

## Original Article



# Effect of vaginal estrogen cream on endometrial thickness in women under embryo transfer by applying long protocol method

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## Abstract

**Background and aims:** Endometrial thickness (ET) is one of the main parameters in the success of assisted reproductive technology (ART) methods, which is influenced by sex steroids. This study investigated the effect of vaginal estrogen cream on ET in women undergoing embryo transfer.

**Methods:** In this clinical trial, 100 infertile women candidates for embryo-fetal transfusion were enrolled in the study by a simple sampling method and then randomly divided into two treatment groups. For patients in group A, estradiol tablets were administered alone (control) and group B received the estradiol plus vaginal estrogen cream (intervention). The thickness of the endometrium was measured by ultrasound in both groups and recorded in the checklist. The rate of positive bHCG, pregnancy, and canceled cycles was evaluated in both groups as well. Finally, the data were analyzed using SPSS software.

**Results:** Both groups were matched in terms of age ( $P=0.129$ ). There was no significant difference between the groups regarding ET ( $P=0.651$ ) and bHCG frequency ( $P=0.418$ ) and the pregnancy rate was similar in two groups. Based on the results, the frequency of the canceled cycles was 32% and 50% in intervention and control groups ( $P=0.031$ ). Eventually, no significant relationship was observed between pregnancy outcome (0.637) and the bHCG (0.553) test with ET.

**Conclusion:** Overall, the administration of vaginal estrogen cream has no effect on ET and pregnancy rate in women under embryo transfer by applying the long protocol method in patients who referred to endometrium and endometriosis research center but it significantly reduces the eliminated cycles.

**Keywords:** Infertility, Embryonic transfer, Estradiol

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## Introduction

Infertility is one of the common health problems that affects many people worldwide (1) and imposes stupendous costs for women, their families, and health care systems (2). In addition, it is a prevalent disorder which is attributed to several consequences such as exclusion, aggression, social stigma, and emotional-psychological problems that ultimately have negative effects on the quality of life (3-5). Further, infertility is a multi-factorial disorder and thin endometrium is considered as one of its important causes (6). The thin endometrium is responsible for early miscarriages or implantation failure due to the lack of blood supply (7). Endometrial thickness (ET) is one of the factors that plays an important role in infertility (8). According to Mahajan and Sharma (9), women with <7 mm ET at the time of embryo implantation are categorized into suboptimal in assisted reproductive

technologies (ARTs). Given successful implementation in ARTs, ET should be taken into account to increase fertility likelihood (8,10) since it is under-recognized by fertility specialists in treating infertility (11). Hormone-based treatments are effective and regarded as a side-effect-free approach for treating thin ET in folliculogenesis and ovulation (12). However, low-dose estrogen-progestin therapy was not associated with significant changes in ET (13). On the other hand, specific molecular and cellular changes in the endometrium are needed for the ability to attach the embryo to the endometrial wall, especially the luminal epithelium. Furthermore, the lining should be made and these changes must be induced by steroid hormones (i.e., estrogen and progesterone). Any anomaly in these hormones can disrupt uterine admission, and ultimately, result in fetal implantation (14). Although there are several treatments for increasing ET, the efficacies

of these treatments require further discussion (15). Most studies in this area are retrospective, and this causes bias and prejudice during the comparisons. Likewise, such studies failed to consider the preferences of infertile patients thus it is necessary to conduct randomized controlled studies (16-18). Therefore, the aim of this study was to evaluate the effect of conjugated estrogens vaginal cream on ET in women who referred to the endometrial and endometriosis research center and underwent in-vitro fertilization and ET with long-term protocols.

**Materials and Methods**

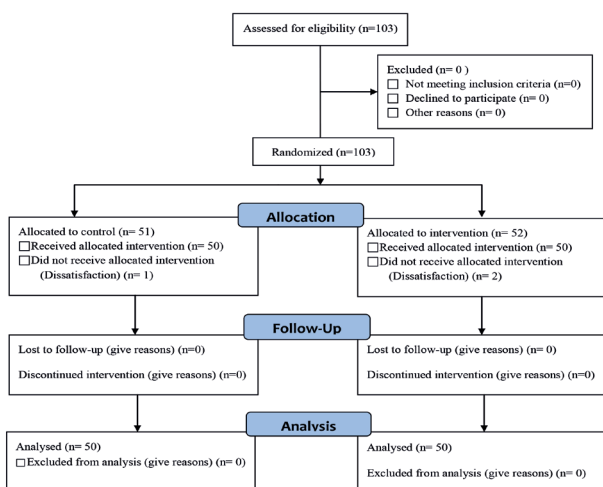
In this clinical trial study, 100 infertile women referring to endometrial and endometriosis research center for frozen fetal transmission were selected and randomly divided into two treatment groups. The inclusion criteria included infertile women with infertility and endometrial problems, the lack of underlying disease, no contraindication for estradiol usage, the absence of uterine problems such as myoma, polyps and adhesion, and finally, patient satisfaction with participation in the study.

