



Role of Sildenafil in Improvement of Endometrial Receptivity Markers in Fresh ICSI Cycles

Rabab Alwan Taher^{*1}, Manal Taha Al-Obaidi¹, Thuraya Husamuldeen Abdullah²

¹Department of Reproductive Physiology in High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad, Iraq

²Department of Gynecology and obstetrics, Al-Emamain AL-Kadimain Teaching Hospital, Baghdad, Iraq

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ABSTRACT

Receptivity of endometrial is a series of events which enhance implantation of the embryo. It is controlled by several growth factors, cytokines and steroid hormones. The current study aims to assess the influences of vaginally managed sildenafil in ICSI patients on the thickness of endometrial, sub endometrial resistance index and pulsatility index, serum level of vascular endothelial growth factor (VEGF) and pregnancy outcome. The study was planned as a controlled randomized trial (interventional experimental study) involved a total of sixty women who attend High Institute of Infertility Diagnosis and Assisted Reproductive Technologies who undergo ICSI cycle. Enrolled patients divided into two groups: 1) Study group: thirty (30) infertile women had received sildenafil 25mg tablet intravaginally four times daily from the day of stopping blood of menstrual cycle to the day of hCG injection. 2) Control group: thirty (30) infertile women had not treated by sildenafil. In our study, we found the following results: the level of vascular endothelial growth factor was highly significant in the study group. The sub endometrial Doppler study, the mean pulsatility index was significantly lower in the study group, while no significant difference in the resistant index and mean systolic to the diastolic ratio between study and control groups. The thickness of endometrial on the same day of ova pickup was higher in the study group, although statistically not significant. The pregnancy rate of sildenafil group was higher. However, the difference was not statistically significant. We concluded the using vaginal sildenafil during fresh ICSI cycle improve endometrial receptivity by enhancing sub endometrial blood flow through reducing pulsatility index and increasing vascular endothelial growth factor.



*Corresponding Author

Name: Rabab Alwan Taher
Phone: 07823336597
Email: rbabalasdy17@gmail.com

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INTRODUCTION

The good quality embryo, receptive endometrial, and perfect crosstalk between them is requiring for successful of in vitro fertilization and embryo transfer cycles. A weakening of any one of these issues may cause implantation failure (Harper and Harton, 2010).

Endometrial receptivity might be defined as a series of influences which prepare the endometrium to implantation of the embryo. The implantation window or "window of receptivity is a limited time

during which interactions between the embryo and the uterus must have occurred. During this time, any breach in the communication between the endometrium and the embryo leads to implantation failure (Mišan and Severinski, 2016).

Endometrial receptivity depends on the several factors including morphological factors (endometrial thickness, pattern and sub endometrial blood supply) and biochemical factors (endometrial adhesion and anti-adhesion molecules, cytokines, growth factors like (Vascular Endothelial Growth factor (VEGF) and Insulin-like growth factor (IGF), endometrial immune factors, leukemia inhibitory factor (LIF) and endometrial glycodelin) (Bartosch et al., 2011).

Vascular endothelial growth factor (VEGF), is belonging to proteins binding heparin that attach to endothelial cells, and which lead to proliferation and new blood vessel formation. The VEGF also stimulates the release of various cytokines by the endothelial cells leading to dilatation of blood vessels. It acts as an angiogenic factor to promote angiogenesis in different tissues. This explains the critical role of angiogenesis in a variety of women reproductive activities, like the growth of a dominant follicle, development of a corpus luteum, endometrial progress, and implantation (Nardo, 2005).

Ultrasonography evaluation of the endometrial morphology, including endometrial thickness and pattern and Doppler ultrasound of sub endometrial blood flow toward providing a good non-invasive tool for the evaluation of endometrial receptivity. Vascularization of the endometrium is regulated by the vascular endothelial growth factor, which is a strong angiogenic issue which was first described as a significant growth factor for endothelial cells of vessels (Rahmatullah et al., 2020).

Sildenafil is an effective and highly selective inhibitor of phosphodiesterase type 5 (PDE₅). It enhances the vasodilatory influences of NO on vascular smooth muscle by stopping the degradation of cGMP (Wareing et al., 2004).

MATERIALS AND METHODS

Patients, materials and methods

The study was designed as a controlled randomized trial (interventional experimental study) conducted in High Institute of Infertility Diagnosis and Assisted Reproductive Technologies / AL-Nahrian University during the time from November 2018 till the end of April 2019.

Sixty infertile pairs have been included in this study who undergo ICSI cycle, they were told about the

study and signed a printed informed agreement.

