Original Article

ANATOLIAN CURRENT MEDICAL

DOI: 10.38053/acmj.1209514

Anatolian Curr Med J 2023; 5(1); 71-75

Effects of sequential and fixed-dose estradiol valerate administration on premature progesterone rise in frozen-thawed embryo transfer cycles

Ayşe Şeyma Küçükakça[1], Mehmet Ağar[2], Nur Dokuzeylül Güngör [3], Tuğba Gürbüz[4]

[1]Department of Gynecology and Obstetric Clinic, İstanbul Medipol University Çamlıca Hospital, İstanbul, Turkey
[2]Private Clinic, Şanlıurfa, Turkey
[3]Department of Obstetrics and Gynecology IVF Clinic, Bahçeşehir University Göztepe Medikal Park Hospital, İstanbul Turkey
[4]Gynecology and Obstetric Clinic, Medistate Hospital, İstanbul, Turkey

Cite this article as: Küçükakça AŞ, Ağar M, Dokuzeylül Güngör N, Gürbüz T. Effects of sequential and fixed-dose estradiol valerate administration on premature progesterone rise in frozen-thawed embryo transfer cycles. Anatolian Curr Med J 2023; 5(1); 71-75.

ABSTRACT

Aim: This study investigated the risk of premature progesterone (P4) rise in the fixed and sequential estradiol valerate (EV) administration for frozen embryo transfer (FET) cycles.

Material and Method: In this cross-sectional case-control study, 1272 cycles of FET were analyzed retrospectively from computer records between January 2015 to August 2020. EV was administrated in 795 patients with a fixed dose and in 477 patients with a sequential dose. P4 values were measured on the day when the endometrial thickness reached 8 mm in the patients.

Results: There were 795 patients in the fixed EV administration group with a mean age of 30.75±3.39 and 477 patients in the sequential EV administration with a mean age of 30.75±3.39. P4 of the sequential-dose group (1.05±0.31) was significantly higher than the fixed-dose group (1.01±0.33). The Pairwise Z-Tests found that the abort rate was significantly higher in the sequential-dose group (p=0.04).

Conclusion: Our results showed a higher P4 and abortion rate in the sequential-dose group. These findings show that premature P4 rise can be considered a risk factor.

Keywords: Estradiol valerate, progesterone, FET

INTRODUCTION

Some infertile couples use in vitro fertilization (IVF) methods to get pregnant, but the implantation rate is still low, even with the transfer of apparently healthy embryos (1). The estradiol (E2) level directly affect the maturation of the oocyte or embryo (2). A high E2 level in the follicular phase has been associated with an increase in the harvest of fertile eggs (3). The effect of high levels of E2 on the outcome of the use of assisted reproductive technologies is still debatable.

In the conducted studies, the harmful effect of high E2 on the receptivity of the endometrium has been proposed (4,5); however, in some studies, this negative effect has not been reported (6,7). The role of E2 in the follicular phase, including the proliferation of stroma and glandular epithelial vessels in the endometrial tissue, has been discussed (5,6). In addition, E2 causes the synthesis of specific proteins, growth factors, estrogen (E), and P4 receptors (3). Although the role of P4 in the implantation of the early stages of pregnancy is crucial (5), the role of E2 in the luteal phase is still not well defined.

In response to the successive release of E and P4 from the ovary, the endometrium proliferates and prepares it for embryo implantation. The changes in endometrial vessels occurs following the coordination between the actions of E and P4, which causes the adequate blood supply to the endometrium to accept the pregnancy (8). Subendometrial and endometrial vascularity has significantly decreased in women with unexplained infertility (6,7). The junction of the endometrium and myometrium is rich in blood vessels and plays a significant role in embryo implantation (9). The effects of E and P4 on the hemodynamics of this region have not been studied so far.

Corresponding Author: Tuğba Gürbüz, drtgurbuz@hotmail.com

Received: 24.11.2022 **Accepted:** 28.12.2022

Both P4 and E2 are necessary to prepare the endometrium for blastocyst implantation and successful pregnancy (6,7). In the cycles following IVF, more mature follicles are formed due to the use of ovarian stimulating drugs, so the level of these hormones can be higher than the physiological state. This increase in level can cause concern about luteal phase disorders and uterine tissue changes (7). E2 first causes hyperplasia and hypertrophy of endometrial epithelial cells (9), but its role in the luteal phase is unclear (8). Although the role of E2 in the coordination and implantation of blastocyst is not clear, the role of P4 in the luteal phase is better defined, and it has been shown that lumpectomy before the seventh week of pregnancy leads to abortion in most cases (10). P4 causes the endometrium prepared with E2 to become secreted tissue and provides an environment ready for ET to uterine tissue (11). Although research has been conducted on the effects of E2 and P4 on implantation, there is little research on their levels in the early luteal phase.