The exclusion criteria were embryo transfer more than three times, which represented an underlying problem but was not related to the treatment method and the patient’s reluctance to continue the treatment. Totally, 100 subjects were randomly assigned to intervention and control groups (Figure 1).

The sample size was calculated according to the sample size formula. For the comparison of the mean in two societies, the values of standard deviation and the mean of Khadem and Ensafi (19) were computed according to the alpha of 0.05 and type 2 error was considered at 20%, and finally, 50 patients were assigned to each group. Moreover, the simple and purposeful sampling method was utilized to select the samples and the sample size continued till saturation.

Additionally, the randomization method was done by 100 envelopes with their inner papers, 50 of which consisted of the therapeutic A and the remaining 50 ones were considered as the B treatment group. The envelopes were provided to the gynecologist and the specialist randomly selected and opened an envelope if she was eligible to enter the study. According to the type of treatment which was recorded in the envelopes, the patient’s name was given to the researcher. In group A, only an oestradiol tablet was prescribed so that patients started on the third day of menstruation, started estradiol valerate tablets, and every three days, the thickness of the endometrium was measured by the ultrasound. In some patients, researchers increased the dose if necessary. Ultrasound was performed with a vaginal probe by a person who was not aware of the type of intervention. Thus, the thickness of the endometrium was 1 cm lower than the uterine fundus by the end-to-end method. When the drug was consumed for at least 11 days and the ET exceeded 8mm, the progesterone was started for the patient and the embryo was transferred according to the stage of division. In B group, in addition to estradiol tablets, the conjugated estrogens vaginal cream was used vaginally after 1-2 days of menstruation and discontinued after embryo transfer. Additionally, the ET was calculated by ultrasound before embryo transfer, and the success of pregnancy after embryo transfer was recorded for two groups and in the checklist which was designed for this purpose. The checklist included demographic characteristics, ET checklist, and intervention outcomes.

After data gathering, SPSS, version 16 was used for statistical analysis. In addition, mean and standard deviation, as well as frequency and table were applied to describe quantitative and qualitative data. Further, a t-test was employed to compare the mean ET in the two groups. Finally, the Chi-square test was used to compare the relationship between pregnancy success rate,



**Figure 1:** Progress through the phases of a parallel randomized trial of two groups

**Table 1.** Comparison of the mean (SD) of Age among infertile women in intervention and control groups

Variable	Group	Number	Mean (SD)	P value
Age	A	52	29.18±5.40	0.129
	B	51	30.82±5.30	
ET	A	50	9.04±1.46	0.651
	B	50	9.17±1.49	

Note:SD: Standard deviation.

**Table 2.** Comparison of positive bHCG frequency in the two groups

bHCG	Scale	Group		P-value
		A	B	
Negative	No.	33	31	0.418
	%	66.0	62.0	
Positive	No.	17	19	
	%	34.0	38.0	
Total	Number	50	50	

Note. HCG: Human chorionic gonadotropin.

positive bHCG, and the abnormal number of cycles and type error was considered as 0.05 in this study.

## Results

In this study, 100 patients were divided into control (A) and intervention (B) groups and received the estradiol tablet, as well as conjugated estrogens vaginal cream + estradiol tablets, respectively. The results demonstrated no significant difference between the two groups regarding the age and ET (Table 1).

The results of the comparison of positive bHCG frequency in the two groups indicated that there was no significant difference between the two groups (Table 2). Similarly, the pregnancy rate was similar between the two groups (Table 3).

As shown in Table 4, the number of canceled cycles in the control group was significantly higher than that in the intervention group ( $P=0.031$ ).

Based on the results of Table 5, no significant relationship was found between pregnancy outcome and bHCG test with ET.

## Discussion

This study aimed to investigate the effect of conjugated estrogens vaginal cream on ET in infertile women. In this study, although the mean ET was higher in the intervention group, this difference was not statistically significant. On the other hand, the number of canceled cycles in the intervention group was significantly lower than that in the control group. In addition, in a study aimed at comparing oral and vaginal estrogen usage in inadequate endometrial patients for frozen-thawed blastocysts transfer, applying vaginal estrogen required more days and higher dosage, but it had thinner endometrium on the day of the transfer (20). Rinaldi et al (21) in their study regarding determining

the effect of predicting ET in pregnancy after in vitro fertilization (IVF) and intracytoplasmic sperm injection, concluded that ET was a predictive variable in IVF fertility assisted reproduction, which is inconsistent with the results of the present study since there was no statistically significant difference between successful and unsuccessful pregnancies and positive bHCG in terms of ET. In another study, the results showed that extended estrogen therapy for 14 to 82 days can increase the pregnancy rate. Therefore, extended estrogen administration followed by frozen-thawed IVF protocol is beneficial for patients with a thin endometrium (22). However, the conception rate demonstrated no significant difference in both groups in this study.

