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Optimal Duration of Progesterone Treatment before Cryopreserved-Thawed Embryo Transfer

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ABSTRACT

Objective: *To investigate the optimal duration of progesterone therapy before cryopreserved-thawed embryo transfer and its impact on clinical pregnancy and live birth rates.*

Methods: *Five hundreds women undergoing cryopreserved-thawed embryo transfer were included in the study. These patients had a total of 500 embryos frozen on day 3 (n = 200), day 4 (n = 100), day 5 (n = 150) and day 6 (n = 50). Artificial endometrial preparation was successfully performed in all participants. If the endometrial thickness reached a minimum of 8 mm or in the presence of a triple-line view, the patients were divided into four different groups and each group into two subgroups according to the estimated duration of progesterone treatment to be used. Group 1 (n = 200): This group consisted of patients with day 3 embryo transfer. While 100 of 200 patients received embryo transfer after 3 days of progesterone treatment, the remaining 100 patients received embryo transfer after 4 days of progesterone treatment. Group 2 (n = 100): This group consisted of patients who underwent day 4 embryo transfer. While 50 of 100 patients had embryo transfer after 4 days of progesterone treatment, the remaining 50 patients received embryo transfer after 5 days of progesterone treatment. Group 3 (n = 150): This group consisted of patients who received day 5 embryo transfer. While 75 of 150 patients received embryo transfer after 5 days of progesterone treatment, the remaining 75 patients received embryo transfer after 6 days of progesterone treatment. Group 4 (n = 50): While 25 of 50 patients received embryo transfer after 6 days of progesterone treatment, the remaining 25 patients received embryo transfer after 7 days of progesterone treatment. The primary outcome measure of our study was to evaluate clinical pregnancy rate (CPR), ongoing pregnancy rate (OPR), live birth rate (LBR) and miscarriage rate per pregnancy.*

Results: *Clinical pregnancy rates were found in 50 of 100 (50%) cases who were given progesterone for 3 days. Of the 100 cases who were given progesterone for 4 days, 40 clinical pregnancy was detected (40%). Both OPR and LBR were found to be significantly lower in patients who received 4 days of progesterone treatment compared to those given 3 days. The rates of miscarraige (9.09%) in patients who received progesterone treatment for 4 days were significantly higher than those who received progesterone for 3 days (5.8%). In Group 2 both OPR and LBR were found to be significantly lower in patients who received 5 days of progesterone treatment compared to those given 4 days. The rate of miscarraige (25.0%) was significantly higher in patients who received progesterone treatment for 5 days compared to those who received progesterone treatment for 4 days (33.3%). When 75 patients in group III who underwent embryo transfer on the fifth day and received progesterone treatment for 5 days and 75 patients who were given progesterone treatment for 6 days were evaluated in terms of CPR, OPR and LBR the difference was statistically significant between the two gruops. When patients in group IV were evaluated in terms of CPR, OPR and LBR the difference was statistically insignificant.*

Conclusions: *Extending the progestereone usage period one day before embryo transfer has been found beneficial in patients who have been transferred for only fifth day.*

Keywords

Introduction

In addition to patient age and embryo quality, the loss of balance between progesterone and estrogen actions appears to be a central theme in the pathogenesis of failed FET cycles. After the endometrium is brought to a certain thickness with estrogen priming, the time to start progesterone treatment and the duration of use is the basic approach for the endometrium to reach the receptive phase and continue this phase for a while. For this reason, artifcial endometrium preparation in FET cycles becomes critical for a successful implantation. In order for the endometrium to pass into the receptive phase, many local and systemic molecules have to work in a combined and coordinated manner. The two essential hormones required for the regular activation of local mechanisms related to receptivity in the endometrium are estrogen and progesterone. These two hormones, which often act as physiological antagonists of each other, play a role in the preparation of the pre-implantation endometrium through the following pathways in both natural and FET cycles. (i) while estrogen increases endometrial inflammation, progesterone exerts an anti-inflammatory effect, (ii) while estrogen increases subendometrail contractions, progesterone directs the uterus to absolute silence, (iii) while estrogen increases epithelial proliferation pogesterone increases the expression of the basic receptivity gene, such as homeobox 10 and 11 [1-4].