This study aimed to compare the effects of sequential and fixed-dose EV administration on P4 values measured on the day when the sufficient endometrial thickness is reached in frozen-thawed embryo transfer (FET) cycles.

MATERIAL AND METHOD

This study was carried out with the permission of Beykoz University Research and Project Development Ethics Committee (Date: 26.10.2020, Decision No:1). In this cross-sectional case-control study, 1272 cycles of FET were analyzed retrospectively from computer records between January 2015 to August 2020. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

In the fixed dose method, 3*2 mg of estradiol valerate (known as estrofem trade name) was used. In the sequential method, 2*2 mg was used for the first 3 days and increased to 3*2 mg in the following days. Estradiol valerate support was continued until the 7th week of pregnancy.

EV was administrated in 795 patients with a fixed dose and in 477 patients with a sequential dose. Therefore, participants were divided into two groups of fixed and sequential doses of EV. Luteinizing hormone (LH) and P4 values were measured on the day when the endometrial thickness reached 8 mm in the patients. The inclusion criteria of patients were as follows: 1) be between 20-35 years old; 2) not having an endometrial factor; 3) A body mass index (BMI) between 18-30. The exclusion criteria for patients were as follows: 1) the presence of an endometrial factor; 2) the presence of other chronic diseases; 3) A BMI of more than 30. Women between the ages of 25 and 39 were included in this study.

Statistical Analysis

The Kolmogorov-Smirnov test was performed to check the normality, and the nonparametric tests were performed given the groups' non-normality before the statistical analyses. Mean and standard deviations (SD) were measured to check each continuous variable, including Age, BMI, total oocytes, MII oocytes, pronuclei (PN), anti mullerian hormone (AMH), prolactin (PRL), free T4 (FT4), thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), LH, E2, endometrial thickness, and P4. The Mann-Whitney U test was performed to study the difference between the two groups. SPSS v22 was used for statistical analyses. A value of $p<0.05$ was accepted as statistically significant.

RESULTS

This study included 1272 age-matched (30.47±3.47) and BMI-matched (23.93±2.21) participants. There were 795 patients in the fixed EV administration group with a mean age of 30.75±3.39 and 477 patients in the sequential EV administration with a mean age of 30.75±3.39. **Table 1** shows the descriptive statistics of maternal characteristics and laboratory parameters.In the present study, we compared laboratory parameters between two groups. We assessed the capability of those parameters to differentiate between sequential and fixed-dose EV administration on premature P4 rise in FET cycles.

Table 1. Descriptive statistics of study parameters in women (n=1272)

Study parameters	Median (range) mean ± SD
Maternal characteristics	
Age	32 (20-35) 30.47±3.47
BMI	24 (19.8-29) 23.93±2.21
Laboratory parameters	
Total oocytes	2 (1-7) 2.76±1.43
MII oocytes	9 (7-16) 10.05±2.37
PN	8 (6-15) 8.89±2.34
AMH	8 (6-13) 8.41±1.94
PRL	15 (8.48-25) 16.92±5.54
FT4	0.99 (0.31-1.62) 1.03±0.28
TSH	1.16 (0.63-2.46) 1.47±0.54
FSH	7.83 (4-12) 7.59±1.48
LH	7 (3.52-13) 6.93±1.53
E2	40 (30-51.2) 40.12±6.66
Endometrial thickness	9 (9-12) 9.9±1.05
Progesterone	0.96 (0.31-4) 1.02±0.32

SD, standard deviation. BMI, body mass index; PN, multi-pronuclei; AMH, Anti-Mullerian hormone; PRL, prolactin ; FT4, Free T4; TSH, thyroid-stimulating hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2; Estradiol.

