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

Mahnaz Yavangi¹, Soghra Rabiei²*

¹Department of Gynecology, Endometrium and Endometriosis Research Center, Hamadan University of Medical Sciences, Hamadan, Iran
²Endometrium and Endometriosis Research Center, Hamadan University of Medical Sciences, Hamadan, Iran

*Corresponding Author: Soghra Rabiei: Endometrium and Endometriosis Research Center, Fatemieh Hospital, Hamadan University of Medical Sciences, Hamadan, Iran. Email: rabieesogol@yahoo.com

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

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,

| 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.

| bHCG | Scale | Group | P-value |
|------|-------|-------|---------|
|      |       | A     | B       |
| Negative | %   | 33    | 31      | 0.418   |
| Positive  | %   | 66.0  | 62.0    |         |
| Total     | Number | 50    | 50      |         |

Note. HCG: Human chorionic gonadotropin.
positive bHCG, and the abnormal number of cycles and type I 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 Emamverdikhan 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 |
|-------|-----------------|-------------------|-------|
|       | No. | %     | Successful | Unsuccessful |       |
| Group A | 17  | 34.0  | 33         | 50          |       |
| Group B | 17  | 34.0  | 33         | 50          |       |
| Total  | 34  | 35.0  | 66         | 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 |
|-------|-----------------|---------------------------|-------|
|       | No. | %     | 0 | 1 | 2 |     |
| Group A | 25  | 50.0  | 17 | 16.0 | 8 | 50 |
| Group B | 34  | 68.0  | 15 | 2.0  | 1 | 50 |
| Total  | 59  | 59.0  | 32 | 9.0  | 9 | 100 |
Mean (SD) 9.21±1.90 9.00±1.21 P value 9.18±1.85

Acknowledgments
Hereby, we gratefully thank the Research and Technology Deputy of the Hamedan University of Medical Sciences and all people who assisted us in conducting this study. This article was obtained from a research project approved at the Research and Technology Deputy of the Hamedan under No. 9606143760.

References
1. Polis CB, Cox CM, Tunçalp Ö, McLain AC, Thoma ME. Estimating infertility prevalence in low-to-middle-income countries: an application of a current duration approach to Demographic and Health Survey data. Hum Reprod. 2017;32(5):1064-74. doi: 10.1093/humrep/dex025.
2. Dyer SJ, Patel M. The economic impact of infertility on women in developing countries: a systematic review. Facts Views Vis Obgyn. 2012;4(2):102-9.
3. Hasanpoor-Azhghdy SB, Simbar M, Vedadhiri A. The social consequences of infertility among Iranian women: a qualitative study. Int J Fertil Steril. 2015;8(4):409-20.
4. Hasanpoor-Azhghdy SB, Simbar M, Vedadhiri A. The emotional-psychological consequences of infertility among infertile women seeking treatment: results of a qualitative study. Iran J Reprod Med. 2014;12(2):131-8.
5. Namdar A, Naghizadheh MM, Zamani M, Yaghmaei F, Sameni MH. Quality of life and general health of infertile women. Health Qual Life Outcomes. 2017;15(1):139. doi: 10.1186/s12955-017-0712-y.
6. Baradwaj S, Shafi D, Baradwan A, Bashir MS, Al-Jaroudi D. The effect of endometrial thickness on pregnancy outcome in patients with Asherman’s syndrome post-hysteroscopic adhesiolysis. Int J Womens Health. 2018;10:77-82. doi: 10.2147/ijwh.s151283.
7. Tissava T, Sheterov A, Kyurkchiev S. Recurrent implantation failure: the role of the endometrium. J Reprod Infertil. 2014;15(4):173-83.
8. Habibzadeh V, Nematalahi 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):41-6.
9. Mahajan N, Sharma S. The endometrium in assisted reproductive technology: How thin is thin? J Hum Reprod Sci. 2016;9(1):3-8. doi: 10.4103/0974-1208.178632.
10. Yavangi M, Rabiee S, Nazari S, Farimani-Sanoei M, Amir Z, Bahmanzadeh M, et al. Comparison of the effect of oestrogen plus Foeniculum vulgare seed and oestrogen alone on increase in endometrial thickness in infertile women. J Clin Diagn Res. 2018;12(1):QC1-QC4. doi:10.7860/JCDR/2018/30164.11020.
11. Wolff EF, Vahidi N, Alford C, Richter K, Widra E. Influences on endometrial development during intrauterine insemination: clinical experience of 2,929 patients with unexplained infertility. Fertil Steril. 2013;100(1):194-9.e1. doi: 10.1016/j.fertnstert.2013.03.023.
12. Satirapod C, Wingrawat S, Jultannamas 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. doi: 10.1111/jog.12130.
13. Christodoulakos GE, Botsis DS, Lambrinoudaki IV, Papagianni VD, Panoulis CP, Creatsa MG, et al. A 5-year study on the effect of hormone therapy, tibolone and raloxifene on vaginal bleeding and endometrial thickness. Maturitas. 2006;53(4):413-23. doi: 10.1016/j.maturitas.2005.07.003.
14. Zhang S, Lin H, Kong S, Wang S, Wang H, Wang H, et al. Physiological and molecular determinants of embryo implantation. Mol Aspects Med. 2013;34(5):939-80. doi: 10.1016/j.mam.2012.12.011.
15. Ye Q, Zhang Y, Fu J, Zou Y, Zhao W, Chen C, et al. Effect of Ligustrazine on Endometrium Injury of Thin Endometrium Rats.
Evidence-based complementary and alternative medicine: eCAM. 2019;2019:7161906. doi: 10.1155/2019/7161906.