The potent inflammatory effect of estrogen enables the endometrium to reach 7-8 mm in FET cycles where artifcal endometrium preparation is performed. In FET cycles, estrogen administration induces decidual development by macrophage activation, as well as edema, neutrophil and glycogen accumulation in the endometrium. Estrogen also regulates immune modulation and angiogenesis in endometrial cells via T and B cells [5-7]. This molecular pathway activation and inflammatory process that occurs with estrogen administration must be balanced with progesterone, a counter-regulatory hormone, after the endometrial thickness reaches 8 mm. Otherwise, endometrial thickening and inflammatory process may reach pathological dimensions and prevent implantation. From this stage on, endometrial inflammation and thickening are kept under control and the expression of integrins, HOXA10, 11 and LIF genes responsible for receptivity are stimulated by starting progesterone administration [1]. Defects in progesterone signaling account for the noted decrease in LIF, HOXA10, and integrin expression [8].

For all these reasons, the use of progesterone before frozen embryo transfer cycles must be kept at an optimal level. Conventionally, the duration of progesterone use before FET is the same as the day of the embryo transferred. To explain briefly, the duration of progesterone use before FET is three days in patients who undergo embryo transfer on the third day [9]. Similarly, the duration of progesterone use is five days in cases with embryo transfer on the fifth day. An error in the duration of progesterone use or a defect

at the receptor and/or postreceptor level due to the underlying disease will cause impairment in the expression of receptivity genes, mainly HOXA and integrins. However, even if attention is paid to the dosage and duration of use of progesterone, there may be a difference between the histological dating of the endometrium and its actual day. If this difference is more than 2 days [10], it is considered as out of phase endometrium and a significant decrease in pregnancy rates after FET in these patients is inevitable.

Materials and Methods

Five hundreds women undergoing cryopreserved-thawed embryo transfer were included in the study. These patients had a total of 500 embryos frozen on day 3 (n = 200), day 4 (n = 100), day 5 (n = 150) and day 6 (n = 50). Blood was drawn from the patients in all groups on the 3rd day of the cycle to determine the basal FSH, LH, E2 and progesterone levels. On the same day, transvaginal USG examination was performed to determine the presence of residual follicle or ovarian cyst, as well as endometrial thickness and endometrial pattern evaluation. In the evaluation made on the 3rd day, if the endometrial thickness is 4 mm or less and the serum E2 level is below 50 pg/mL, the artifical preparation of endometrium was planned. Endometrial preparation was started with estradiol valerate 2 mg twice daily. Additionally, 100 mg of aspirin and 5 mg of folic acid per day were added to the treatment. On the 12th day of the treatment, the patient was called for transvaginal USG control. If the endometrial thickness reached a minimum of 8 mm or in the presence of a triple-line view, the patients were divided into four different groups and each group into two subgroups according to the estimated duration of progesterone treatment to be used. Group 1 (n = 200): This group consisted of patients with day 3 embryo transfer. While 100 of 200 patients received embryo transfer after 3 days of progesterone treatment, the remaining 100 patients received embryo transfer after 4 days of progesterone treatment. Group 2 (n = 100): This group consisted of patients who underwent day 4 embryo transfer. While 50 of 100 patients had embryo transfer after 4 days of progesterone treatment, the remaining 50 patients received embryo transfer after 5 days of progesterone treatment. Group 3 (n = 150): This group consisted of patients who received day 5 embryo transfer. While 75 of 150 patients received embryo transfer after 5 days of progesterone treatment, the remaining 75 patients received embryo transfer after 6 days of progesterone treatment. Group 4 (n = 50): While 25 of 50 patients received embryo transfer after 6 days of progesterone treatment, the remaining 25 patients received embryo transfer after 7 days of progesterone treatment (Table 1). Injectable progesterone 100 mg daily was administered intramuscularly to patients in all groups. Estradiol valerate treatment was continued for another 2-3 days in patients with an endomerile thickness of less than 8 mm. Patients with no increase in endometrial thickness despite the prolongation of treatment were excluded from the study.

