

# Effect of Sildenafil Citrate on the Outcome of In Vitro Fertilization (IVF) after Multiple IVF Failures Attributed to Poor Endometrial Development: A Randomized Controlled Trial

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## ABSTRACT

**Aim of the work:** this study aimed to evaluate the effect of sildenafil citrate on endometrial development in women with history of recurrent implantation failure after IVF.

**Patients and methods:** This randomized controlled study was conducted in Obstetrics and Gynecology Department of Ain Shams University. The study included 80 women with previous two or more failed IVF. Women in **group A** (N=40) took oral sildenafil citrate at dose of 25mg tab /6h daily from day six of induction of ovulation until day of HCG administration. Those in **group B** (N=40) took placebo tablets.

**Results:** The primary outcome was change in endometrial thickness before and after intervention.

Endometrial thickness in both groups was statistically insignificant between the two groups when measured in day 6 with p-value 0.070. Endometrial thickness on day of HCG injection measured and found that it was higher in **group A** (Sildenafil group) than in **group B** (Placebo group) with significantly increased statistically difference between the two groups with p-value <0.001.

**Conclusion:** sildenafil citrate led to smooth muscle relaxation and vasodilation. This may enhance endometrial development and increased pregnancy rate in females undergoing IVF, which may be attributed to the increase in the endometrial thickness.

**Keywords:** Endometrial thickness, IVF, Sildenafil.

## INTRODUCTION

Embryo implantation is dependent on the ovum's quality and endometrial receptivity. Endometrial receptivity is a temporally distinct set of variables that causes the endometrium to be receptive to embryonic implantation. The implantation window is a time when the endometrium is most susceptible to blastocyst implantation <sup>(1)</sup>.

According to the findings, prospective examination of the quality of decidualization response in the endometrium may be a beneficial tool for determining the likelihood of successful implantation and pregnancy outcome. Since its introduction, ultrasound has been widely used in the clinic to investigate uterine features such as endometrial thickness, endometrial pattern, and that may be predictive of pregnancy, particularly in the context of assisted reproductive technology <sup>(2)</sup>.

Sildenafil citrate is a unique type 5-specific PDE inhibitor that inhibits cGMP breakdown while also improving the effects of NO on vascular smooth muscle. Sildenafil has been used successfully in the treatment of vascular smooth muscle via a cGMP-mediated mechanism since its introduction in 1997 <sup>(3)</sup>. The goal of this study was to determine how sildenafil citrate altered endometrial development in women who had experienced repeated implantation failure after IVF.

## METHODS

### Inclusion criteria:

The research included infertile women aged 20 to 40 who had a body mass index (BMI) between 20 and 29 kg/m<sup>2</sup> and had undergone two or more implantation failures due to poor endometrial development.

### Exclusion criteria:

Women with septate, bicornuate, fibroid uterus, or Asherman syndrome were excluded, and those with any contraindication to sildenafil citrate or any of its formulation components, including concurrent use of organic nitrates and nitrites, severe hepatic or renal impairments, hypotension and recent stroke, or heart attack.

Each lady provided informed written permission before being subjected to the following: Personal, menstrual, previous, and obstetric histories are all included in the detailed medical history. Examination of the body: After obtaining a history and meeting inclusion and exclusion criteria, clinical exams were performed, including general abdominal and pelvic examinations. Fasting, postprandial blood sugar, and complete blood count were done as routine laboratory tests.

### Intervention:

Women in group A received oral sildenafil citrate 25 mg tablets every 6 hours from the sixth day of ovulation induction until the day of HCG delivery, whereas those in group B received placebo pills.