16. Kalem Z, Kalem MN, Gürgan T. Methods for endometrial preparation in frozen-thawed embryo transfer cycles. J Turk Ger Gynecol Assoc. 2016;17(3):168-72. doi: 10.5152/jtggaj2016.15214.

17. Dal Prato L, Borini A, Cattoli M, Bonu MA, Sciajno R, Flamigni C. Endometrial preparation for frozen-thawed embryo transfer with or without pretreatment with gonadotropin-releasing hormone agonist. Fertil Steril. 2002;77(5):956-60. doi: 10.1016/s0015-0282(02)02960-6.

18. Groenewoud ER, Cantineau AE, Kollen BJ, Macklon NS, Cohlen BJ. What is the optimal means of preparing the endometrium in frozen-thawed embryo transfer cycles? a systematic review and meta-analysis. Hum Reprod Update. 2013;19(5):458-70. doi: 10.1093/humupd/dmt030.

19. Khadem N, Ensafi P. Modulating the antiestrogenic effects of clomiphene citrate by ethinyl estradiol in patients undergoing intrauterine insemination. J Reprod Infertil. 2003;4(2):129-36. [Persian].

20. Liao X, Li Z, Dong X, Zhang H. Comparison between oral and vaginal estrogen usage in inadequate endometrial patients for frozen-thawed blastocysts transfer. Int J Clin Exp Pathol. 2014;7(5):2533-9. doi: 10.2147/ijcpd.v7i5.10318.

21. Rinaldi L, Lisi F, Floccari A, Lisi R, Pepe G, Fishel S. Endometrial thickness as a predictor of pregnancy after in-vitro fertilization but not after intracytoplasmic sperm injection. Hum Reprod. 1996;11(7):1538-41. doi: 10.1093/oxfordjournals.humrep.a019434.

22. Chen MJ, Yang JH, Peng FH, Chen SU, Ho HN, Yang YS. Extended estrogen administration for women with thin endometrium in frozen-thawed in-vitro fertilization programs. J Assist Reprod Genet. 2006;23(7-8):337-42. doi: 10.1007/s10815-006-9053-1.

23. Kyrou D, Fatemi HM, Popovic-Todorovic B, Van den Abbeel E, Canus M, Devooye P. Vaginal progesterone supplementation has no effect on ongoing pregnancy rate in hCG-induced natural frozen-thawed embryo transfer cycles. Eur J Obstet Gynecol Reprod Biol. 2010;150(2):175-9. doi: 10.1016/j.ejogrb.2010.02.038.

24. Parnan Emamverdikhan A, Golmakani N, ShariﬁSistani N, Taghi Shakeri M, Hasanzade Mofrad M, Sajadi Tabassi A. Comparing two treatment methods of vitamin E suppository and conjugated estrogen vaginal cream on the quality of life in menopausal women with vaginal atrophy. J Midwifery Reproductive Health. 2014;2(4):253-61. doi: 10.22038/jmrh.2014.3246.

25. Krause M, Wheeler TL 2nd, Snyder TE, Richter HE. Local effects of vaginally administered estrogen therapy: a review. J Pelvic Med Surg. 2009;15(3):105-14. doi: 10.1097/SPV.0b013e3181ab4804.

26. Liu SM, Zhou YZ, Wang HB, Sun ZY, Zhen JR, Shen K, et al. Factors associated with effectiveness of treatment and reproductive outcomes in patients with thin endometrium undergoing estrogen treatment. Chin Med J (Engl). 2015;128(23):3173-7. doi: 10.4103/0366-6999.170258.

27. Shahrokh Tehraninejad E, Kabodmehri R, Hosein Rashidi B, Jafarabadi M, Keikha F, Masomi M, et al. Trans dermal estrogen (oestrogel) for endometrial preparation in freeze embryo transfer cycle: An RCT. Int J Reprod Biomed (Yazd). 2018;16(1):51-6.

28. Lindahl SH. Reviewing the options for local estrogen treatment of vaginal atrophy. Int J Womens Health. 2014;6:307-12. doi: 10.2147/ijwh.s52555.

29. Shah M, Karena Z, Patel SV, Parmar N, Singh PK, Sharma A. Treatment of vaginal atrophy with vaginal estrogen cream in menopausal Indian women. Oman Med J. 2017;32(1):15-9. doi: 10.5001/omj.2017.03.

30. Davar R, Janati S, Mohseni F, Khabazkhoob M, Asgari S. A comparison of the effects of transdermal estradiol and estradiol valerate on endometrial receptivity in frozen-thawed embryo transfer cycles: a randomized clinical trial. J Reprod Infertil. 2016;17(2):97-103.

31. Krause M, Wheeler TL 2nd, Richter HE, Snyder TE. Systemic effects of vaginally administered estrogen therapy: a review. Female Pelvic Med Reconstr Surg. 2010;16(3):188-95. doi: 10.1097/SPV.0b013e3181d7e86e.