific [9] improvement in uterine blood flow and, in conjunction with estrogen, led to the estrogen- [8] thickening. Endometrium and develop a sustaining blood supply, which requires the following genes to produce the necessary proteins for digesting the endometrial cellular matrix, to regulate cell growth, and to induce angiogenesis: Sildenafil citrate could lead to an Sildenafil citrate enhances uterine blood flow and increases endometrial induced proliferation of the

endometrial lining [10].

The achieved implantation depends on the blastocyst's ability to invade the [7,11,12] Sildenafil citrate was enhanced markedly in p53 and stimulated angiogenic responses [13,14] with increased VEGF. Citrate on ultrasonographic endometrial thickness and pattern and to investigate the estrogen level on the day of progesterone administration, the implantation rate and chemical pregnancy rate in frozen embryo transfer cycles.

The aim of work was to assess the effect of sildenafil citrate on endometrial thickness and pregnancy rate of frozen-thawed embryo transfer cycles.

## Methods

A total of 40 patients with history of poor endometrial response to frozen embryo transfer were included in this study. Inclusion criteria were Age less than 40 years. One IVF failure before and High-quality frozen embryos. Exclusion criteria were A history of endocrine diseases like thyroid, DM, Presence of chronic diseases; like cardiovascular, renal and liver diseases and Contraindication to Sildenafil like; hypotension stress, MI.

Patients were randomly allocated into two equal groups. Group A received estradiol valerat 2mg/tab, three tablets from the first to the 12<sup>th</sup> day of the menstrual. Group B treated by the same protocol plus sildenafil citrate tablets (50mg) daily from the first day of the cycle until the day of starting progesterone to be stopped 3 days before embryo transfer.

Starting on the 11<sup>th</sup> day of the menstrual cycle, the endometrial thickness was estimated by transvaginal ultrasonography every other day. The evaluations were performed by a single investigator. Once endometrial thickness were more than 8mm, 100mg of progesterone was injected intramuscularly. Endometrial thickness was assessed again on day 3 and day 5 of progesterone treatment. Transfer of frozen embryos occurred in day 3 of progesterone treatment. Pregnancy test was done to assess pregnancy rate in two groups.

## Results

The Age in group I ranged from 22-38 with mean value  $29.7 \pm 5.038$  and in group II ranged from 21-37 with mean value  $29.55 \pm 5.286$ . There was no statistical significant difference between two studied groups regarding the age ( $P > 0.05$ ).

The IVF attempt. IVE attempt in group I was  $2.45 \pm 0.510$  and in group II was  $2.35 \pm 0.489$ . There was no statistical significant difference between two studied groups regarding the IVF attempt ( $P > 0.05$ ). Agonist in group I was 13(65%) and in group II was 12(60%) while antagonist was 7(35%) and 8(40%) in both groups respectively. There was no statistical significant difference between two studied groups regarding the protocol used ( $P > 0.05$ ). Endometrial thickness in group I ranged from 7.4-9.5 with mean value  $8.875 \pm 0.535$  and in group II ranged from 7.5-9.4 with mean value  $8.85 \pm 0.630$ . There was no statistical significant difference between two studied groups regarding endometrial thickness ( $P > 0.05$ ) (Table 1).

The number of embryo in group I was  $3.3 \pm 0.733$  and in group II was  $3.25 \pm 0.716$ . There was no statistical significant difference between two studied groups regarding number of embryo ( $P > 0.05$ ).

**Table 1:** Comparison between the two studied groups regarding basic characteristics.

	Group I	Group II	P value
<b>IVF</b>			0.265
Range	2-3	2-3	
Mean $\pm$ S.D.	$2.45 \pm 0.510$	$2.35 \pm 0.489$	
<b>Protocol used</b>			0.725 N.S.
Agonist	13 (65.0%)	12 (60.0%)	
Antagonist	7 (35.0%)	8 (40.0%)	
<b>Endometrial thickness</b>			0.446
Range	7.4-9.5	7.5-9.4	
Mean $\pm$ S.D.	$8.875 \pm 0.535$	$8.85 \pm 0.630$	

**Table 2:** Comparison between the two studied groups regarding out come parameters.

	Group I	Group II	P value
<b>Number of embryo</b>			0.421
Range	2-5	2-4	
Mean $\pm$ S.D.	$3.3 \pm 0.733$	$3.25 \pm 0.716$	
<b>Quality of embryo</b>			1
Grade A	20 (100.0%)	20 (100.0%)	
Grade B	0(0.0%)	0 (0.0%)	
<b>Pregnancy</b>			0.107
Positive	9, 45.0	13,65.0	
Negative	11, 55.0	7,5.0	

The quality of embryo show that the grade A in both groups was 20(100%) and there was no Grade B. Positive pregnancy in group I was 9(45%) and in group II was 13(65%) while Negative pregnancy was 11(55%) and 7(35%) in both groups respectively. There was no statistical significant difference between two studied groups regarding the pregnancy rate ( $P > 0.05$ ) (Table 2).

