

Comparison of Endometrial Thickness with Concomitant Administration of Sildenafil Citrate and Ethinyl Estradiol vs Ethinyl Estradiol Alone for Frozen Embryo Transfer

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ABSTRACT

Introduction: Infertility is one of the common medical issues in today's world. One of the common causes of infertility is the lack of implantation of the embryo.

Aim: To compare the effect of Ethinyl Estradiol (E2) alone on Endometrial Thickness (ET) with that of sildenafil citrate and E2 combination.

Materials and Methods: In this clinical trial, 70 infertile women undergoing treatment with Assisted Reproductive Techniques (ARTs) were randomly divided into two groups of 35 each. One group received E2 and vaginal sildenafil and the other E2 alone. On the seventh day of the cycle and, if necessary, until ET reached 8 mm, ET in both groups was measured by transvaginal ultrasound. After the embryo transfer, Beta-Human Chorionic Gonadotropin (BHCG) was performed in both groups.

The measured thickness of uterus and BHCG were recorded in the checklist for both groups. Data were analysed by SPSS and $p < 0.05$ was considered significant.

Results: The mean duration (in days) to reach an 8 mm ET in infertile women in the E2+sildenafil group (13.59 ± 3.07) was lower than that in the E2 group (14.88 ± 4.78), yet insignificant ($p = 0.21$). Although the mean dose (mg) of E2 and duration (day) to reach an 8 mm ET in the E2+sildenafil group was lower than those in the E2 group, significance (p) levels were 0.02 and 0.21, respectively.

Conclusion: In infertile women undergoing ARTs, co-administration of sildenafil citrate and E2 increased ET relatively and reduced the dose of E2 and the duration of its administration.

Keywords: Endometrial thickness, Ethiniosuronidol, Infertility

INTRODUCTION

One of the most common healthcare problems that affect couples across the world, including Iran, is infertility [1-3]. It also imposes a high burden on families and the health system in developing countries [4]. To achieve successful implantation in Assisted Reproductive Technology (ART), ET should be taken into account to increase the likelihood of fertility [5,6].

Several therapies are available to bring the ET to the best possible thickness during the implantation window. For example, estrogen, by increasing blood supply to the endometrium, induces endometrial tissue proliferation and increases ET [7,8]. Sildenafil citrate also increases the uterine blood flow and, by binding to estrogen, leads to an increase in the efficacy of estrogen-induced proliferation in the endometrium lining [9]. Also, E2 is one of the popular therapies for ET [8]. However, despite the fact that there are several treatments for increasing ET, the efficacy of these treatments remains to be definitely established [8]. Therefore, the present study was done with the aim to compare the effect of E2 alone on ET with that of sildenafil citrate and E2 combination for Frozen Embryo Transfer (FET) in infertile women undergoing ARTs.

MATERIALS AND METHODS

This prospective clinical trial was conducted on 70 infertile women undergoing ARTs {In Vitro Fertilisation (IVF) and intracytoplasmic sperm injection} referred to the Infertility Clinic of Hamadan Fatemiyeh Hospital (Hamadan Endometriosis Centre). This study was begun in February 2016 and was ended in March 2017.

The protocol of the present study was registered as the IRCT201704119014N156 at the Iranian Registry of Clinical Trials. According to a study conducted in this field [10], the sample size was determined as 28 for each group according to a formula for comparing two ratios that were evaluated on the basis of the same ET, and to account for the dropout of 20% of the samples, the result was multiplied by 1.25, and therefore final sample size in each group was calculated as 35 people. Samples were entered into the study by census. The sampling was performed by randomised block consisting of 10 columns and 7 rows in which the treatment groups were randomly assigned the letters A and B, that were randomly allocated to the treatment groups by the prescribing person.

Inclusion criteria included age under 40 years, idiopathic infertility, ovulation problems that did not respond to several periods of ovulation induction and Intrauterine Insemination (IUI), fallopian tube and endometriosis problems, lack of using a Nitric Oxide (NO)-lowering drug, the presence of at least one embryo with grade A or B, and the lack of concomitant use of other ARTs. After the detection of the cause of infertility following examinations and analysis of the tests including hormone analysis, sperm analysis, and hysterosalpingography, the type of treatment for patients was selected and patients who had the indication for the use of ARTs were included in the study. The presence of hydrosalpinx in one or two fallopian tubes, having embryos with grade C or lower, and lack of providing consent to participate in the study were determined as the exclusion criteria.

