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Evaluation the effect of sildenafil on nitric oxide secretion and improvement of endometrial receptivity in fresh ICSI cycles

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Abstract
Introduction: Endometrial receptivity is a chain of events that promotes embryo implantation and is regulated by a variety of substances, such as cytokines, growth factors, and steroid hormones. The study's objective is to assess how vaginally delivered sildenafil in ICSI patients affects pregnancy outcome, endometrial thickness, subendometrial resistance index, and pulsatility index.
Methods: The sixty women who participated in this controlled randomised trial (interventional experimental research) at the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies underwent IVF/ICSI cycles. Patients were split into two groups: Study group: From the day of the last menstrual period to the day of the hCG injection, thirty (30) infertile women got four (4) doses of vaginal sildenafil 25mg tablets daily. Thirty (30) infertile ladies who weren't given sildenafil therapy made up the control group.
Results: Nitric oxide levels in the study group's serum were significantly significant. In the subendometrial Doppler research, the mean pulsatility index was considerably lower in the study group whereas the mean resistance index and mean systolic to diastolic ratio did not change statistically between the control and study groups. Between the control and study groups, there was no statistically significant difference in the mean endometrial thickness. The sildenafil group had a greater pregnancy rate, although the difference was not statistically significant.
Conclusion: By enhancing subendometrial blood flow by lowering the pulsatility index and raising the nitric oxide (NO) factor, the use of vaginal sildenafil in a new ICSI cycle improves endometrial receptivity.

Keywords: Sildenafil, endometrial receptivity, fresh ICSI cycle

Introduction

Fertility issues affect 7-15% of women of reproductive age, and assisted reproductive techniques are used to treat them. Successful *in vitro* fertilization and embryo transfer cycles require good quality embryos, receptive endometrial, and efficient crosstalk between receptive endometrium and the embryo [2]. Implantation failure might result from a flaw in any of these elements or biological processes [3, 4]. The ability to successfully implant remains a fundamental limitation of assisted reproductive technologies, and endomtrial receptivity is crucial. Endometrial perfusion is one of the variables that regulates endometrial receptivity [5]. To increase endometrial receptivity in IVF cycles, a number of therapeutic methods were investigated. These might include (oral or vaginal sildenafil, extended oestrogen administration, vitamin E, pentoxifylline, and GnRH-a adminstration during luteal phase) [6]. Sildenafil citrate enhances the effects of NO by inhibiting phosphodiesterase type 5 (PDE5), which breaks down cGMP. As a consequence, when sildenafil is used, cGMP levels remain high, improving endometrial thickness and relaxing blood vessels [22]. Using the transvaginal colour Doppler technology, researchers may examine how various drugs affect uterine perfusion [8]. The study's objective is to assess how vaginally delivered sildenafil in ICSI patients affects pregnancy outcome, endometrial thickness, sub endometrial resistance index, and pulsatility index.

Method

The research was carried out at the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies / AL-Nahrian University between October 2018 and the end of May 2019. It was intended as a controlled randomised trial (interventional experimental study). This research includes 60 infertile couples that undergo an ICSI cycle.

Inclusion Criteria

- The ladies ranged in age from 19 to 40.
- Infertility caused by female causes, such as irregular menstrual cycles, tubal obstruction, minor endometriosis (confirmed either clinically or laparoscopically), and unexplained infertility.
- Male-related infertility.
- All instances in which ICSI trials have previously failed

Exclusion Criteria

1. A medical reason why PDES-5 inhibitors shouldn't be used.
2. Women who have severe uterine disease or uterine congenital abnormality.