In a clinical trial conducted by Khadem and Ensafi (19) on infertile women who were candidates for intrauterine insemination, the results of the treatment of clomiphene with ethinyl estradiol compared to clomiphene alone showed an increase in the frequency of pregnancy, while there was a reduction in the number of eliminated cycles. However, as regards abortion in the first- and third-trimester abortion, the results represented no significant effect. The number of abortions was not surveyed in our study. The result of the above-mentioned study in which the number of eliminated cycles was in the intervention group and less compared to the control group, is in line with the findings of our study. Nevertheless, there was no statistically significant difference between the two groups in terms of the number of pregnancies and the eliminated cycles.

Likewise, Kyrou et al, in their study regarding the effect of vaginal estrogen and progesterone supplements on the success rate of pregnancy by HCG-induced natural frozen-thawed embryo transfer cycles, reported that there was no significant difference between the success rate of pregnancy in the estrogen receptor group with different doses and progesterone group (23), which corroborates with the results of the present study indicating no correlation between ET and pregnancy success. In addition, Parnan Emamverdikhani et al compared two treatment methods of vitamin E suppository and conjugated estrogens vaginal cream on the quality of life of menopausal women with vaginal atrophy. Based on their results, there was no significant difference between the two groups based on vitamin E suppository and estrogen cream therapy

**Table 3.** Comparison of Pregnancy Rate in the Two Groups

Group	Variable Levels	Pregnancy Outcome		Total
		Successful	Unsuccessful	
Group A	No.	17	33	50
	%	34.0	66.0	100
Group B	No.	17	33	50
	%	34.0	66.0	100
Total	No.	34	66	100
	%	35	65	100

**Table 4.** Comparison of the number of canceled cycles due to the inappropriate growth of endometrium in two groups

Group	Variable Levels	Number of Canceled Cycles			Total
		0	1	2	
Group A	No.	25	17	8	50
	%	50	34.0	16.0	100
Group B	No.	34	15	1	50
	%	68.0	30.0	2.0	100
Total	No.	59	32	9	100
	%	59.0	32.0	9.0	100

**Table 5.** Comparison of the mean ET in terms of the success of ART

Success Standard	Scale	Mean (SD)	P value
bHCG	Positive	9.21±1.90	0.553
	Negative	9.00±1.21	
Pregnancy	Positive	9.18±1.85	0.637
	Negative	9.01±1.22	

Note. ET: Endometrial thickness; ART: Assisted reproductive technology; SD: Standard deviation; HCG: Human chorionic gonadotropin.

(24). Applying hormone replacement therapy, the individualization of various estrogen treatment types and amounts must be done depending on the patient's specific needs (25). Accordingly, differences in patient population and characteristics, low dose administration, treatment adherence, and low sample size in this study can be due to such contradictions. Further, the rate of clinical pregnancy in infertile patients was correlated with the number of good-quality embryos which were transferred in both frozen-thawed and fresh embryo transfer cycles (26).

It seems that the type of estrogen administration and its dose can have a direct effect on the success rate of fertility and its outcomes (27). On the other hand, estrogen can increase the ET even in low doses with no side effect and it can be well-tolerated and reduced when observing the symptom of vaginal atrophy. In fact, estrogens vaginal cream increases endometrial proliferation (28-30). It is assumed that endometrial cancer risk elevation is attributed to intravaginal estrogen and thus some researchers recommend periodic progesterone instead of vaginal estrogen (31). Moreover, abnormal placentation and periportal complication must be considered in estrogen therapy (22). Therefore, clinicians should be aware of the potential risks to women at high risk.

## Conclusion

In general, the administration of vaginal estrogen cream had no effect on ET and pregnancy rate in women under embryo transfer by applying long protocol methods in patients who referred to endometrium and endometriosis research center, whereas it significantly reduced the eliminated cycles. Therefore, it is recommended that future studies consider different doses and types of drug administration in order to determine the effect of vaginal estrogen on the ET.

## Conflicts of interests

The authors have no conflict of interests.

## Ethical Considerations

The study protocol was approved by the Ethics Committee of Hamadan University of Medical Sciences (number: IR.UMSHA.REC.1395.251).

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