The 70 patients who were included in the study were divided into two groups of 35 each; the patients in the group 1 received 25 mg vaginal sildenafil four times a day+6 mg E2 from the second or third day of the cycle, and the group 2 received only 6 mg E2; and on the seventh day, transvaginal ultrasound was performed, and according to the status of endometrium, we investigated whether ET had reached 8 mm or higher, and also the dosage of drugs was gradually increased until ET reached 8 mm or higher. The researcher who measured the ET was blind to the grouping of the patients.

Ovulation stimulation was performed by one of the appropriate protocols for the patient. After the egg was collected, IVF or microinjection was performed on oocytes and the fertilised embryos were transferred to the uterus at the appropriate time [11].

It should be noted that in order to eliminate the risk of excessive ovarian hyperstimulation syndrome, none of the patients whose number of follicles was more than 11 were included in the study. The causes of frozen embryo in our study included cervical stenosis, inappropriate endometrium, high number of foetuses, and systemic disorders [12]. The result of the treatment cycle was followed-up by performing Beta-Human Chorionic Gonadotropin (BHCG) on day 14 after embryo transfer. The data were then recorded in the checklist of the outcomes of the intervention.

STATISTICAL ANALYSIS

After data collection, the SPSS version 16 was used to analyse the data. To describe the quantitative data, mean (\pm Standard Deviation) and quadrant were used and to describe qualitative data, frequency was used. To compare average ET between the two groups, parametric t-test was used.

RESULTS

In this study, 70 infertile women undergoing ARTs were assigned to two groups of 35 each, underwent treatment with E2 and sildenafil citrate, and then studied.

There was no statistically significant difference in the number of transferred embryos, number of previous pregnancies, number of abortions, number of cycles, number of retrieved eggs, and number of fertilised eggs between the E2 group and the E2+sildenafil citrate group ($p>0.05$).

There was also no significant difference in intervention type and ART between the average ET on the seventh day of the cycle ($p=0.85$). There was no significant difference in the duration to reach an ET of 8 mm between the two groups ($p=0.83$) [Table/Fig-1].

Treatment group	Days				
	7-10	11-13	14-17	18 \leq	Total
	n (%)	n (%)	n (%)	n (%)	n (%)
E2	3 (8.6)	15 (42.8)	8 (22.9)	9 (25.7)	35 (100)
E2+Sildenafil citrate	2 (5.7)	13 (37.1)	10 (28.6)	10 (28.6)	35 (100)
Total	5 (7.1)	28 (40)	18 (25.7)	19 (27.1)	70 (100)

[Table/Fig-1]: Comparison of the duration to reach an endometrial thickness of 8 mm based on treatment. Fisher's-exact test: $\chi^2=0.62$, $p=0.083$

The mean duration (day) to reach an 8 mm ET in infertile women in the E2+sildenafil group (13.59 ± 3.07) was lower than that in the E2 group (14.88 ± 4.78), yet insignificant ($p=0.21$). The mean dose (mg) of E2 needed to reach an 8 mm ET in the E2+ sildenafil group (108.59 ± 85.42) was lower than that (131.71 ± 49.69) in the E2 group ($p=0.2$).

There was no significant difference between mean age of infertile women treated with E2 (31.54 ± 5.41) and those treated with E2+sildenafil citrate (31.34 ± 5.26) ($p=0.876$) and the two groups were matched by age. There was no significant difference in the mean ET score between infertile women treated with E2 alone and those treated with E2+ sildenafil citrate ($p>0.05$) [Table/Fig-2].

Treatment group	Age group	Number	Mean \pm SD	p-value
E2	<30	12	6.72 \pm 1.15	0.36
	\leq 30	23	5.95 \pm 1.61	
	Total	35	6.23 \pm 1.49	
E2+Sildenafil citrate	<30	11	6.57 \pm 0.95	0.96
	\leq 30	24	6.32 \pm 1.12	
	Total	35	6.64 \pm 1.07	

[Table/Fig-2]: Comparison of mean endometrial thickness score on the seventh day between the ethinyl estradiol group and the sildenafil citrate+ethinyl estradiol group by age.

