Value of Vaginal Sildenafil Citrate for Endometrial Preparation and Outcome in Frozen Thawed Embryo Transfer Cycles

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Abstract

**Background:** Vaginal Sildenafil Citrate may increase endometrial thickness, improving endometrial blood flow and affect the outcome of frozen-thawed embryo transfer cycles.

**Objective:** To assess the effect of vaginal sildenafil citrate in improving endometrial central line (trilaminar) pattern, implantation rate and chemical pregnancy rate in frozen embryo transfer cycles.

**Methods:** This study was carried out at ART unit in The International Islamic Center for Population Studies and Research (IICPSR) at Al-Azhar University in addition to private in vitro-fertilization (IVF) center during the period between June 2015 and Dec. 2016. On the 21st day of l cycle proceeding the treatment, all infertile women (90) who had an antecedent of poor endometrial response and frozen embryos. Endometrial thickness, pattern and vascularity and frozen embryos were included in this study. Group A, 45 Women were included and given sildenafil citrate 25mg vaginally every 6 hours starting from day 2-14 of the cycle in addition to oral 2mg of estradiol valerat 6-8 hourly from the day 2-14 of the menstrual cycle. This was discontinued 48-72 hours prior to the embryo transfer. Group B, 45 women were given oral estradiol valerat tablets 2mg 6-8 hourly from the day 2-14 of the cycle to prepare the endometrium. Women were evaluated by using trans-vaginal sonography (TVS) on day 8 of cycle for assessment endometrial thickness and pattern and by using two dimension power Doppler for assessment endometrial vascularity.

**Results:** The endometrial thickness on cycle day 8 was significantly higher in women of the sildenafil citrate group (p<0.001). There was a significantly higher proportion of women who had triple line pattern (trilaminar) of the endometrial on cycle day 8 among women of sildenafil citrate group, when compared to those of control group (p<0.001). Finally, the biochemical pregnancy rates were higher in the sildenafil citrate group but not significantly.

**Conclusion:** Transvaginal of sildenafil citrate is a significant to improve the endometrial thickness, pattern and vascularity in women undergoing frozen-thawed embryo transfer cycle. Further studies on larger scale patients are needed to prove value in improving pregnancy outcome.

**Key Words:** Vaginal sildenafil citrate – Endometrial preparation – Embryo transfer.

Introduction

THOUGH there are many articles correlating endometrial blood flow with in vitro-fertilization (IVF) success rates, there is a paucity of data in frozen embryo transfer (FET) cycles which contribute to about 25% of all births achieved by assisted reproductive techniques (ART) [1]. Therefore, more attention should be focused on to improve the endometrial thickness, pattern and vascularity in women undergoing frozen-thawed embryo transfer cycle. Implantation remains a major limiting step of ART and uterine receptivity is essential for successful implantation in all species. Successful implantation requires good embryo quality, appropriately timed and 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 biological processes may result in implantation failure [2].

Endometrial receptivity during the implantation window (a self-limited period within the cycle days 20 and 24) depends on various factors, the morphological markers are endometrial thickness, endometrial echogenic pattern, endometrial and sub endometrial blood flow [3]. To improve the implantation, there are 14 treatment options: (a) blastocyst transfer, (b) assisted hatching, (c) coculture, (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 treatment, (m) allogenic lymphocyte therapy, and (n) IV immunoglobin therapy [4].
Endometrial receptivity (ER) is essential for conception in natural and infertility treatment cycles. The altered hormone levels could mediate an asynchrony between the endometrium and the transferred embryos leading to an endometrial environment that could be responsible for implantation failure [8].

To date, with the advances of the embryo cryopreservation techniques, the quality of the frozen embryos and their potential of implantation are similar to the observed with fresh embryos [6]. Some studies have shown good results with the cryopreservation of all embryos and subsequent frozen embryo transfers (FET) in patients with an increased risk ovarian hyper stimulation syndrome (OHSS) [7]. In FET, endometrial priming may be achieved with the use of estrogen and progesterone, and the endometrial development can be controlled more precisely than in cycles of controlled ovarian hyper stimulation with gonadotropins [8]. Estrogen induced endometrial proliferation is in large part dependent upon blood flow to the basal endometrium [9].

