Effect of Sildenafil Citrate on the Outcome of *in vitro* Fertilization 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.

Setting: This study was conducted in obstetrics and gynecology department of Ain Shams University.

Patients and methods: this is a randomized controlled trial on 80 women with previous two or more failed IVF. Women in **group A** (N=40) took oral sildenafil citrate at dose 25mg tab /6h daily from day six of induction of ovulation until day of HCG administration; while those in **group B** (N=40) took placebo tablets.

Outcome measuers: The primary outcome was change in endometrial thickness before and after intervention.

Results: 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 HMG injection measured and found that it was higher in **group A**(Sildenafil Group) than **group B**(Placebo Group) with significantly increased statistically difference between the two groups with p-value <0.001.

Conclusion: sildenafil citrate leads 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 depends on the quality of the ovum and endometrial receptivity. Endometrial receptivity is a temporally unique sequence of factors that make the endometrium receptive to embryonic implantation. Implantation window is a period during which the endometrium is optimally receptive to implanting blastocyst[1].

Observations suggested that prospective assessment of the quality of decidualization response in the endometrium may be an important tool for predicting the likelihood of successful implantation and pregnancy outcome. Since its introduction into the clinic, ultrasound has been used widely to assess uterine features such as endometrial thickness; endometrial pattern and that may be predictive of pregnancy, especially in the context of assisted reproductive technology [2]. Sildenafil citrate is a newly developed, type 5-specific PDE inhibitor that prevents the breakdown of cGMP and potentiates the effects of NO on the vascular smooth muscle. Since its introduction in 1997, sildenafil has been used with great success in treatment of the vascular smooth muscle through a cGMP-mediated pathway [3]. This work aimed to evaluate the effect of sildenafil citrate on endometrial development in women with history of recurrent implantation failure after IVF.

METHODOLOGY

All participants were subjected to the following:

A) Detailed medical history including:

➢ Personal history.
➢ Menstrual history.
➢ Past and obstetric history.

B) Physical examination:

After history taking and fulfillment of both inclusions and exclusion criteria, clinical examination were done including: general abdominal and pelvic examination.

C) Laboratory investigations:

Routine investigations including: fasting and postprandial blood sugar and complete blood count.

D) Intervention:

Women in **group A** took oral sildenafil citrate at dose 25mg tab /6h daily from day six of induction of ovulation until day of HCG administration; while those in **group B** took placebo tablets.

E) Induction of ovulation:

- On day 3 of spontaneous cycles, all patients had basal hormonal profile (FSH, LH, E2, TSH and prolactin).
- Transvaginal (TV) ultrasound (U/S) on day 3 of non-stimulated cycles was done by transvaginal probe of 5-9 MHZ. Any patient found to have uterine abnormalities was excluded.
- Ovarian hyper stimulation protocol was held according to a long GnRH agonist protocol starting from midluteal phase by daily subcutaneous

injection of triptoreline acetate (Decapeptyl 0.05 mg, Ferring Pharmaceutical, Kid, Germany). Then on day 3 of next cycle ovarian hyper stimulation was started by daily injection of HMG (Menogon 75 lU/amp "Ferring Pharmceutical, Kid, Germany "or Merional 75 IU/amp" IBSA, Switzerland"). The starting dose of gonadotropines was prescribed according to the age and body weight of the subjects, then the dose was adjusted according to the ovarian response that was assessed by transvaginal folliculometry which was done on cycle day six.

- According to the ovarian response, every other day TV U/S was performed and at the moment when the leading follicle reaches 16mm, daily TV U/S was performed till the largest follicle reached a diameter of >18mm. The maximum duration of HMG was not allowed to exceed day 16.
- HCG (Choriomon 10,000 lU/amp. "IBSA, Switzerland") was administered for triggering ovulation.

F) Sonography:
Transvaginal sonography was performed in the day of HCG administration to measure endometrial thickness and pattern

- The endometrium pattern was classified as:
    1. Proliferative when echogenicity is hypo echoic in relation to the myometrium.
    2. Peri-ovulatory when it is trilaminar.
    3. Secretory when it is hyperechoic.

G) Ovum pick up:
- 36 hours after HCG injection, the transducer was connected to the ultrasound system. The direction of the guide beam was checked. The puncturing needle was connected to an aspiration apparatus attached by a fixation ring to the front and rear ends of the vaginal transducer, thereby defining the direction of puncture corresponding to the guide beam on the ultrasound image.
- The aspiration was checked using test tubes. The uterus, both ovaries and iliac vessels were identified by the visualization in both planes. The distance between the upper pole of the vagina and the ovary was closely evaluated (care was taken to avoid intestinal or vascular interposition).
- Depth localization of the closest accessible follicle (distance from the upper vaginal pole to the center of the follicle) was done. Needle was pushed forcefully to the center of the follicle (Aspiration pressure 90-100mmHg).