Artificial endometrial preparation was successfully performed in all participants. On the planned day of transfer, frozen embryos were thawed and embryo qualities were evaluated. The transfer was performed if at least one healthy embryo of good quality was

Groups	Transferred embryo	Progesterone usage time	The number of patients	Progesterone preparation	Estrogen preparation	Transfer day
I (n=200)	Day 3	3 days	100	IM	EV	4th day
		4 days	100	IM	EV	5th day
II (n=100)	Day 4	4 days	50	IM	EV	5th day
		5 days	50	IM	EV	6th day
III (n=150)	Day 5	5 days	75	IM	EV	6th day
		6 days	75	IM	EV	7th day
IV (n=50)	Day 6	6 days	25	IM	EV	7th day
		7 days	25	IM	EV	8th day

Table 1: Characteristics of ecah group in terms of the number of transferred embryo and progesterone usage time.

detected as a result of this evaluation. Micronized progesterone was initiated vaginally for luteal support. Estradiol valerate treatment was continued 3 times a day. The primary outcome measure of our study was to evaluate pregnancy rates. In this context, clinical pregnancy rate, ongoing pregnancy rate, live birth rate and miscarriage rate per pregnancy were recorded. Clinical pregnancy rate defined as evidence of a gestational sac, confirmed by ultrasound examination. Ongoing pregnancy rate defined as evidence of a gestational sac with fetal heart motion at 12 weeks, confirmed with ultrasound examination. Live birth rate defined as delivery of a live fetus after 24 completed weeks of gestational age. Serum beta-hCG levels were measured in all patients on the 12th day of embryo transfer. In the presence of a positive pregnancy test, luteal support was continued and USG was performed at the 4th week of the transfer and the presence of gestational sac and thus clinical pregnancy was confirmed. Patients with an endometril thickness <7mm on the day of progesterone initiation, patients older than 38 years of age, those with recurrent implantation failure or endometriosis/endometrioma diagnosis, those given long-term agonist suppression therapy, and hydrosalpinx cases were not included in the study.

Statistical analysis
SPSS 23.0 (IBM Corporation, Armonk, NY, USA) was used for the statistical analysis. Shapiro Wilk test was used to confirm the distribution of the data. The quantitative data were expressed as mean ± standard deviation (SD), and percentage (%). In the comparison of the groups, the one-way analysis of variance (ANOVA) test was used with the corresponding Tukey contrast test. The chi-square or Fisher's exact tests were performed to compare the frequencies of the categorical variables, as appropriate.

Results
A total of 500 patients were enrolled in the study, 200 in group I, 100 in group II, 150 in group III and 50 in group IV. There was no significant difference in four groups with regard to age, endometrial thickness at the time of embryo transfer, and the number of embryos transferred. When 100 patients in group I who underwent embryo transfer on the third day and received progesterone treatment for 3 days and 100 patients who were given progesterone treatment for 4 days were evaluated in terms of CPR, OPR and LBR the difference was statistically significant.

Clinical pregnancy rates were found in 50 of 100 (50%) cases who were given progesterone for 3 days. Of the 100 cases who were given progesterone for 4 days, 40 clinical pregnancy was detected (40%). OPR and LBR were detected in 44 (44.0%) of 100 patients who were given progesterone for 3 days and in 34 (34.0%) of 100 patients who received progesterone treatment for 4 days. Both OPR and LBR were found to be significantly lower in patients who received 4 days of progesterone treatment compared to those given 3 days (p<.001). The rates of miscarraige (9.09%) in patients who received progesterone treatment for 4 days were significantly higher (p<.003) than those who received progesterone for 3 days (5.8%).