For the cause of infertility in the group treated with E2, the most common cause was male factor {n: 18 (51.4%)} followed by uterus {n: 7 (20%)} and idiopathic {n: 10 (28.6%)}; and in the group treated with E2+sildenafil citrate, the most common cause was male factor {n: 21 (60%)} followed by uterus {n: 5 (14.3%)} and idiopathic {n: 9 (25.7%)}. The mean ET score on the seventh day was not significantly different between the E2+sildenafil citrate group and the E2 group ($p=0.367$) [Table/Fig-3].

Treatment group	Infertility causes	Mean \pm SD
E2	Male	6.45 \pm 1.41
	Ovarian	6.50 \pm 1.06
	Unknown	5.97 \pm 0.51
	Total	6.32 \pm 1.12
E2+Sildenafil citrate	Male	6.21 \pm 1.01
	Ovarian	6.47 \pm 1.66
	Unknown	1.29 \pm 2.49
	Total	5.93 \pm 1.61
Total	Male	6.32 \pm 1.21
	Ovarian	6.49 \pm 1.24
	Unknown	5.53 \pm 1.64
	Total	6.15 \pm 1.36

[Table/Fig-3]: Comparison of mean endometrial thickness score on the seventh day between the ethinyl estradiol group and the sildenafil citrate+ethinyl estradiol group by the cause of infertility.

There was no significant difference in the duration of infertility (year) between infertile women treated with E2 (7.83 ± 4.18) and those treated with E2+sildenafil citrate (4.12 ± 5.80) ($p=0.117$). There was also no significant difference in embryo grade between infertile women treated with E2 and those treated with E2+sildenafil citrate ($p>0.05$) [Table/Fig-4].

The number of BHCG positive cases in the sildenafil citrate+E2 group was 9 (25.7%) and in the E2 group 11 (31.4%) ($\chi^2=0.28$, $p=0.396$) [Table/Fig-5].

Embryo grade	Treatment group		p-value
	E2	E2+Sildenafil citrate	
Morella	16	13	0.94
Blast	8	3	0.34
4Cell A	2	0	0.25
4Cell B	1	0	0.75
6Cell A	0	2	0.25
6Cell B	0	0	<0.001
8Cell A	13	9	0.22
8Cell B	7	4	0.36
16Cell A	2	6	0.13
16Cell B	0	2	0.25
18Cell A	0	0	<0.001
18Cell B	0	13	0.94

[Table/Fig-4]: Comparison of embryo grade between treatment groups.

Test result	Variable levels	Treatment group		Total
		E2	E2+Sildenafil citrate	
Negative	Number	24	26	50
	Percent	68.6	74.3	71.4
Positive	Number	11	9	20
	Percent	31.4	25.7	28.6
Total	Number	35	35	70
	Percent	100	100	100

[Table/Fig-5]: Comparison of beta-human chorionic gonadotropin results in studied infertile women by treatment.

DISCUSSION

In the present study there are no effect on embryo grade and positive BHCG was observed after concomitant use of E2 and sildenafil citrate. In a study that evaluated the effect of sildenafil citrate on ET in the control and sildenafil groups, the results showed that the drug could significantly increase ET and triple-line patterns in the endometrium.

However, intermediate patterns did not differ between the two groups [13]. In another study by Malinova M et al., the results indicated that vaginal consumption of sildenafil citrate and serophene in infertile women, by increasing uterine blood flow, increased endometrial thickness and can be used as an effective treatment method for ovulation induction [14]. In another study, it was found that vaginal administration of sildenafil, in addition to a 70% increase in ET in the studied women, also caused pregnancy in infertile women to persist [15].

The study of Soliman et al., showed that thermosensitive sildenafil vaginal gel significantly improved ET and uterine blood flow (without any side effects). Sildenafil vaginal ointment also reduced the dose and duration of treatment [16]. Sildenafil also had positive outcomes with respect to increase in ET in two women with Asherman's syndrome [17]. In infertile women with lower ET, treatment with vitamin E, L-arginine, or sildenafil citrate could increase ET [18]. Sildenafil can increase the ET by inducing the vasodilator property [19]. Recently, NO synthase isoforms have been identified in the uterus and the role of NO as a uterine blood flow modulator has been identified [17]. It has been shown that NO plays a role in relaxing vascular smooth muscle through the cyclic Guanyl Monophosphate (cGMP) mediated pathway.