Nitric oxide (NO) is a key signaling molecule involved in the vasodilator response of smooth muscle cells. NO activates the cyclic guanosine monophosphate (cGMP)/protein kinase G (PKG) pathway within smooth muscle cells to promote smooth muscle cell relaxation. Sildenafil citrate inhibits phosphodiesterase 5 (PDE5) maintaining activation of cGMP and PKG and maximizing the effect of existing NO, thus facilitating smooth muscle cell relaxation. The potent vasodilator action of sildenafil has led researchers to evaluate sildenafil as a treatment in assisted reproduction where low uterine blood flow is perceived to be a contributor to implantation failure [3,9]. The aim of this study to assess the effect of vaginal sildenafil citrate on endometrial thickness, pattern, endometrial vascularity, biochemical pregnancy rate and pregnancy outcome in frozen embryo transfer cycles.

Material and Methods

This was a prospective randomized clinical study carried out at ART unit in The International Islamic Center for Population Studies and Research (IICPSR) at Al-Azhar University in addition to private IVF center during the period between June 2015 and Dec. 2016. Ethical approval was not required as the study followed standard departmental clinical practice and no additional visits or procedures were imposed on the patients. Informed written consent was taken from all participants after explaining the purpose and procedures of the study.

Each patient had been subjected to the following: Detailed history taking, careful examination, infertility work up and hormone profile. Also, sonographic markers were included the endometrial thickness and pattern for evaluating poor endometrial receptivity as manifest by a thin endometrium (thickness <5mm) and no ovarian cyst was seen to confirm fulfilling all inclusion and exclusion criteria [10].

Inclusion criteria included:
- The age less than 38 years old.
- Undergoing their first FET cycle. All cases have at least two good quality embryos, where vitrification technique was used for freezing and were available for transfer after thawing.

Exclusion criteria included:
- History of endocrine diseases.
- Uterine structural abnormalities multiple myomas, severe intrauterine adhesions, hydrosalpinx or pyosalpinx.
- Cardiovascular, renal and liver diseases.
- Hypotension (blood pressure <90/50mmHg).
- History of stroke or myocardial infarction.

A total of 90 subfertile women who met these conditions and had previously undergone intra-cytoplasmic sperm injection (ICSI) when the embryos were surplus for fresh cycle or when fresh transfer was not possible due to risk of ovarian hyperstimulation syndrome were entered the study. Women were included and divided by simple randomized into two groups:

Group A: 45 Women were included and given sildenafil citrate 25mg vaginally every 6 hours (silden 25mg tablet by EIPICO) starting from day 2-14 of the cycle, in addition to oral 2mg of estradiol valerate tablets 6-8 hourly from the day 2-14 of the menstrual cycle. Estrogen and progesterone (prontogest 400mg pessaries by EIPICO) were given vaginally 3 days prior embryo transfer. Sildenafil was discontinued 48-72 hours prior to the embryo transfer due sildenafil may have some detrimental effects on endometrium in the implantation window [3].

Group B: 45 women were given oral estradiol valerate tablets 2mg 6-8 hourly from the day 2-14 of the cycle to prepare the endometrium, all women were evaluated by using trans-vaginal sonography (TVS) (5-9 MHz) on Voluson E8 Expert (GE med-
ical systems) on day 14 of cycle for assessment endometrial thickness and pattern after obtaining a true longitudinal view of uterus on B-mode. Endometrial morphology was classified as triple line, echogenic and intermediate. For assessing endometrial vascularity (on day 14 of cycle), two dimension power Doppler characteristics as normal quality of color, color gain-3.4, pulse repetition frequency of 600Hz and wall motion filter of 50Hz were applied in all examinations. We have followed Applebaum’s zones of vascularity for categorizing endometrial vascularity: Zone 1 vascularity-When blood vessels reached the hypocoelic endometriomymetria junction, Zone 2 vascularity when the vessels reached the outer hyperechoic line of endometrium, Zone 3 vascularity when it reached the intervening hypocoelic area, Zone 4 vascularity when the vessels were seen reaching the central echogenic line [3,11].