Inclusion Criteria

Patients at age group (19-40) years, infertility due to: tubal blockage, anovulatory cycles, unexplained infertility, cases of mild endometriosis whether diagnosed clinically or laparoscopically, due to male factors and couples with unexplained infertility

Exclusion Criteria

Patients with congenital uterine malformation, with extensive uterine pathology and medical contraindication of PDES-5 inhibitors.

Thirty (30) of patients had received sildenafil 25mg intravaginally four times daily from the day of stopping blood of menstrual cycle to the day of HCG injection.

IVF/ICSI procedures

Controlled ovarian hyperstimulation (COH), oocyte retrieval (OCR), fertilization and embryo culture, embryo quality, embryo transfer (ET).

Two different types of controlled ovarian hyperstimulation (COH) were used according to the demographic parameters of patients. First, a long agonist protocol started on cycle day 21 of the previous cycle, and patients were treated by GnRH agonist (Decapeptyl[®] 0.1 mg, Ferring Co., Germany); then, on the cycle day 2, follicular stimulation began with an rFSH (follitropin alfa, Gonal F[®], Merck Serono) in a daily dose range of (150-450 IU).

Then secondly, the option used flexible antagonist protocol by starting with a rFSH (follitropin alfa, Gonal F[®], Merck Serono) in a daily dose range between (150-450 IU). When follicles reached 13-14 mm in size, a GnRH antagonist was started (Cetrotide[®], Merck Serono). Patients were monitored by transvaginal sonography (TVS). HCG (Ovitrelle[®] 250 microgram, Merck Serono) was given once follicular size reached a diameter of 18 mm in three or more follicles.

Oocytes pick up was done using a transvaginal probe 34-36 hours following the HCG injection, immediately before the rupture of follicles. Oocytes were aspirated by transvaginal ultrasound-guided oocyte retrieval (TUGOR); oocytes at retrieval can be either the germinal vesicle (GV) or from metaphase I (MI). The absence of a polar body indicates it to be at the MI stage, which is an intermediate stage between the GV and MII (mature) stages, or metaphase II oocyte (MII), which is mature. Generally, before OCR, a semen sample is prepared. The patient's serum was obtained for measuring VEGF serum level also two-dimensional transvaginal ultrasound scan

(two-dimensional) were done to evaluate endometrial width, regularity, and echogenicity, and sub-endometrial blood flow colour Doppler indices (RI, PI and Vs/Vd) were calculated.

At IVF laboratory, aspirated follicles were examined. Flushing was performed, and the follicles are kept one-to-two hours in a 37°C/CO₂ incubator. Then, all oocytes underwent denudation and grading in a laminar flow cabinet. After that, a needle was carefully inserted through the shell of the egg into its cytoplasm, put in the CO₂ incubator during waiting for the cell division. After insemination, zygotes were observed after 18-20 hours to examine for the occurrence of two pronuclei, then for 25-29 hours to confirm the presence of the first cleavage, which correlated with higher implantation rates. At day one, the presence of two pronuclei was considered a good prognostic sign. Embryo transfer was done at day two-or-three post-OCR, according to the number and grading of the embryos.

RESULTS AND DISCUSSION

Demographic characteristics and baseline hormonal levels of infertile women

Our study showed no significant differences in the patient features and baseline hormonal levels in both study and control groups (Table 1). Our study was comparative study, so we selected females nearly comparable regarding the age, BMI, type, duration and causes of infertility to avoid the effect of these parameters on the outcome results.

Endometrial characteristics at the day of oocyte retrieval

The endometrial thickness on the day of ova pickup was higher in the study group but statically no significant than the control group ($P = 0.599$), 10.07 ± 1.42 mm versus 9.84 ± 1.91 mm, respectively, also no significant difference in endometrial pattern. However, [El-Maghrabi et al. \(2020\)](#) found that the endometrial thickness and trilaminar pattern of endometrium were comparable in both groups. [Firouzabadi et al. \(2013\)](#) found that patients with previous unsuccessful ICSI cycles due to thin endometrial thickness showed good result after oral sildenafil treatment. [Fetih et al. \(2017\)](#) recommended that uterine blood flow and endometrial thickness significantly increased after treatment with a vaginal gel of sildenafil, in women with low endometrium as a result of clomiphene citrate (CC). In our study, we didn't select the sildenafil group according to previous IVF failure due to poor endometrial thickness.