When 50 patients in group II who underwent embryo transfer on the fourth day and received progesterone treatment for 4 days and 50 patients who were given progesterone treatment for 5 days were evaluated in terms of CPR, OPR and LBR the difference was statistically significant. Clinical pregnancies were detected in 18 of 50 cases (36%) who were given progesterone for four days. Of the 50 cases who were given progesterone for five days, 10 of them had clinical pregnancy (20%). OPR and LBR were detected in 12 (24.0%) of 50 patients who were given progesterone for 4 days and in 6 (12.0%) of 50 patients who received progesterone treatment for 5 days. Both OPR and LBR were found to be significantly lower in patients who received 5 days of progesterone treatment compared to those given 4 days (p<.002). The rate of miscarraige (25.0%) was significantly higher in patients who received progesterone treatment for 5 days (p<.02) compared to those who received progesterone treatment for 4 days (33.3%).

When 75 patients in group III who underwent embryo transfer on the fifth day and received progesterone treatment for 5 days and 75 patients who were given progesterone treatment for 6 days were evaluated in terms of CPR, OPR and LBR the difference was statistically significant between the two gruops. Clinical pregnancies were detected in 40 of 75 cases (53.3%) who were given progesterone for 5 days. Of the 75 cases who were given progesterone for 6 days, 44 clinical pregnancies were detected (58.6%). OPR were detected in 36 of (48%) of 75 patients who were given progesterone for 5 days and in 40 (53.4%) of 75 patients who received progesterone treatment for 6 days. The difference between the two groups was statistically significant

Groups	Transferred embryo	Progesterone usage time	CPR %	OPR %	LBR %	MİSSED
I (n=200)	Day 3	3 days	50	44	44	4 (9.09%)
		4 days	40	34	34	2 (5.8%)
II (n=100)	Day 4	4 days	36	24	24	3 (25%)
		5 days	20	12	12	2 (33.3%)
III (n=150)	Day 5	5 days	53.3	48	52.7	17 (22.6%)
		6 days	58.6	53.4	60	16 (21.0%)
IV (n=50)	Day 6	6 days	24	20	16	2 (8%)
		7 days	28	24	20	2 (8%)

Table 2: CPR, OPR, LBR and miscaariage rate of each group of participant.

(p<.03). LBR were detected in 19 of (52.7%) of 75 patients who were given progesterone for 5 days and in 24 (60.0%) of 75 patients who received progesterone treatment for 6 days. The difference between the two groups was statistically significant (p<.002). The rate of miscarriage was 21.0% in patients who received progesterone treatment for 6 days and 22.6% in patients who received progesterone for 5 days. But the difference failed to show statistical significance (p<.34, Table 2).

When 25 patients in group IV who underwent embryo transfer on the sixth day and received progesterone treatment for 6 days and 25 patients who were given progesterone treatment for 7 days were evaluated in terms of CPR, OPR and LBR the difference was statistically insignificant between the two gruops (p<.54). Clinical pregnancy was achieved in 6 of 25 patients (24%) who received progesterone support for 6 days. The number of ongoing pregnancies was 5 (20%). Four of the five cases resulted in live birth (16%). Clinical pregnancy was achieved in 7 of 25 patients (28%) who were given progesterone support for 7 days. The number of ongoing pregnancies was 6 (24%). Five of these six cases resulted in live birth (20%). Abortus was detected in 2 of the 6 patients (8%) who underwent embryo transfer on the 6th day and who were given progesterone treatment for 6 days. Likewise, abortus was detected in 2 of the 7 patients (%8) who underwent embryo transfer on the 7th day and who were given progesterone treatment for 7 days. The difference between two groups in terms of miscarriage was similar (p<.71).