H) IVF- ICSI:
- Intracytoplasmic sperm injection was performed on metaphase II oocytes using the direct penetration technique, fertilization results were assessed 16 to 19 hours after ICSI. Fertilization

was considered normal by the presence of two pronuclei. Oocyte degeneration was identified by collapse of cytoplasmic contents and separation from the zona. Failed fertilization was defined by the absence of the pronuclei.

I) Embryo transfer:
- Embryo transfer was done on day 5 using cook catheter under ultrasound guide at a distance about 1-1.5 cm from the fundus by the same gynecologist.
- Number of embryos transferred 2-3 embryos.

j) Finally:
A serum βhCG was performed 12 days after embryo transfer and repeated after 48h; followed by US 6 weeks after embryo transfer.

Inclusion criteria
Cases of infertility, older than 20 years of age and not older than 40 years.
(1) Body mass index (BMI):20-29.
(2) Women have experienced two or more implantation failure attributed to inadequate endometrial development.

Exclusion criteria
Uterine abnormalities (e.g. septate, bicornuate and fibroid uterus, Asherman Syndrome).
(1) Any contraindication to sildenafil citrate or any of the components of its formulation including:
- Concurrent use of organic nitrites and nitrates.
- Severe hepatic impairment.
- Severe renal impairment.
- Hypotension.
- Recent stroke or heart attack.

The study was done after approval of ethical board of Ain Shams university and an informed written consent was taken from each participant in the study.

Sample size justifications: data were analyzed using Data were analyzed using Stata® version 14.2 (StataCorp LLC, College Station, TX, USA). Normality of numerical data distribution was examined using the Shapiro-Wilk test. Normally distributed numerical variables were presented as mean and SD and inter-group differences were compared using the independent samples t test. Non-normally distributed numerical data were presented as median and interquartile and intergroup differences were compared using the Wlicoxon rank sum test. Categorical data were presented as number and percentage and differences were compared using Fisher's exact test (for nominal data) or the chi-squared test for trend (for ordinal data).

➢ P-value <.05 was considered statistically significant.

RESULTS

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^2)	21.1 ± 1.6	22.1 ± 1.8	.060

- Data are mean ± SD.
- Unpaired t test.

In our study we found that age and BMI were statistically insignificant between the two groups with p-value 0.071 and 0.060 respectively.

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.

- We found that type of infertility was statistically insignificant increase between the two groups with p-value 0.099.
- Cause of infertility in our study mainly was unexplained in **group A** (Sildenafil Group) and in **group B** (Placebo Group), cause of infertility was insignificant between two groups with p-value 0.196.
- Patients included in both groups had history of IVF and it was statistically insignificant between two groups with p-value.094.

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.

- Endometrial thickness in both groups showed statistically insignificant decrease between two groups when measured in day 6 with p-value 0.070 but when measured in day of HMG injection was statistically significant increased with p-value 0.001.
- Percentage of change in endometrial thickness was statistically significant between two groups with p-value <0.0001.

DISCUSSION

In the present study we evaluated the efficacy of the orally administered sildenafil citrate on the endometrial thickness and pattern, number of follicles, and pregnancy rate in infertility patients undergoing IVF.

Age of the women was important factor due to the observed age related decline infertility as women approaches age 40 [4] . In our study age was found to be insignificant between the two groups.In our study the BMI was insignificant between both groups this comparable with **Bozdag et al.** [5] as they found that BMI was insignificant between the studied groups. **Zollner et al**. [6] agreed with our result as they found that BMI was insignificant in the two groups .Duration of infertility and type of infertility in our study were insignificant between the two groups with p-value 0.057, 0.099 respectively; this agrees with results of **Ataalla et al**.[7] as they found that duration and type of infertility primary and secondary were insignificant between two groups with p-values 0.4, 0.77, 0.816 respectively . In our study we evaluate the efficacy of the orally administered sildenafil citrate on the endometrial thickness in infertility patients undergoing IVF. In our study, measurments on day 6 and day of HMG injection showed that on day 6 follicular numbers were insignificant between two groups also on day of HMG injection there was insignificantly difference in follicular number between the two groups.

This agrees with results of **Ataalla et al**. [7] they found that number of mature follicles were insignificant between two the groups (Sildenafil group, Placebo group) with p-value 0.561

The percentage of changes in endometrial thickness between the two groups in our study after administration of sildenafil it was found to be significantly differences between two groups with improvement in group A with p-value <0.0001.

Fahmy et al. [8] agreed with our result as they found that there was a significant statistical difference in the endometrial thickness between treatment and placebo group which was higher in the treated group. Thicker endometrium might be attributed to the vasodilator effect of sildenafil citrate which leads to increase uterine blood flow.

Moreover, **Fisch et al**. [9] found that sildenafil significantly increased endometrial thickness to >7mm when it was used from day 8 to day 13 of the cycle. The effect of sildenafil citrate on endometrial thickness had also been reported using different route.

CONCLUSION

We revealed that Sildenafil citrate leads 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 endometrial thickness.

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