The mean pulsatility index in the study group was

significantly lower than that in the control group ($P = 0.042$), 0.72 ± 0.27 versus 0.99 ± 0.64 ., respectively. Also, no significant difference in resistant index ($P = 0.350$), 0.59 ± 0.20 versus 0.54 ± 0.15 , respectively and in mean systolic to diastolic ratio between control and study groups $P = 0.256$), 2.99 ± 2.59 versus 2.42 ± 0.90 , respectively (Table 2). [Yahia et al. \(2019\)](#) found (RI, PI and S/D ratio) was significantly lower in patients used sildenafil. [Malinova et al. \(2013\)](#) recommended that both PI and RI in patients managed with 25mg sildenafil citrate significant decrease. While [Moini et al. \(2020\)](#) study found there were no differences in PI and RI between left and right uterine artery I in the three intervention groups (sildenafil, sildenafil+placebo and placebo group).

Embryological characteristics

Our study found no significant difference regarding the oocytes number, 8.57 ± 5.31 versus 9.57 ± 5.15 , respectively ($P = 0.642$), metaphase II (MII) oocytes, 4.30 ± 2.83 versus 5.47 ± 2.99 , respectively ($P = 0.126$), fertilization rate %, 74.36 ± 23.03 versus 67.83 ± 21.88 , respectively ($P = 0.256$), in number of transferred embryos, 2.83 ± 1.09 versus 2.87 ± 0.97 , respectively ($P = 0.901$), and number of grade I embryos between control and study group, 2.23 ± 0.97 versus 2.33 ± 1.06 , respectively ($P = 0.705$), (Table 3). This agrees with results of [Ataalla et al. \(2016\)](#), they found no statistically significant difference between (Sildenafil group, Placebo group) in the total number of oocytes retrieved, of mature follicles, in the maturation and fertilization rate and a whole number of embryos obtained. The cause that the sildenafil does not improve ovarian parameters might be that several of these poor responding women have a diminished ovarian reserve and will not advance from improving ovarian blood supply. While [Yahia et al. \(2019\)](#) found the women treated with sildenafil in ICSI cycle had higher total number of retrieved oocyte than the non-sildenafil group, these increase was statically significant. Sildenafil has vasodilator outcome augment the blood flow to the ovary and accordingly increase ovarian response to stimulation.

Serum level of Vascular Endothelial Growth Factor (VEGF) in study and control groups

Serum level of VEGF was significantly higher, ($P < 0.001$), in the study group, 126.48 (193.44) ng/L than in control group, 51.69 (52.96) ng/L, as shown in (Table 4). This finding in agreement with [Constantinescu et al. \(2017\)](#) was found that expression of VEGFR-3 was highly significantly after sildenafil treatment compared to the control group. Another study described of sildenafil effect in patients with

Table 1: Demographic characteristics and baseline hormonal levels of infertile women

Characteristic	Control group = 30	Study group = 30	P
Age (years)	30.53 ±6.20	31.13 ±6.76	0.721 †
BMI (kg/m ²)	28.00 ±4.68	26.64 ±4.64	0.261 †
Duration of infertility (years)	7.13 ±4.48	6.93 ±3.98	0.856 †
Primary infertility, n (%)	22 (73.3 %)	25 (83.3 %)	0.347 ‡
Secondary infertility, n (%)	8 (26.7 %)	5 (16.7 %)	
FSH	7.49 ±3.61	6.29 ±2.91	0.164
LH	5.09 ±2.49	4.65 ±2.77	0.515
E ₂	37.01 ±15.35	30.61 ±14.07	0.097
Prolactin	18.27 ±11.08	19.13 ±10.19	0.755
TSH	2.18 ±1.25	2.18 ±0.88	0.986

Data are mean ± SD. n: cases number; SD: standard deviation; BMI: Body mass index; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone; E₂: Estradiol; TSH: Thyroid-stimulating hormone; †: Independent samples t-test; ‡: not significant at $P > 0.05$.

Table 2: Endometrial Characteristics at the day of ova pickup

Characteristic	Control group = 30	Study group = 30	P
Endometrial Thickness DOP	9.84 ±1.91	10.07 ±1.42	0.599 †
Endometrial Pattern DOP			
Trilaminar, n (%)	30 (100.0 %)	29 (96.7 %)	1.000 F
Hyperechogenic, n (%)	0 (0.0 %)	1 (3.3 %)	
Resistant index (RI)	0.59 ±0.20	0.54 ±0.15	0.350 †
Pulsatility index (PI)	0.99 ±0.64	0.72 ±0.27	0.042 †
Systolic /diastolic ratio	2.99 ±2.59	2.42 ±0.90	0.256 †

Data are mean ± SD. n: cases number; DOP: day of ova pickup †: Independent samples t-test; F: Fischer exact test; M: Mann Whitney U test; NS: not significant at $P > 0.05$; S: significant at $P \leq 0.05$

