EFFECT OF VAGINAL SILDENAFIL ON ENDOMETRIAL PREPARATION AND OUTCOME IN FROZEN EMBRYO TRANSFER CYCLES IN GENERAL POPULATION: A RANDOMIZED CLINICAL TRIAL

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Abstract

Successful implantation requires good embryo quality, appropriately timed and arranged endometrial receptivity, and efficient matching between the embryo and the receptive endometrium.

The aim of the study: was to assess the effect of sildenafil citrate on improving endometrial thickness, pattern and increasing pregnancy rate of frozen embryo transfer cycles.

Materials and method: A prospective randomized clinical study was conducted in ART unit in Kasr Al Ainy hospital at Cairo University in addition to private IVF center during period between March 2019 and March 2020 and carried out on 100 infertile women divided randomly into two groups. Group A, 50 women were given oral estradiol valerate tablets 2mg 2 tablets twice daily starting from day 2 of the cycle. Group B 50 women were given the same protocol plus sildenafil 50mg vaginally every 12hours starting from day 2 of the cycle until the day of starting prontogest to be stopped 3 days before embryo transfer. Women were evaluated by using trans-vaginal sonography (TVS) on day 10 of cycle for assessment of endometrial thickness and pattern.

Results: The endometrial thickness and triple line pattern was significantly higher in women of the sildenafil citrate group when compared to those of control group ($p<0.001$). Finally, the implantation and chemical pregnancy rates were higher in the sildenafil citrate group but not significantly.

Conclusion: that Transvaginal sildenafil citrate was a significant to improve the endometrial thickness, pattern and vascularity in women undergoing frozen embryo transfer cycle and need further studies to prove value in improving pregnancy outcome.

Keywords: Sildenafil citrate, Estradiol valerate, Endometrial thickness, embryo transfer

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1. INTRODUCTION

Despite the improvement in ovarian stimulation protocols and subsequent access to a number of dominant follicles, mature oocytes, and embryos for transfer, in vitro fertilization (IVF) has successfully reached a plateau. Therefore, more attention should be focused on implantation and endometrial receptivity. Implantation remains a major limiting step of assisted reproductive technology (ART) and uterine receptivity is essential for successful implantation in all species[1].

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].

The endometrium is normally a non-receptive environment for an embryo, except during the window. Implantation window is a period during which the endometrium is optimally receptive to implanting blastocyst within the cycle days 20 and 24. It is characterized by a refractory endometrial status [3].

Endometrial receptivity during the implantation window depends on many morphological factors like

Endometrial thickness, pattern, Endometrial and sub endometrial blood flows [4].

Also, some biochemical markers may affect receptivity like endometrial adhesion molecules, endometrial anti-adhesion molecules, endometrial cytokines, Growth factors and endometrial immune markers. During implantation window, the endometrial epithelium encompasses four cell types: microvilli-rich cells, pinopode cells, vesiculated cells, and ciliated cells [5].

There are many treatment options for improving the implantation like blastocyst transfer, assisted hatching, co-culture, preimplantation genetic screening, hysteroscopy, sildenafil citrate, salpingectomy for tubal disease, antiphospholipid antibodies (APA) testing and treatment, allogenic lymphocyte therapy, and IV immunoglobin therapy[6].

One of the strongest predictors of implantation is endometrial thickness. embryo implantation and clinical pregnancy rates (PRs) are significantly higher in patients with an endometrial thickness >9mm. Thin endometrium, generally measuring <7mm, are thought to be less able to support implantation and pregnancy[7].

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[8].

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)[9].

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 (Shapiro et al., 2009). Estrogen induced endometrial proliferation is in large part dependent upon blood flow to the basal endometrium10. 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 is a potent and selective inhibitor of cGMP specific phosphodiesterase type 5 (PDE5) that prevents the breakdown of cGMP and potentiates the effect of nitric oxide on vascular smooth muscles[11].

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[12].

we aimed to Evaluate the effect of vaginal sildenafil on ultrasonographic endometrial thickness, pattern, vascularity and outcome regarding the implantation rate, chemical and clinical pregnancy rate in frozen embryo transfer cycles.