## Discussion

In this study the age in group I ranged from 22-38 with mean value  $29.7 \pm 5.038$  and in group II ranged from 21-37 with mean value  $29.55 \pm 5.286$ . There was no statistical significant difference between two studied groups regarding the age, the similarity of age in the two studied groups was important to eliminate the effect of age on the outcome results. In agreement with our study, Kortam et al., 2018, study the effect sildenafil on endometrial thickness and pregnancy rate, the mean age in the two studied groups was around 28.0 years, [15] effect of vaginal sildenafil on outcome of fertilization after multiple IVF failure, the patients [16] there was no significant difference between the two groups regarding age. The IVE attempt in group I ranged from 2-3 with mean value  $2.45 \pm 0.510$  and in group II ranged from 2-3 with mean value  $2.35 \pm 0.489$ . There was no statistical significant difference between two studied groups regarding the IVF attempt. This results was in inclusion criteria and selection of the patients must at least one IVF failure before, and the non significant difference between the two groups exclude the effect of multi IVE on the net results of the study. This results was agreement with study of Sher et al., 2002, they study the included in this study in the two

groups show mean previous IFV 2.7, 2.4 respectively.

In our results, endometrial thickness in group I ranged from 7.4-9.5 with mean value  $8.875 \pm 0.535$  and in group II ranged from 7.5-9.4 with mean value  $8.85 \pm 0.630$ . There was no statistical significant difference between two studied groups regarding endometrial thickness ( $P > 0.05$ ). In agreement with our study, Mangal et al, 2016, found that the endometrial thickness and trilaminar pattern of endometrium were comparable in both groups [17].

In contrast to our study Firouzabadi et al., improves uterine arterial blood flow and endometrial thickening. Endometrial thickness was significantly higher in the sildenafil citrate group ( $P < 0.0001$ ). In their study, it was shown that oral sildenafil citrate was efficient in improving endometrial receptivity. Therefore, they recommend the routine use of oral sildenafil citrate in patients with poor endometrial thickness.

A study failed to demonstrate the beneficial effect of sildenafil on thin endometrium, has been reported in women treated with estrogen and sildenafil citrate. ET was compared between the groups. Neither vaginal estrogen nor sildenafil significantly improved ET or blood flow in the subsequent frozen Embryo Transfer (ET) cycle. Thus, the use of sildenafil cannot be expected to help all patients with a thin endometrial lining. Women with intractable damage to the basal endometrium may be less likely to respond to increased uterine blood marginally increased pregnancy outcome in patients undergoing IUI [18].

Two small cohort studies denoted that the use of vaginal sildenafil improved uterine flow. Response to sildenafil is also predicated on an adequate serum estrogen level. Artery blood flow and sonographic endometrial appearance [19,20].

Sildenafil was reported to augment baseline and estrogen-induced uterine blood flow in surgically menopausal ovine model. Another effect of sildenafil has been described in women with recurrent miscarriages; it has shown to reduce natural killer cell activity in addition to the endometrial growth [21] 9(45%) while Negative pregnancy was 7(35%) and 11(55%) in both groups respectively. There was no statistical significant difference between two studied groups regarding the pregnancy rate ( $P > 0.05$ ). Although the pregnancy rate in study group was higher than control group, but this increasing was insignificant.

In study carried out by Kortam et al., this study depicted that chemical pregnancy occurred more in the study group than control group, yet the difference was not statistically [14] that showed non-statistical significant difference in pregnancy rate between both groups; the pregnancy rate in the treatment group was higher by 2.5 than the placebo group. This could be due to the small number of cases in their study and the non-significant difference in patient's demographics in both groups. Another reason is that the endometrium was significantly thicker on day of HCG in the [22].

In contrast to our results, Kansouh et al., biochemical pregnancy rate in women of sildenafil group when compared to those of control group; the difference was, however, statistically insignificant [15 (33.3) vs, 9 respectively,  $p = 0.15$ ]. Adding sildenafil citrate raised the biochemical pregnancy rate by 13.3%, giving a Number Needed To Treat (NNT) of 8 [i.e. in every 8 women who receive sildenafil citrate

in addition to the standard treatment, 1 would benefit by getting pregnant, while 7 would take it [23] facilitating effect.

In our study the positive pregnancy in group I was 13(65%) and in group II was different ( $P > 0.05$ ).

This goes with the results of Fahmy et al., [22] sildenafil group. The thickness was within the acceptable range in both groups [24]. This is consistent with what Firouzabadi et al., current study is close to agree with the results by Mangal et al., Sharma et al., and [25,26] Barker.

## Conclusion

In this study we measured endometrial thickness by transvaginal ultrasound on the day of progesterone administration and there were no differences between the two groups. Although there was a clinical increase in pregnancy rate in study group more than the control group, but this increasing not significant.

## Recommendation

We must consider other variables in assessment of endometrial thickness such as timing of measurement or method of measurement for example Doppler Ultrasound Assessment, which will allow us to have more difference between the two groups.

This study was limited by the small sample size; therefore, further case-controlled prospective research on the correlations between vascular perfusion and endometrial receptivity with a predictable factor of implantation in the FET cycle is needed.

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