This drug inhibits cGMP specific phosphodiesterase type 5 (PDE5) by inhibiting cGMP degradation, thereby enhancing the effect of NO and ultimately increasing blood flow and ET [13,15]. On the other hand, implantation success depends on the ability of the blastocyst to penetrate the endometrium and to create a source of blood that requires certain genes such as Plasminogen Activator Inhibitor 1 (PAI-1), tumour suppressor factor (p53), and Vascular Endothelial Growth Factor (VEGF) for the production of proteins that are required for digestion of the endometrial cell matrix, regulation of cell growth, and induction of angiogenesis; sildenafil can enhance the angiogenesis by increasing the expression of p53 and VEGF [20-22].

In a study, it was shown that Clomiphene Citrate (CC) could have adverse effects, including decreased ET and uterine volume growth at the follicular phase. E2 neutralised these malignant effects and increased uterine development at the follicular phase [23]. Other study also found that adding vaginal E2 to CC during the stimulation period could increase ET on ovulation day and the following day. However, this increase in ET did not lead to an increase in fertility [24,25].

For the foetus to be able to attach to the endometrial wall, specific molecular and cellular changes in the endometrium, especially the luminal epithelium and its glands, should be induced. The induction is mediated by steroid hormones (oestrogen and progesterone).

Any disorder in these hormones can disrupt uterine acceptance and ultimately result in unsuccessful implantation of the foetus [26,27].

LIMITATION

Lack of examination of the effects of the drugs that were used in the study on ovulation was the limitation of the present study.

CONCLUSION

In infertile women undergoing ARTs, co-administration of sildenafil citrate and E2 increased ET relatively and reduced the dose of E2 and the duration of its administration. However, after co-administration, no effect on the embryo grade and the number of positive BHCG cases were observed.