2. MATERIALS AND METHODS

A prospective randomized clinical study was conducted in ART unit in Kasr Al Ainy hospital at Cairo University in addition to private IVF center during period between March 2019 and March 2020. A total of 100 infertile women who met the inclusion criteria and their embryos were frozen were included in this study. Informed written consent was obtained from all patients involved in the study after explaining the purpose and procedures of the study.

- **Inclusion and exclusion criteria**

we included women Younger than 40 years old, undergoing their first frozen et cycle where all frozen embryos were DE frozen and transferred on 5th day and Have at least two high quality frozen embryos grade (a) based upon cleavage stage embryo development.

on the other hand, we excluded women with the history of endocrine diseases which may affect ART success rate (as DM, thyroid diseases, adrenal gland disorders), A history of hysteroscopic surgeries, uterine structural abnormalities, multiple myomas, severe intrauterine adhesions and hydrosalpinx or pyosalpinx, Cardiovascular, renal and liver diseases, Hypotension (blood pressure <90/50mmHg) and A history of stroke or myocardial infarction.

- **Intervention**

Detailed history on admission including personal, menstrual, obstetric, contraceptive, past and family history, physical examination including general, abdominal and local pelvic examination and Infertility works up and hormonal profile, Other investigations to exclude patients susceptible for side effects from adding sildenafil (as ECG , Echo , liver enzymes , kidney functions , thyroid hormones , FBS and 2hpp) and The patients of study had undergone embryo freezing for a reason such as prevention of OHSS , presence of fluid in the uterus on day of ET

- **Treatment protocols:**

A total of 100 Patients who met these conditions and entered the study were divided into two groups based on randomized tables.

Group (A): 50 women were given oral estradiol valerate tablets 2mg 2 tablets twice daily starting from day 2 of the cycle to prepare the endometrium.

Group (B): 50 women were given sildenafil 50mg vaginally every 12 hours (50mg tablet was crushed and dissolved in 2cc of distilled water and injected into vagina) starting from day 2 of the cycle,

in addition to oral 2mg of estradiol valerate 2 tablets twice daily from day 2 of the menstrual cycle.

All women were evaluated on the 10th day of the menstrual cycle; the endometrial thickness and pattern were estimated by transvaginal

ultrasonography. Endometrial morphology was classified as triple line, echogenic and intermediate.

If the endometrial thickness was more than 8mm, prontogest is started. progesterone (prontogest 400mg pessaries) was given 3 days or 5 days (according to day of embryo freezing) prior to embryo transfer. Sildenafil was discontinued 48-72 hours prior to the embryo transfer as sildenafil may have some detrimental effects on endometrium in the implantation window.

If the endometrium thickness was still below 8 mm, increasing dose of estradiol valerate might be done to become 3 tablets 2mg twice daily then assessment by transvaginal ultrasonography after 3 days.

For assessing endometrial vascularity (on day 10 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. By following Applebaum's scoring for categorizing endometrial vascularity into zones: Zone 1 vascularity when blood vessels reach the hypoechoic endometrial- myometrial junction. Zone 2 vascularity when the vessels reach the outer hyperechoic line of endometrium. Zone 3 vascularity when it reaches the internal endometrial hypoechoic area. Zone 4 vascularity when the vessels are seen reaching the endometrial cavity.

All patients had a luteal phase support by giving a daily doses of estradiol valerate 2mg oral daily and progesterone (prontogest 400mg pessary) which continued two weeks after the embryos transfer then Pregnancy test was done to assess pregnancy rate in two groups. In case BHCG was tested and proved to be positive, estradiol valerate and progesterone were continued until the 11th week of pregnancy. Then, four weeks after the embryo transfer, the number of gestational sacs was determined by vaginal ultrasound.

STATISTICAL METHODS:

Data were coded and entered using the statistical package for the Social Sciences (SPSS) version 26 (*IBM Corp., Armonk, NY, USA*). Data was summarized using mean and standard deviation for quantitative variables and frequencies (number of cases) and relative frequencies (percentages) for categorical variables. Comparisons between groups were done using unpaired t test. For comparing categorical data, Chi square (x^2) test was performed. Exact test was used instead when the expected frequency is less than 5. P- values less than 0.05 were considered as statistically significant and less than 0.01 were considered highly significant.

3. RESULTS

We approached 96 participants to join in the study. We excluded eight of them did not meet inclusion criteria, finally 88 patients included in our study, it was conducted in ART unit in Kasr Al Ainy hospital at Cairo University in addition to private IVF center.