Table 3: Embryological characteristics

Characteristic	Control group = 30	Study group = 30	†
Oocyte number	8.57 ±5.31	9.57 ±5.15	0.462
Metaphase II (MII)	4.30 ±2.83	5.47 ±2.99	0.126
Fertilization rate %	74.36 ±23.03	67.83 ±21.88	0.265
Grade I embryo	2.23 ±0.97	2.33 ±1.06	0.705 †

Data are mean ± SD; †: Independent samples t-test; NS: not significant at $P > 0.05$

Table 4: Serum level of Vascular endothelial growth factor (VEGF) in control and study groups

Characteristic	Control group = 30	Study group = 30	€
VEGF (ng/L) Median (IQR)	51.69 (52.96)	126.48 (193.44)	< 0.001 HS

IQR: interquartile range; €: Mann Whitney U test; HS: highly significant at $P \leq 0.01$

Table 5: VGFR level according to pregnancy outcome

Characteristic	Positive pregnancy = 16	Negative pregnancy = 44	P
VEGF (ng/L) Median (IQR)	277.76 (270.84)	53.43 (57.77)	< 0.001 †HS

IQR:inter-quartile range; VEGF: vascular endothelial growth factor; †: MannWhitenyU test; HS: Highly significant at $P \leq 0.01$

recurrent miscarriages; it has decreased natural killer cell activity as well as the facilitating effect on endometrial development. VEGF contributes to angiogenesis and improved blood vessels permeability during the mid-secretory phase, that is important for implantation (Jerzak *et al.*, 2008).

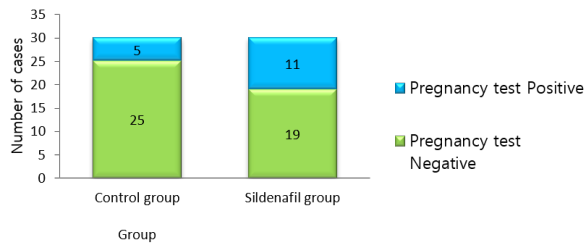


Figure 1: Bar chart showing the positive pregnancy rate in sildenafil and control groups

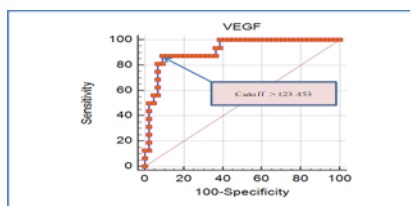


Figure 2: Receiver operator characteristic (ROC) curve evaluation to obtain the best VEGF cutoff value that can predict positive pregnancy outcome

Serum level of Vascular Endothelial Growth Factor (VEGF) according to pregnancy outcome

The VEGF was highly significant in pregnant 277.76 (270.84) than non-pregnant women; 53.43 (57.77), (Table 5). The best VEGF cutoff value was > 123.453, with an accuracy level of 91.9 %, a sensitivity level of 87.5 % and a specificity level of 90.9 %, as shown in (Figure 2). Sugino *et al.* (2002) revealed that the appearance of VEGF and its receptor were higher during the mid-secretory phase throughout regular menstrual cycles. Also, there was an elevated appearance of VEGF in decidual cells in early pregnancy. The authors concluded that VEGF enhances the successful implantation and preservation of pregnancy by improving vascular permeability or by building the vascular network in the decidua. zhi Gao *et al.* (2012) were measured the concentrations of VEGF, endocrine gland derived vascular endothelial growth factor (EG-VEGF) and transforming growth factor-beta 1 (TGF- β 1) in follicular fluid sample (FF) collected during oocyte retrieval (OR) and in serum sample collected two days after OR. There was a positive correlation between the serum concentration of the three growth factors and embryo quality. In contrast, only serum concentrations of EG-VEGF were positively associated

with the pregnancy outcome. While Asimakopoulos *et al.* (2005) compared serum VEGF levels in women entering the ICSI cycle according to the pregnancy outcome and found no significantly different between the pregnant and non-pregnant group.

The pregnancy rate in study and control groups

In the current study the pregnancy rate of the study group, 11/30 (36.7 %) was higher than that of the control group, 5/30 (16.7) but, the difference is not significant, (Figure 1). This result in agreement (Moini *et al.*, 2020) study showed no significant difference in the clinical pregnancy rate between study groups (sildenafil+placebo) and placebo group. In contrast to our study, Sher and Fisch (2002) study showed ongoing pregnancy rates were highly significant for sildenafil (45%) when compared with non-sildenafil groups.

CONCLUSION

Using vaginal sildenafil during fresh ICSI cycle improve endometrial receptivity by enhancing sub endometrial blood flow through reducing pulsatility index and increasing vascular endothelial growth factor.

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Conflict Interest

The authors declare that they have no conflict of interest for this study.

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