Total of sixty patients were evaluated following these steps: Comprehensive medical, surgical, and obstetrical history; evaluation of height & weight to determine BMI. Clinical and gynecological exams to rule out any uterine abnormalities or infections. FSH, LH, E2, Testosterone, Prolactin, and TSH hormone analyses for female partners on day 2 of the menstrual cycle. Analysis of seminal fluid was evaluated in accordance with WHO 2010. The flexible antagonist Controlled Ovarian Hyper stimulation (COH) protocol was used. From the day of the menstrual cycle's last blood until the day of the HCG injection, thirty (30) participants received sildenafil 25mg intravaginal four times daily. IVF/ICSI procedures: 1. Controlled ovarian hyper stimulation (COH), 2. Oocyte retrieval (OCR), 3. Fertilization and embryo culture, 4. Embryo quality, 5. Embryo transfer (ET). The flexible antagonist regimen used for controlled ovarian hyper-stimulation (COH) began with a daily dosage of 150–450 IU rFSH (follitropin alfa, Gonal F®, Merck Serono), and when the follicles reached 13–14 mm in size, a GnGH antagonist (Cetrotide®, Merck Serono) was initiated. Transvaginal sonography was used to monitor the patients (TVS). When three or more follicles had grown to a diameter of 18 mm, HCG (Ovitrelle® 250 microgram, Merck Serono) was administered. A transvaginal probe was used to retrieve the oocytes 34–36 hours after the HCG injection, just before the follicles burst. Transvaginal ultrasound-guided oocyte retrieval was used to aspirate oocytes (TUGOR). In addition to evaluating the patient's endometrial thickness using two-dimensional transvaginal ultrasound scans, the color Doppler indices (PI, RI, and Vs/Vd) of the patient's sub-endometrial blood flow were measured.

Results

Demographic characteristics and baseline hormonal levels of infertile women enrolled in this study

There were no significant differences in the patient characteristics and in baseline hormonal levels in both study and control groups (Table 1). Endometrial characteristics at day of ova pickup: There was no significant difference in mean endometrial thickness on day of ova pickup between control group and study group (P = 0.599). However, the mean pulsatility index was significantly lower in study group than that in control group (P = 0.042). There was also no significant difference in mean resistant index (P = 0.350) and in mean systolic to diastolic ratio between control and study groups (P = 0.256), (Table 2)

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Embryological characteristics

There were no significant difference in mean number of oocytes (P = 0.642), mean metaphase II (MII) oocytes (P = 0.126), mean fertilization rate% (P = 0.256), in mean number of transferred embryos (P = 0.901), and mean number of grade I embryos between control and study group (P = 0.705), (Table 3).

Nitric Oxide (NO) levels in control and study groups

The level of NO was higher in study group than in control groups and the difference was highly significant (P < 0.001), as shown in (Table 3).

Pregnancy rate in control and study groups: The pregnancy rate of study group was higher than that of control group, 11/30 (36.7%) versus 5/30 (16.7%), respectively however, the difference did not reach a statistical significance, (Figure 1).

The level of nitric oxide according to pregnancy outcome

There was highly significant difference in NO between pregnant and non-pregnant women; being higher in pregnant ladies, (Table 4). The best NO cutoff value was > 29.502 with an accuracy level of 78.8%, a sensitivity level of 68.8% and a specificity level of 81.8%, as shown in (Figure 2).

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

Characteristic	Control group N = 30	Study group N = 30	*P*
Age (years)	30.53 ±6.20	31.13 ±6.76	0.721 † NS
BMI (kg/m^2)	28.00 ±4.68	26.64 ±4.64	0.261 † NS
Duration of infertility (years)	7.13 ±4.48	6.93 ±3.98	0.856 † NS
Primary infertility, *n* (%)	22 (73.3%)	25 (83.3%)	0.347 ¥ NS
Secondary infertility, *n* (%)	8 (26.7%)	5 (16.7%)	
FSH	7.49 ±3.61	6.29 ±2.91	0.164 NS
LH	5.09 ±2.49	4.65 ±2.77	0.515 NS
E_2	37.01 ±15.35	30.61 ±14.07	0.097 NS
Prolactin	18.27 ±11.08	19.13 ±10.19	0.755 NS
TSH	2.18 ±1.25	2.18 ±0.88	0.986 NS