After 5 days when the endometrial pattern was triple line pattern (trilaminar) and endometrial thickness was more than 8mm,embryos will be transferred. All patients had a luteal phase support by giving a daily doses of estradiol valerat 2mg oral daily and 100mg progesterone (prontogest 100mg ampoule by EIPICO)as an intramuscular which will be continued two weeks after the embryos transfer. In case BHCG was tested and proved to be positive, estradiol valerat and progesterone which will be continued two weeks after the embryo transfer cycle.

The primary and secondary outcomes of the study were biochemical pregnancy and abortion rate, number of gestational sacs respectively.

**Statistical Methods:**

In the present study, the data was collected on Microsoft Excel Sheet 2010. Data were statistically described in terms of mean ± standard deviation (±SD), frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using t-test. Data was collected, tabulated, then analyzed using IBM® SPSS® Statistics version 22 (IBM®).

**Results**

There were no significant differences in age, duration of infertility, no of previous attempt, no of success in previous fresh embryo transfer cycle and no of transferred embryo between groups. The duration of treatment cycle was significantly shorter in women of Sildenafil group (p<0.001). Sildenafil group had a higher trend of biochemical pregnancy rate without reaching statistical significance between groups. There were no significant differences in abortion rates and multiple pregnancy between two groups.

Endometrial thickness was significantly higher in the sildenafil citrate group (p<0.001) on cycle day 8. The triple line pattern of the endometrium was significantly higher in the sildenafil citrate group (p<0.001) while the intermediate pattern of the endometrium and the echogenic pattern were higher significantly in control group (p<0.001). The endometrial vascularity on zone 3 and zone 4 significantly higher in sildenafil group while zone 1 and zone 2 were higher significantly in control group (p<0.001).

<table>
<thead>
<tr>
<th>Table (1): Clinical Characteristics of women in the two groups.</th>
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<tbody>
<tr>
<td><strong>Age (years)</strong></td>
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<td>28.42±3.79</td>
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<tr>
<td><strong>Duration of Infertility (years)</strong></td>
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<tr>
<td><strong>No of previous attempt</strong></td>
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<td><strong>No of success in previous fresh embryo transfer cycle</strong></td>
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<td><strong>No of transferred embryo</strong></td>
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<tr>
<td><strong>Duration of Treatment Cycle (days)</strong></td>
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<td><strong>Biochemical pregnancy</strong></td>
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<td><strong>Abortion rate</strong></td>
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<td><strong>Multiple pregnancy</strong></td>
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</table>

*p<0.05 considered as significant.
*Data presented as mean ±SD. **Data presented as n (%)

<table>
<thead>
<tr>
<th>Table (2): Difference between Groups regarding endometrial Thickness, pattern and vascularity on day 8 of cycle.</th>
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</thead>
<tbody>
<tr>
<td><strong>Endometrial Thickness on Cycle Day 8 (mm)</strong></td>
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<tr>
<td>8.13±1.04</td>
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<tr>
<td><strong>Endometrial Pattern on Cycle Day 8</strong>*:</td>
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<tr>
<td><strong>Triple line pattern (Trilaminar)</strong></td>
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<tr>
<td><strong>Intermediate</strong></td>
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<td><strong>Echogenic</strong></td>
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<td><strong>Zone 1</strong></td>
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<td><strong>Zone 2</strong></td>
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<td><strong>Zone 3</strong></td>
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<td><strong>Zone 4</strong></td>
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*p<0.05 considered as significant.
*Data presented as mean ±SD. **Data presented as n (%)


Discussion

In our study, we evaluated the effect of sildenafil citrate on the outcome of frozen-thawed embryo transfer cycles. Endometrial growth seems to be dependent on uterine artery blood flow, and the importance of endometrial development in the pregnancy outcome has been already reported [12,13]. A positive linear correlation between endometrial thickness measured on the hCG day and pregnancy rate (PR) was identified [14]. A good correlation has been found between endometrial thickness and the prevalence of conception. An endometrial thickness of \( \geq 9 \text{mm} \) in the late proliferative phase, as determined by vaginal ultrasound, correlates well with the chance of pregnancy after IVF, whereas a thinner endometrium is associated with poorer prognosis for success [12].