Table 2 shows the comparison of the study parameters of the two groups. As stated in **Table 2**, a Mann-Whitney test did not find a statistically significant association between the two treatment groups regarding total and MII oocytes (p>0.05). No significant difference was observed between the two treatment groups regarding PN, PRL, and FT4 (p>0.05). The serum AMH, TSH, FSH, LH, and E2 levels were similar between the two treatment groups (p>0.05). There was no statistically significant difference between groups in terms of E2 and endometrial thickness (p>0.05). P4 of the fixed-dose group (Mean±SD = 1.01±0.33) was significantly lower than the sequential-dose group (Mean±SD = 1.05±0.31). The Mann-Whitney test indicated that this difference was statistically significant (p<0.05).

Table 2. Comparison of study parameters of fixed-dose and sequential-dose groups

Study parameters	Fixed-dose group (n=795) mean ± SD	Sequential-dose group (n=477) mean ± SD	p
Age	32 (20-35) 30.46±3.5	32 (20-35) 30.49±3.41	0.903
BMI	24 (19.8-29) 23.92±2.22	24 (19.8-29) 23.94±2.19	0.830
AMH	2 (1-7) 2.77±1.5	2 (1-7) 2.74±1.31	0.099
Total oocytes (n)	9 (7-16) 10.05±2.36	9 (7-16) 10.05±2.37	0.994
MII oocytes (n)	8 (6-15) 8.89±2.34	8 (6-15) 8.9±2.35	0.961
PN	8 (6-13) 8.4±1.94	8 (6-13) 8.43±1.95	0.741
Prolactin	15 (8.48-25) 16.91±5.54	15 (8.48-25) 16.93±5.54	0.919
FT4	0.99 (0.31-1.62) 1.04±0.28	0.99 (0.31-1.62) 1.03±0.28	0.862
TSH	1.21 (0.63-2.46) 1.48±0.54	1.16 (0.63-2.46) 1.47±0.53	0.761
FSH	7.83 (4-12) 7.58±1.48	7.83 (4-12) 7.61±1.49	0.827
LH	7 (3.52-9.64) 6.93±1.49	7 (3.52-13) 6.91±1.6	0.555
E2	40 (30-51.2) 40.13±6.67	39.4 (30-51.2) 40.11±6.67	0.996
Endometrial thickness	9 (9-12) 9.9±1.05	9 (9-12) 9.9±1.05	0.926
Progesterone	0.95 (0.31-4) 1.01±0.33	1 (0.31-2) 1.05±0.31	0.001

M, Mean; N, number of subjects; AMH, Anti-Mullerian hormone; PN, multi-pronuclei; FT4, Free T4; TSH, thyroid-stimulating hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2; Estradiol. All variables were tested by a Mann-Whitney U test.

Figure 1 shows the difference between two treatment groups (fixed-dose and sequential-dose) on premature P4.

The relationship between pregnancy results and the two treatment groups is shown in **Table 3**. As presented in **Table 3**, a chi-square test found no statistically significant association between the ongoing pregnancy rate and the two treatment groups (p>0.05). No significant difference was observed between the two treatment groups regarding cycle cancellation rate (p>0.05).

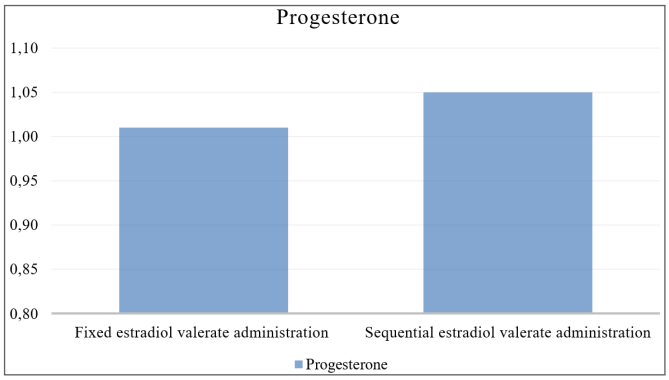

Figure 1. Progesterone of fixed-dose and sequential-dose groups

Table 3. The relationship between pregnancy results and two treatment groups

Variables		Fixed-dose group (n=795) n (%)	Sequential-dose group (n=477) n(%)	p
Pregnancy results Bhcg (+) (%)	Yes	390 (49.1)	254 (53.2)	0.148*
	No	405(50.9)	223 (46.8)	
Ongoing pregnancy rate (%)	Yes	360 (45.3)	206 (43.2)	0.392*
	No	435 (54.7)	271 (56.8)	
Cycle cancellation rate (%)	Yes	58 (7.3)	42 (8.8)	0.098*
	No	737 (92.7)	435 (91.2)	

*A Chi-square test.