Data are mean ± SD. *N*: number of cases; SD: standard deviation; BMI: Body mass index; FSH: Follicle stimulating hormone; LH: Luteinizing hormone; E_2: Estradiol; TSH: Thyroid stimulating hormone; †: Independent samples t-test; NS: not significant at $P > 0.05$

Table 2: Characteristics of infertile women at day of ova pickup

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

Data are mean ± SD. *n*: number of cases; 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 $P \leq 0.05$

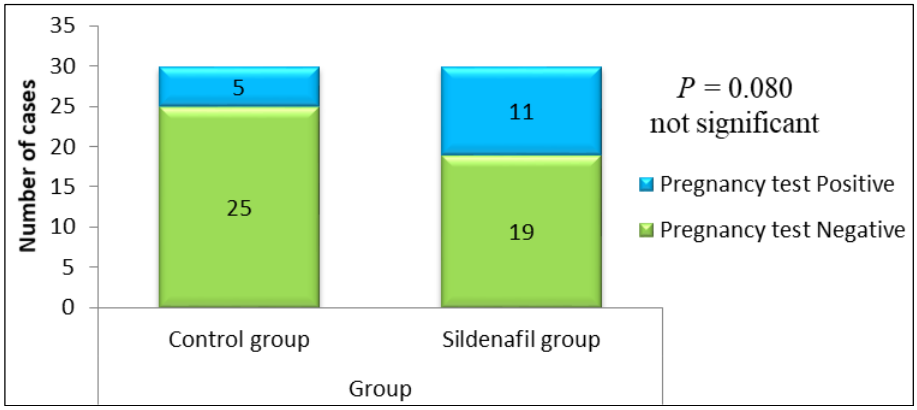

Fig 1: Bar chart showing the positive pregnancy rate in study and control groups

Table 3: Nitric Oxide (NO) level in control and study groups

Characteristic	Control Group *N* = 30	Study Group *N* = 30	*P* €
Nitric oxide (µmol/L) Median (IQR)	12.27 (20.31)	31.98 (80.69)	< 0.001 HS

N: Number of Cases; IQR: Inter-Quartile Range; €: Mann Whitney U test; HS: Highly Significant at $p \leq 0.01$

Table 4: Nitric Oxide (NO) level according to pregnancy outcome

Characteristic	Positive Pregnancy *N* = 16	Negative Pregnancy *N* = 44	*P*
Nitric oxide (µmol/L) Median (IQR)	35.19 (183.72)	18.63 (17.11)	< 0.001 † HS

IQR: Inter-Quartile Range; VEGF: Vascular Endothelial Growth Factor; *N*: number of cases;
†: Mann Whiteny U test; HS: Highly significant at $p \leq 0.01$.

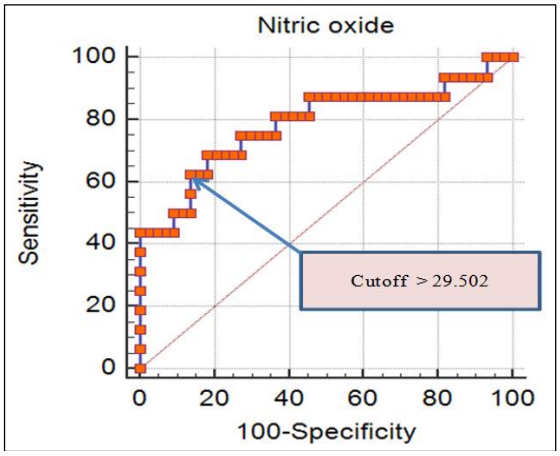

Fig 2: Receiver operator characteristic (ROC) curve analysis to find the best nitric oxide (NO) cutoff value that can predict positive pregnancy outcome