### Induction of ovulation:

All patients exhibited a baseline hormonal profile on the third day of their spontaneous cycles (FSH, LH, E2, TSH and prolactin). Transvaginal (TV) ultrasonography (U/S) was performed on the third day of non-stimulated cycles using a 5-9 MHz transvaginal probe. Any patient who had uterine anomalies was ruled out. A long GnRH agonist regimen was used to stimulate the ovaries beginning in midluteal phase with daily subcutaneous

injections of triptoreline acetate (Decapeptyl 0.05 mg, Ferring Pharmaceutical, Kid, Germany). On day 3 of the following cycle, ovarian hyper stimulation was initiated with a daily injection of HCG (Menogon 75 IU/amp "Ferring Pharmaceutical, Kid, Germany" or Merional 75 IU/amp "IBSA, Switzerland"). The first dose of gonadotrophins was prescribed based on the women's age and weight, and the dose was later raised depending on the ovarian response as determined by transvaginal folliculometry, which began on the sixth day of the cycle. Based on the ovarian response, TV U/S was done every other day, and when the leading follicle reached 16mm, daily TV U/S was performed until the largest follicle attained a diameter of >18mm. The maximal HMG duration was not to exceed 16 days. HCG (Choriomon 10,000 IU/amp. "IBSA, Switzerland") was used to trigger ovulation.

**Sonography:** Transvaginal sonography was used to assess endometrial thickness and pattern on the day after HCG injection. The endometrial pattern was classed as: **proliferative:** when echogenicity is hypo echoic in relation to the myometrium, **peri-ovulatory:** when it is trilaminar and **secretory:** when it is hyperechoic.

**Ovum pick up:**

The transducer was linked to the ultrasound system thirty-six hours following the HCG injection. The guidance beam's direction was verified. A fixation ring connected the puncturing needle to an aspiration equipment attached to the front and back ends of the vaginal transducer, establishing the direction of puncture matching to the guidance beam on the ultrasound picture. Test tubes were used to assess the aspiration. The visualization in both planes indicated the uterus, both ovaries, and iliac arteries. The distance between the vaginal top pole and the ovary was carefully measured (care was taken to avoid intestinal or vascular interposition). The nearest available follicle was depth localized (distance from the upper vaginal pole to the center of the follicle). The needle was inserted firmly into the follicle's core (Aspiration pressure 90-100mmHg).

**IVF- ICSI:**

The direct penetration approach was used to deliver intracytoplasmic sperm into metaphase II oocytes, and fertilization outcomes were examined 16 to 19 hours after ICSI. The presence of two pronuclei indicated proper fertilization. The collapse of cytoplasmic contents and detachment from the zona were used to identify oocyte degeneration. The lack of pronuclei defined failed fertilization.

**Embryo transfer:** The same physician performed the embryo transfer on day 5 using a cook catheter under ultrasound guidance at a distance of roughly 1-1.5 cm from the fundus. Approximately 2-3 embryos were transferred.

**Finally:** A blood hCG test was conducted 12 days after embryo transfer and repeated 48 hours later, followed by US 6 weeks after embryo transfer.

**Statistical Analysis**

The data were analyzed with Stata® 14.2 software (Stata Corp LLC, College Station, TX, USA). It was decided whether the distribution of numerical data was normal using the Shapiro-Wilk test. The independent samples t test was used to look at inter-group differences after reporting the mean and standard deviation of numerical data that had a normally distributed distribution. Statistical significance was defined as P-values ≤ 0.05.

**RESULTS**

No significant difference was found between both groups age, BMI, cause and duration of infertility and number of previous trials (**table 1**).

**Table (1):** Demographic characteristics of the two study groups

| Variable                 | Sildenafil (n=40) | Placebo (n=40) | p-value¶ |
|--------------------------|-------------------|----------------|----------|
| Age (years)              | 29.5 ± 3.4        | 28.0 ± 3.7     | .071     |
| BMI (kg/m <sup>2</sup> ) | 21.1 ± 1.6        | 22.1 ± 1.8     | .060     |