In the present study, the duration of treatment cycle was the duration from the day of starting endometrial preparation either by estradiol valerat alone or estradiol valerat with sildenafil citrate until the day of 14 of menstrual cycle. It was significantly shorter in women used estradiol valerat combined with sildenafil citrate compared with women used estradiol valerat alone \( (p<0.001) \). This is consistent with what Firouzabadi RD et al., found that the duration of treatment cycle was significantly shorter in women of sildenafil group \( (p<0.001) \) [15].

The present study revealed that the mean endometrial thickness on cycle day 8 was significantly higher in women used sildenafil citrate in addition to estradiol valerat than women used estradiol valerat alone \( (p<0.001) \). This is consistent with what Firouzabadi et al., found that the mean endometrial thickness on cycle day 8 was significantly higher in women of sildenafil group \( (p<0.001) \) [15].

This agreed with several studies such Aisaka et al., [16], Takasaki et al., [14], Morad et al., [17] and confirmed by most recently Eid ME found that 22 patients were administered vaginal sildenafil with an endometrial thickness (EMT) of 7mm and elevated pulsatility index (PI>0.3) for a total of 7 days between ovulation trigger and embryo transfer. Sixty-eight percent (15/22) of patients experienced improvement in endometrial thickness and a decrease in their PI. Implantation rates and pregnancy rates were higher in patients who responded to treatment \( (26\% \text{ vs. } 7\% \text{ and } 40\% \text{ vs. } 14\% \text{ respectively} \) [18].

On other hand, Check et al., found that neither sildenafil nor estradiol improved endometrial thickness in women with poor endometrial response [19].

In our study, sildenafil citrate was a significantly higher triple line pattern (trilaminar) of the endometrium but decreased its echogen pattern when compared with other group \( (p<0.001) \). A combined analysis of endometrial thickness and pattern on cycle day 8 the day proved to be a better predictor of the outcome of IVF/ICSI-ET and may be more helpful for patient counseling than a separate analysis [20]. This is consistent with Firouzabadi RD et al., [15].

These results are agreement with what Sher & Fisch, and Al-Assadi et al., described in their studies when they found that the use of vaginal sildenafil citrate improved endometrial thickness and pattern and uterine artery blood flow with prior failed assisted reproductive cycle due to poor endometrial response [9,21].

The current study found that the endometrial vascularity on zone 3 and zone 4 were significantly higher in sildenafil group while zone 1 and zone
2 were higher significantly in control group \((p<0.001)\), therefore sildenafil citrate improves the endometrial perfusion and uterine blood flow with a prior failed assisted reproductive cycle due to poor endometrial response \([9,13,22]\).

On other hand endometrial thickness and blood flow indices of endometrium and uterus measured by transvaginal sonography and Doppler velocimetry measurements are not an effective predictor of pregnancy outcome in frozen-thawed embryo transfer cycles \([23]\).

In this study, there was a higher 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\, [20] 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 needlessly]. This is consistent with what Firouzabadi et al. \([15]\). The data concluded from the current study is close to agree with the results by Mangal et al., Sharma et al., and Barker \([24,25,26]\).

In contrast to our study, Merce et al. \([27]\) and Rashidi et al. \([28]\) found that there was no observed significant differences in the endometrial thickness or pattern between the pregnant and nonpregnant groups. Zachova et al., also concluded that measurement of power Doppler indices using 3 D ultrasound on the day of the FET does not provides, any additional information concerning the outcome of the cycle \([29]\).

In our study there was a higher abortion rate in pregnant women of control group when compared to those of sildenafil group; the difference was statistically insignificant \([1\, (11.11\, vs.\, 1\, (6.67)\, respectively, \(p=0.8]\). This agree with Ng et al., who found that the endometrial and subendometrial vascularity were significantly higher in pregnant patients with live birth than in those who suffered a miscarriage \([30]\).