Discussion

The mean endometrial thickness in the current research was larger, but there was statistically no difference between the control and study groups, nor was there a significant difference according to endometrial pattern. El-Maghrabi, *et al.* [9] discovered that the endometrium's thickness and pattern in the study and control groups were equivalent. Oral sildenafil treatment was recommended by Dehghani Firouzabadi, *et al.* [10] for individuals whose prior IVF cycles had failed due to insufficient endometrial thickness. Additionally, Yahia A, *et al.* found that there was a statistically significant difference in endometrial thickness between the study and control groups, with the study group having a higher endometrial thickness. This finding may be related to sildenafil citrate's vasodilator effect, which increases uterine blood flow. Between the control and study groups in our research, there were no statistically significant variations in the sub-endometrial RI and S/D ratio means.

However, compared to the control group, the mean pulsatility index was considerably lower in the study group [11]. Malinova, *et al.* [12] reported that sildenafil citrate-using patients' PI and RI of the uterine artery significantly decreased. The Moini A, *et al.* [13] research found no differences between the sildenafil, sildenafil plus placebo, and placebo groups in the right and left uterine arteries, RI and PI. In the current investigation, there was a significantly significant (P 0.001) difference in the amount of NO between the sildenafil and non-sildenafil groups. Sildenafil enhances accumulate NO through a cGMP pathway, leading in higher concentrations of NO resulting in better blood flow in the uterine endometrial and improving endometrial thickness prior to implantation. This finding is in agreement with Ensieh, *et al.* [14] study; however, their study included 72 patients who were used 100 mg vaginal sildenafil suppositories daily beginning on day 3 of menstruation and continued until human chorionic gonado. Another experimental *in vitro* investigation that evaluated the impact of different sildenafil doses on endometrial biopsies (1, 10, and 20 M) discovered a moderate proliferative effect on human endometrial epithelial cells, although NO level was unaffected. Although the study group's pregnancy rate was greater than the control groups, the difference failed to approach statistical significance (P = 0.080). In the sildenafil group, the AbdelKader Fahmy, *et al.* [16] research reported a 2.5 times increase in pregnancy, although this difference was not statistically significant. In contrast to our research, Yahia A, *et al.* [11] and Geoffrey Sher, *et al.* [17] found that sildenafil groups had considerably greater pregnancy rates than non-sildenafil groups. Pregnant women had greater levels of NO, which was a very significant difference between them and non-pregnant women (P = 0.001). According to Battaglia, *et al.* [18], NO has a beneficial impact on women who have a poor ovarian response to COS. According to Ohl, *et al.* [19], individuals undergoing IVF cycles with a history of prior implantation failures discovered that the NTG therapy administered the day before embryo transfer was not significantly more beneficial than a placebo at increasing implantation or pregnancy rates. This outcome did not support our findings, however the length of the NTG patch therapy may be to blame for the absence of an improvement in the pregnancy rate. The early follicular phase is when the NO donor patches ought to have started. If this is the case, ovarian response or, in the absence of it, uterine receptivity should be positively impacted. In patients with peritoneal or tubal factor infertility, Tsung, *et al.* [20] discovered that up-regulated NO was linked to unsuccessful IVF/ICSI therapy. Female partners from marriages with male factor infertility did not discover such an effect. A greater amount of embryo fragmentation was linked to elevated follicular NO levels. Further research is required to understand how NO production is regulated, and patients who are infertile due to peritoneal or tubal factors should avoid having their NO production upregulated. To prevent negative effects of NO at the level of the endometrium during the implantation window, sildenafil medication was begun in the current trial on the day that the menstrual cycle stopped until the day of the HCG injection. A cause of implantation failure has been identified as the NO enhanced release of cytokines from activated natural killer cells, such as tumour necrosis factor-Alfa [21].

Conclusion

Using vaginal sildenafil in fresh ICSI cycle enhance endometrial receptivity by increasing subendometrial blood flow through reducing pulsatility index and increasing nitric oxide (NO) factor.

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