**Table (2):** Duration, type, and cause of infertility and number of previous IVF trials in the two studied groups

| Variable                        | Sildenafil (n=40) | Placebo (n=40) | p-value |
|---------------------------------|-------------------|----------------|---------|
| Duration of infertility (years) | 7.2 ± 1.9         | 6.0 ± 1.9      | .057¶   |
| Type of infertility             |                   |                | .099§   |
| Primary                         | 35 (87.5%)        | 28 (70.0%)     |         |
| Secondary                       | 5 (12.5%)         | 12 (30.0%)     |         |
| Cause of infertility            |                   |                |         |
| Male factor                     | 3 (7.5%)          | 3 (7.5%)       | 1.000§  |
| Anovulation                     | 5 (12.5%)         | 2 (5.0%)       | .432§   |
| Tubal factor                    | 5 (12.5%)         | 1 (2.5%)       | .201§   |
| Endometriosis                   | 0 (0.0%)          | 1 (2.5%)       | 1.000§  |
| Unexplained                     | 27 (67.5%)        | 33 (82.5%)     | .196§   |
| Number of previous IVF trials   |                   |                | .094#   |
| Two                             | 23 (57.5%)        | 34 (85.0%)     |         |
| Three                           | 16 (40.0%)        | 6 (15.0%)      |         |
| Four                            | 1 (2.5%)          | 0 (0.0%)       |         |

Data are mean ± SD or number (%) - Unpaired t test - Fisher's exact test - Chi-squared test for trend

Endometrial thickness was significantly higher in sildenafil group (table 3).

**Table (3):** Endometrial thickness before and after test drug in both study groups

| Variable  | Sildenafil (n=40)  | Placebo (n=40) | p-value |
|---|--------------------|----------------|---------|
| - Endometrial thickness before test drug (mm)       | 7 ± 1              | 8 ± 1          | .070¶   |
| - Endometrial thickness after test drug (mm)        | 9 ± 1              | 8 ± 1          | .001¶   |
| - Increase in endometrial thickness (% of baseline) | 12.5 (11.8 – 28.6) | 0 (0 – 12.5)   | .0001 § |

Data are mean ± SD or median (interquartile range) - Unpaired t test - Wilcoxon rank sum test

Both biochemical pregnancy rate and clinical pregnancy rate was significantly higher in sildenafil group (table 4)

**Table (4):** Biochemical and clinical pregnancy rates in both study groups

|                            | Sildenafil (n=40) | Placebo (n=40) | p value |
|----------------------------|-------------------|----------------|---------|
| Biochemical pregnancy rate | 23 (57.5%)        | 13 (32.5%)     | 0.028   |
| Clinical pregnancy rate    | 22 (55%)          | 12 (30%)       | 0.031   |

**DISCUSSION**

In the present study, we evaluated the efficacy of the orally administered sildenafil citrate on the endometrial thickness and pattern, in infertility patients that underwent IVF with previous history of recurrent implantation failure due to poor endometrial development. Age of the women was important factor due to the observed age-related decline infertility as women approaches age of 40 (4).

In our study, there was no significant difference between study groups regarding age and BMI. In our study, there was no difference between study groups regarding duration and type of infertility with p-value 0.057 and 0.099 respectively. The number of previous trials showed no significant difference between the study groups.

In our study endometrial thickness was assessed by TVUS on day 6 induction of ovulation, there was no significant difference between studied groups. The mean endometrial thickness in sildenafil group was 7 mm while in the other group was 8mm.

After administration of sildenafil orally 25 mg/6h from 6<sup>th</sup> day induction of ovulation till HCG trigger day there was improvement in endometrial thickness in sildenafil group with mean endometrial thickness of 9 mm, which was statistically significant compared to endometrial thickness in the placebo group with mean endometrial thickness 8mm.

In our investigation, the proportion of changes in endometrial thickness following the administration of sildenafil was statistically significantly different across study groups, with group A showing a substantial improvement with a p-value of 0.0001. Fahmy et al. (5) identified a significant statistical difference in endometrial thickness between the treatment and placebo groups, with the treated group having a greater endometrial thickness. Thicker endometrium might be related to sildenafil citrate's vasodilator action, which increased uterine blood flow. Furthermore, Fisch and colleagues (6) discovered that using sildenafil from day 8 to day 13 of the cycle significantly increased endometrial thickness >7mm. Sildenafil citrate's effect on endometrial thickness has also been documented via a different ways.