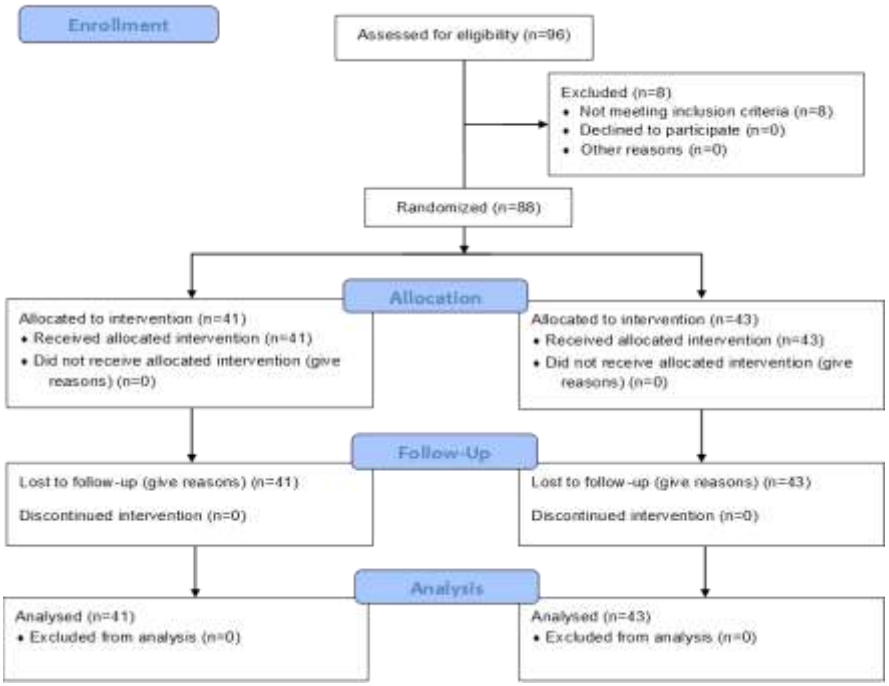

Figure 1: shows the study flow chart.

Table (1): baseline characteristics of the included studies

		Control group (41)	Sildenafil group (43)	P-value
Age		29.44±3.33	28.90±2.78	0.381
duration of infertility		3.34±1.71	2.90±0.97	0.117
N. of previous attempts		1.16±0.82	1.22±0.76	0.705
N. of transferred embryo		2.64±0.56	2.70±0.46	0.562
Duration of treatment cycle (days)		12.10±1.30	10.64±1.22	< 0.001
No. of success in previous Fresh ET cycle	**Yes**	16 (39%)	18 (42%)	0.673
	No	25 (61%)	25 (58%)	

There were no significant differences in age, duration of infertility, no of previous attempt, no of success in previous fresh embryo transfer cycles and no of transferred embryos between groups. The duration of treatment cycle was significantly shorter in women of Sildenafil group with mean (p<0.001), Sildenafil group had a higher percentage of chemical pregnancy rate without reaching statistical significance between the 2 groups. Also, implantation rate was higher in sildenafil group but not significantly **Table (1)**

Table (2): Outcome of the included studies

		Control group (41)	Sildenafil group (43)	P-value
chemical pregnancy rate	**Yes**	11(26.8%)	18(41.8%)	0.123
	No	39(95%)	32(74.5%)	
implantation rate	**Yes**	5(12.2%)	8(18.6%)	1
	No	6(14.6%)	10(23.3%)	
multiple pregnancy	**Yes**	2(5%)	2(4.65%)	0.622
	No	9(22%)	16(37.2%)	
Endometrial thickness on cycle day 10 in mm		7.44±1.2	9.30±1.16	< 0.001
endometrial pattern on cycle day 10	**Echogen**	6(14.6%)	2(4.65%)	< 0.001
	intermediate	28(68.3%)	14(32.5%)	
	triple line pattern	16(39%)	34(79%)	
endometrial vascularity on cycle day 10	**zone 1 vascularity**	22 (53.6%)	11(25.6%)	0.008
	zone 2 vascularity	12(29.3%)	8(18.6%)	
	zone 3 vascularity	16(39%)	27 (62.8%)	
	zone 4 vascularity	0	4(9.3%)	

Endometrial thickness was significantly higher in the sildenafil group (p<0.001) on cycle day 10, 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), and 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<0.05) **Table (2)**.