Also there was multiple pregnancy in pregnant women of sildenafil group when compared to those of control group; the difference was, statistically insignificant \([2\, (13.13)\, vs.\, 1\, (11.11\, respectively, \(p=0.6]\). Therefore, a single or double ET can be attempted when good endometrial blood flow (zone 3 or 4) is observed in sildenafil group for preventing associated complications of higher order of multiple pregnancies \([11]\).

As regard to improve pregnancy rates in FET cycles, we can also help to reduce the risk of OHSS by using gonadotropin releasing hormone (GnRH) agonist on day 21 of previous cycle before starting treatment and/or by elective cryopreservation of embryos in high risk cases. However, this analysis needs to be complemented by research correlating the study protocol with live birth rate \([11]\).

One limitation of our study could be the small number of patients studied. Another limitation is the observed difference in age in the study groups, suggesting a possible effect on endometrial blood flow. Possibly larger studies are required to reach definite conclusions.

**Conclusion:**

Transvagal of sildenafil citrate is a significant to improve the endometrial thickness, pattern and vascularity in women undergoing frozen-thawed embryo transfer cycle. We recommend the routine use of vaginal sildenafil citrate in women with a previous failure of assisted reproduction technology cycles due to poor endometrial thickness although this improvement in endometrial blood flow has a weak positive feedback on pregnancy rate and outcome of frozen embryo transfer cycle. Further studies on larger scale patients are needed to prove value in improving pregnancy outcome.

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سيئات السيليديبينافيل المهبلية لإعداد بطاقة الرحم في دورات نقل الأجنة المذابة بعد تجميدها ونتائجها

هدف البحث: تقييم تأثير سيرات السيليديبينافيل المهبلية في تحسين كل من سرعة ونسبة نجاح نقل الرحم في دورات نقل الأجنة المجمدة.

وقد اعتمدت البحث دراسة عشوائية مستقبلية من 90 سيدة تعاني من تأخر الحمل تم الكشف عليها وأخذ تاريخ مرضي من كل واحدة منهن وعمل كل الفحوصات المعملية في وحدة أطفال الأيباد مركز الإخصاب السداسي لكلية الطب جامعة الأزهر وبعض المراكز الخاصة. وكانت كل سيدة تحتفظ ببطاقة مجمدة. تم تقسيم السيدات إلى مجموعتين بالتساوي المجموعة الأولى تم تناول السيرات المهبلية، والثانية تم تناول السيرات المهبلية بالكامل. وتم تقييم السيدات ببطاقة مجمدة. بينما المجموعة الثانية حصلت على سيرات المهبلية فاتحة كتوحيد لبطاقة الرحم. وقد تم تقييم نسب بطاقة الرحم وحسابها من قبل الموجات فوق الصوتية عبر المهبل وعمل دور نقل ثاني بعدد.<br>

وقد أظهرت نتائج البحث أن هناك نسبة أعلى بكثير من النساء اللواتي لديهن في اليوس الثاني من دورات نقل الرحم سرعة ونسبة نجاح نقل الرحم في المجموعة الأولى.<br>

الخلاصة: يمكن استئناف سيرات السيليديبينافيل المهبلية لتحسين سرعة ونسبة نجاح نقل الرحم في السيدات التي ينضمن عن ثورة نقل الأجنة المذابة بعد تجميدها. هذا التحسن في بطاقة الرحم يعود بمرور إيجابي ضعيف على معدل نتائج الحمل ونتائج نورة نقل الأجنة المجمدة. ومع ذلك تحتاج هذه النتائج للتحقق منها إجراء مزيد من الدراسات الواسعة في مراكز متعددة وشوع مختلفة.

الكلمات الرئيسية: سيرات السيليديبينافيل المهبلية، إعداد بطاقة الرحم، معدل الحمل، نتائج الحمل، نقل الأجنة المذابة بعد تجميدها.