The relationship between the abort rate of the two treatment groups is shown in **Table 4**. As stated in **Table 4**, a chi-square test found a statistically significant association between the abort rate and two treatment groups (p<0.05). The Pairwise Z-Tests found that the abort rate was significantly higher in the sequential-dose group (p=0.04).

Table 4. The relationship between abort rate of the two treatment groups

Variable		Fixed-dose group (n=390) n (%)	Sequential-dose group (n=254) n (%)	p-value
Abort rate (%)	Yes	30 (7.7)	48 (18.9)**	0.04*
	No	360 (92.3)	206 (81.1)	

*A Chi-square test. **The Pairwise Z-Tests.

DISCUSSION

Considering the importance of treatment for couples who suffer not only mental and emotional injuries due to infertility problems but a high cost to achieve the desired result, it is necessary to conduct more studies in this field to achieve the desired result and increase the pregnancy rate of infertile couples. In this study, we investigated the effect of EVadministration in two forms, fixed and sequential, in FET cycles. The results showed that the P4 level in the sequential-dose group was significantly higher than in the fixed-dose group (p=0.001). Also, the abortion rate in the sequential-dose group was significantly higher (p=0.04). Interesting interpretations can be made from

this result. One of the significant problems in the success of IVF methods is implantation failure, one of the critical signs of which is endometrial acceptance. Hormones also influence endometrial acceptance, and P4 is one of the essential hormones in this process (12). Since P4 and E are necessary to prepare the endometrium for blastocyst implantation and successful pregnancy, there should be more mature follicles in IVF cycles due to the use of ovarian-stimulating drugs. Therefore, the level of these hormones can be higher than the physiological state, and this increase can cause concern about the occurrence of luteal phase disorder and uterine tissue changes (13).

Out of 1272 women using the FET method in this study, 644 (50.62%) women became pregnant, and 628 (49.37%) women did not, and there was no significant difference between the two groups in terms of variables related to pregnancy. Among variables related to pregnancy, only progesterone had a significant difference between the two groups (p=0.001). This result was consistent with other studies (14,15). In Pabuccu et al. (16), which compared the ratio of E2 to P4 on the day of ET in pregnant and non-pregnant women, no significant difference was observed in the two groups of patients regarding FSH, the number of embryos transferred, and the amount of ampoule consumed. However, P4, the number of oocytes obtained, and the number of embryos formed in the two groups were significantly different from each other. It seems that demographic characteristics cannot significantly impact the outcome of pregnancy.

Pais et al. (17) showed no significant increase in P4 levels in pregnant women with the sequential-dose administration method. This result was despite the fact that the ratio of serum E2 to P4 on the day of ET was higher in pregnant women than in non-pregnant women, which was statistically significant. In Pan et al. (18), the increase in E2 levels in the transfer of one or two embryos on the day of HCG consumption had harmful effects on implantation, but this effect disappeared when three embryos were transferred. Although this relationship was not evaluated in the study, examining the level ratio between E2 and P4 on the day of embryo transfer, their lack of relationship was seen, which requires further investigation in this field.

Our results showed that the abort rate was significantly higher in the sequential-dose group. This finding is consistent with the findings of some studies (19,20). Considering the higher rate of abortion in the sequential-does group, it can be concluded that the P4 level in women whose pregnancy had an abortion was higher compared to women who had a successful pregnancy, which is consistent with the findings of previous studies (21,22). Regarding the explanation of this result, Xu et al. (23) reported that when the E2 level in the luteal phase is significantly reduced, it disrupts endometrial receptivity. It is unclear whether using E2 in these women after ovulation will improve their condition, which needs more research. In previous studies, high E2 levels had side effects on the environment of the endometrium but did not affect the quality of the fetus. Also, the high ratio of P4 to E2 level does not affect the pregnancy rate (23,24).

This study also had limitations. Due to the study's retrospective nature, E2 levels were not measured in women whose pregnancies were terminated due to miscarriage. Also, there was no measurement of the ratio of E2 to P4 in these women and its comparison with women with a successful pregnancy, which can be considered in future studies.

CONCLUSION

This study compared the risk of premature P4 rise in the fixed and sequential EV administration for FET cycles. Our results showed a higher P4 and abortion rate in the sequential-dose group. These findings show that premature P4 rise can be considered a risk factor.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was carried out with the permission of Beykoz University Research and Project Development Ethics Committee (Date: 26.10.2020, Decision No:1).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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