4. DISCUSSION

In order for successful implantation to occur, an adequately prepared endometrium has to be built up during the menstrual cycle. Endometrial development is regulated by steroid hormones and various growth factors and cytokines. Some of these factors are produced locally and act via paracrine mechanisms. Other have to be transferred to the endometrium. Sufficient blood supply is required for these factors to reach the endometrium especially the functional layer [13].

A good correlation has been found between endometrial thickness and the prevalence of conception. An endometrial thickness of ≥9mm 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 [14].

Similar study by **Kortam et al.,** [15] showed the effect of sildenafil on endometrial thickness and pregnancy rate, the mean age in the two studied groups was around 28.0 years. **Sher et al.,** [16] showed in their study effect of vaginal sildenafil on outcome of fertilization after multiple IVF failure there was no significant difference between the two groups regarding age. Regarding previous IVF

attempts in our study in control group ranged with mean value 1.16±0.82 and in sildenafil group ranged with mean value 1.22±0.76. There was no statistically significant difference between two studied groups regarding the previous IVF attempts.

Firouzabadi et al., [17] showed in their study increased uterine arterial blood flow and endometrial thickening. Which was significantly higher in the sildenafil citrate group (P<0.0001). In their study, it was shown that oral sildenafil citrate was efficient in improving endometrial receptivity. Several studies agreed to this as **Aisaka et al.,** [18]; **Takasaki et al.,** [19] and **Morad et al.,** [20], found that neither sildenafil nor estradiol improved endometrial thickness in women with poor endometrial response Regarding endometrial pattern on day 10 in our study , 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). In **Firouzabadi et al[16].**, study triple line pattern was found significantly higher with sildenafil and estradiol valerate as compared to estradiol alone and this finding is similar to that reported by **Sher et al.,** [10]. In contrast to our study, **Mangal et al.,** [21], found that the endometrial thickness and trilaminar pattern of endometrium were not significantly different in both groups Also, **Merce et al.,** [22] and **Rashidi et al.,** [23] found that there were no observed significant differences in the endometrial thickness or pattern between both groups. Regarding endometrial vascularity on day 10 by following Applebaum's scoring for categorizing endometrial vascularity into zones we found that zone 3 and zone 4 were significantly higher in sildenafil group while zone 1 and zone 2 were higher significantly in control group (p<0.05). **Malinova et al., 24** showed in their study that vaginal administration of sildenafil citrate in infertile women increased uterine blood flow and endometrial thickness. Also, **Zinger et al.,** [25] showed that vaginal administration of sildenafil citrate improved endometrial growth and pregnancy rates in patients with a thin endometrium by increasing the uterine blood flow.

Regarding pregnancy outcome in our study Sildenafil group had a higher percentage of chemical pregnancy rate (18 out of 50) 36% without reaching statistical significance between the 2 groups. Also, implantation rate was higher in sildenafil group (8 out of 18) 44.4% but not significantly the results by **Firouzabadi et al.,** [17]; **Mangal et al.,** [21]; **Sharma et al.,** [26] and **Barker et al.,** [27] agreed to higher pregnancy and implantation rates among sildenafil group.

In study carried out by **Kortam et al.,** [15] showed that chemical pregnancy occurred more in the study group than control group by 2.5 , yet the difference was not statistically significant Another study by **Kansouh et al.,** [28] showed that biochemical pregnancy rate in women of sildenafil group when compared to those of control group; the difference was statistically insignificant [15 (33.3%) vs 9 respectively, p=0.15]. From the results of this study, it is evident that adding vaginal sildenafil for endometrial preparation in frozen embryo transfer cycles can improve endometrial blood flow to enable achieving adequate endometrial growth and receptivity thus achieving a better pregnancy rate and outcome.

5. CONCLUSION

We recommend the routine use of vaginal sildenafil citrate as an adjuvant therapy in women with previous failure of assisted reproduction technology cycles due to poor endometrial thickness although this improvement in endometrial blood flow has weak positive feedback on pregnancy and implantation rates of frozen embryo transfer cycles. Further studies on larger scale patients are needed to prove value in improving pregnancy outcome and evaluate sildenafil effect on fetal outcome in pregnant women.

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