As regards the biochemical pregnancy rate, clinical pregnancy were 57.5 and 55% in group A, which were significantly higher than those in group B, namely 32.5 and 30%, respectively (p<0.05).

Sher and Fisch (7) conducted a cohort study on 105 infertile women aged <40 years, with normal ovarian reserve and at least two consecutive prior IVF failures attributed to inadequate endometrial development. Of 105 patients, 73 (70%; Group A), attained an endometrial thickness of ≥ 9 mm, whereas 32 (30%; Group B) did not. Implantation and ongoing pregnancy rates were significantly higher for group A (29% and 45%) than for group B (2% and 0). Of 11 women in group B who had embryos transferred in that cycle, only one conception occurred, which resulted in a miscarriage. In group B, 59% of women had a history of endometritis, compared to 44% in group A.

Firouzabadi et al. (8) conducted a randomized controlled trial on 80 patients who had an antecedent of poor endometrial response and frozen embryos. 40 patients were given estradiol by a step up method with menstruation to prepare the endometrium, and the other 40 were given sildenafil citrate tablets (50 mg) daily in addition to the above treatment protocol from the first day of the cycle until the day progesterone was started. This was discontinued 48-72 hours prior to the embryo transfer.

The endometrial thickness was significantly higher in the sildenafil citrate group (p<0.0001), the triple line patterns of the endometrium were significantly higher in the sildenafil citrate group (p<0.0001), while the intermediate patterns of the endometrium were not significantly different in the two groups. The echogenic patterns of the endometrium were significantly higher in control group (p<0.0001). Finally, implantation rate and the chemical pregnancy rates were higher in the sildenafil citrate group but not significantly (8).

## CONCLUSION

We revealed that sildenafil citrate led to smooth muscle relaxation and vasodilation. It enhanced endometrial development, thickness and increased pregnancy rate in females undergoing IVF.

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**Conflict of interest:** None declared

**Ethical approval:** The study was approved by the Institutional Ethics Committee.

## REFERENCES

1. **Aboubakr M, ElnasharG ,Aboul-Eneinli (2004):** Endometrial receptivity. Middle East Fertility Society J., 9 (1): 10-24.
2. **Abdallah Y, Naji O, Saso S, Pexsters A, Stalder C, Sur S, Bourne T (2012):** Ultrasound assessment of the peri-implantation uterus: a review. Ultrasound in obstetrics&gynecology, 39(6): 612-619.
3. **Ballard S, Gingell C, Tang K, Turner L, Price M,Naylor A (1998):** Effects of sildenafil on the relaxation of human corpus cavernosum tissue in vitro and on the activities of cyclic nucleotide phosphodiesterase isozymes. The Journal of urology, 159(6): 2164-2171.
4. **Practice Committee of the American Society for Reproductive Medicine(2015):** Diagnostic evaluation of the infertile female: a committee opinion. Fertility and sterility, 103(6):e44-50.
5. **Fahmy A , El Sokyary M, Sayed S (2015):** The value of oral sildenafil in the treatment of female infertility: a randomized clinical trial. Life Sci. J, 12(4): 78-82.
6. **Fisch P, Casper R , Brown Set al.(1989):** Unexplained infertility: evaluation of treatment with clomiphene citrate and human chorionic gonadotropin. Fertility and sterility, 51(5): 828-833.
7. **Sher G, Fisch J(2002):.** Effect of vaginal sildenafil on the outcome of in vitro fertilization (IVF) after multiple IVF failures attributed to poor endometrial development. Fertility and Sterility, 78(5):1073-6.
8. **Firouzabadi R, Davar R, Hojjat F, Mahdavi M (2013):** Effect of sildenafil citrate on endometrial preparation and outcome of frozen-thawed embryo transfer cycles: a randomized clinical trial. Iranian journal of reproductive medicine, 11(2):151.