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REFERENCES

- [1] Mascarenhas MN, Flaxman SR, Boerma T, Vanderpoel S, Stevens GA. National, regional, and global trends in infertility prevalence since 1990: a systematic analysis of 277 health surveys. *PLoS Med.* 2012;9(12):e1001356.
- [2] Hasanpoor-Azghdy SB, Simbar M, Vedadhir A. The social consequences of infertility among Iranian women: a qualitative study. *Int J Fertil Steril.* 2015;8(4):409-20.
- [3] Hasanpoor-Azghdy SB, Simbar M, Vedadhir A. The emotional-psychological consequences of infertility among infertile women seeking treatment: Results of a qualitative study. *Iran J Reprod Med.* 2014;12(2):131-38.
- [4] Dyer SJ, Patel M. The economic impact of infertility on women in developing countries a systematic review. *Facts Views Vis Obgyn.* 2012;4(2):102-09.
- [5] Habibzadeh V, Nematollahi Mahani SN, Kamyab H. The correlation of factors affecting the endometrial thickness with pregnancy outcome in the IUI cycles. *Iran J Reprod Med.* 2011;9(1):41-46.
- [6] Momeni M, Rahbar MH, Kovanci E. A meta-analysis of the relationship between endometrial thickness and outcome of in vitro fertilization cycles. *J Hum Reprod Sci.* 2011;4(3):130-37.
- [7] Satirapod C, Wingprawat S, Jultanas R, Rattanasiri S, Jirawatnotai S, Choktanasiri W. Effect of estradiol valerate on endometrium thickness during clomiphene citrate-stimulated ovulation. *J Obstet Gynaecol Res.* 2014;40(1):96-101.
- [8] Mahajan N, Sharma S. The endometrium in assisted reproductive technology: How thin is thin? *J Hum Reprod Sci.* 2016;9(1):3-8.
- [9] Fetih AN, Habib DM, Abdelalil II, Hussein M, Fetih GN, Othman ER. Adding sildenafil vaginal gel to clomiphene citrate in infertile women with prior clomiphene citrate failure due to thin endometrium: a prospective self-controlled clinical trial. *Facts, Views and Vision in ObGyn.* 2017;9(1):21-27.
- [10] Sher G, Fisch JD. Vaginal sildenafil (Viagra): a preliminary report of a novel method to improve uterine artery blood flow and endometrial development in patients undergoing IVF. *Hum Reprod.* 2000;15(4):806-09.
- [11] Kalem Z, Kalem MN, Gurgan T. Methods for endometrial preparation in frozen-thawed embryo transfer cycles. *J Turk Ger Gynecol Assoc.* 2016;17(3):168-72.
- [12] Guo YX, Yin YJ, Tian L. Freeze-all embryo transfer awareness and acceptance of IVF-ET patients in China. *Medicine.* 2016;95(47):e5171.
- [13] Dehghani Firouzabadi R, Davar R, Hojjat F, Mahdavi M. Effect of sildenafil citrate on endometrial preparation and outcome of frozen-thawed embryo transfer cycles: a randomized clinical trial. *Iran J Reprod Med.* 2013;11(2):151-58.
- [14] Malinova M, Abouyta T, Krasteva M. The effect of vaginal sildenafil citrate on uterine blood flow and endometrium in the infertile women. *Akush Ginekol (Sofia).* 2013;52(Suppl 1):26-30.
- [15] Sher G, Fisch JD. Effect of vaginal sildenafil on the outcome of in vitro fertilization (IVF) after multiple IVF failures attributed to poor endometrial development. *Fertil Steril.* 2002;78(5):1073-76.
- [16] Soliman GM, Fetih G, Abbas AM. Thermosensitive bioadhesive gels for the vaginal delivery of sildenafil citrate: in vitro characterization and clinical evaluation in women using clomiphene citrate for induction of ovulation. *Drug Dev Ind Pharm.* 2017;43(3):399-408.
- [17] Zinger M, Liu JH, Thomas MA. Successful use of vaginal sildenafil citrate in two infertility patients with Asherman's syndrome. *J Womens Health (Larchmt).* 2006;15(4):442-44.
- [18] Takasaki A, Tamura H, Miwa I, Taketani T, Shimamura K, Sugino N. Endometrial growth and uterine blood flow: a pilot study for improving endometrial thickness in the patients with a thin endometrium. *Fertil Steril.* 2010;93(6):1851-58.
- [19] Hale SA, Jones CW, Osol G, Schonberg A, Badger GJ, Bernstein IM. Sildenafil increases uterine blood flow in nonpregnant nulliparous women. *Reprod Sci.* 2010;17(4):358-65.
- [20] Di X, Gennings C, Bear HD, Graham LJ, Sheth CM, White KL, Jr., et al. Influence of the phosphodiesterase-5 inhibitor, sildenafil, on sensitivity to chemotherapy in breast tumour cells. *Breast Cancer Res Treat.* 2010;124(2):349-60.
- [21] Pyriochou A, Zhou Z, Koika V, Petrou C, Cordopatis P, Sessa WC, et al. The phosphodiesterase 5 inhibitor sildenafil stimulates angiogenesis through a protein kinase G/MAPK pathway. *J Cell Physiol.* 2007;211(1):197-204.

- [22] Goodman C, Jeyendran RS, Coulam CB. P53 tumour suppressor factor, plasminogen activator inhibitor, and vascular endothelial growth factor gene polymorphisms and recurrent implantation failure. *Fertil Steril*. 2009;92(2):494-98.
- [23] Yagel S, Ben-Chetrit A, Anteby E, Zacad D, Hochner-Celnikier D, Ron M. The effect of ethinyl estradiol on endometrial thickness and uterine volume during ovulation induction by clomiphene citrate. *Fertil Steril*. 1992;57(1):33-36.
- [24] Çetinkaya K, Kadanali S. The effect of administering vaginal estrogen to clomiphene citrate stimulated cycles on endometrial thickness and pregnancy rates in unexplained infertility. *J Turk Ger Gynecol Assoc*. 2012;13(3):157-61.
- [25] Gerli S, Gholami H, Manna C, Di Frega AS, Vitiello C, Unfer V. Use of ethinyl estradiol to reverse the antiestrogenic effects of clomiphene citrate in patients undergoing intrauterine insemination: a comparative, randomized study. *Fertil Steril*. 2000;73(1):85-89.
- [26] Bartel C, Tichy A, Schoenkypf S, Aurich C, Walter I. Effects of steroid hormones on differentiated glandular epithelial and stromal cells in a three dimensional cell culture model of the canine endometrium. *BMC Vet Res*. 2013;9:86.
- [27] Singh MM, Chauhan SC, Trivedi RN, Maitra SC, Kamboj VP. Correlation of pinopod development on uterine luminal epithelial surface with hormonal events and endometrial sensitivity in rat. *Eur J Endocrinol*. 1996;135(1):107-17.

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