Discussion
In cycles in which the endometrium is hormonally prepared for FET estrogen application is continued until the endometrial thickness measured in ultrasonography reaches 8 mm and above, and then progesterone is added. The duration of progesterone use is conventionally proportional to the number of days until the embryo is frozen. To be more precise, in the presence of an embryo frozen on day 3, the duration of progesterone use before transfer is minimum 3 days. Similarly, before the transfer of a frozen embryo on the fourth day of its development, the progesterone usage period will be 4 days [9].

In our study, in patients who underwent embryo transfer on the 3rd day, OPR and LBR were found to be significantly higher in those who were given P for 3 days before the transfer compared to those who received progesterone for 4 days. In other words, increasing the use of P one day before transfer did not contribute to pregnancy rates. Interestingly, abortion rates were significantly higher in those given 4 days P than those given 3 days P. Similarly, OPR and LBR were found to be significantly higher in patients who underwent embryo transfer on the 4th day and received 4-day P compared to those given 5-day P. In summary, giving 5 days P on 4th day embryo transfers does not provide any extra gain in terms of pregnancy rates. On the other hand, extending the P duration by 1 day led to a significant increase in miscarriage rates in this patient group. In contrast to embryo transfers on the third and fourth day, OPR and LBR were found to be significantly higher in patients who underwent embryo transfer on the 5th day and received 6-day P compared to those who received 5-day P. In this group of patients, extending the P treatment period by 1 day increased pregnancy rates, but did not cause an increase in abortion rates. In embryos transferred on the 6th day, extending the P period by 1 day does not contribute to pregnancy rates and miscarriage rates.

We can summarize the reasons for higher clinical pregnancy and LBR in cases with third day or fourth embryo transfer and whose duration of P use was not extended before the transfer compared to those whose P treatment was extended one day. Applying P after estrogen in endometrium prepared artificially for FET causes both functional and morphological changes in the endometrium and provides transition to the secretory phase [10,11]. However, as both estrogen receptor (ER) and progesterone receptor levels are down-regulated during the implantation window, it is not beneficial to extend the P usage period [12]. Giving P treatment in a period when PR level decreases does not increase the endometrial thickness, but only leads to a denser endometrial appearance. For these reasons, extending the P application period by 1 day in FET cycles on the 3rd and 4th days may not contribute to endometrial receptivity and thus pregnancy rates. It is not possible to say anything clearly about the reason for the increase in the abortion rates seen in those with extended P use. Miscarriages may be related to the genetic structure of the embryo, as well as other factors that play a role in the etiology of abortion. What we will say about the mechanism by which prolonging P treatment causes abortion will not go beyond speculation. Prolonged P treatment may contribute to the increase

in abortion by densifying the endometrium in the presence of decreased PR. However, this idea needs to be confirmed.

Both OPR and LBR rates were found to be higher in patients who underwent embio transfer on the fifth day and the duration of P use was extended by 1 day before the transfer. This finding is the opposite of cases where embryo transfer was performed on the third or fourth day and the P period was extended. Extending the P treatment one day on the third or fourth day transfers decreases the pregnancy rates, while extending the P treatment one day on the 5th day increases the pregnancy rates significantly. In the sixth day transfers, no positive or negative result was found in extending the P treatment. To summarize, extending the P usage period one day before transfer has been found beneficial in patients who have been transferred for only 5 days. In patients who were transferred on the third, fourth or sixth day, prolonging the duration of P use did not increase pregnancy rates and caused an increase in miscarriage rates. Of course, it is not very logical and scientific to think that the reason for the increase in abortion rates in this group of patients is the prolongation of the P period alone. Because P is one of the main drug categories used in the treatment of abortion, and its administration in the luteal phase contributes significantly to pregnancy rates. The reason that increasing the P usage time one day before the transfer leads to an increase in the pregnancy rates in the 5th day embryos may be due to the fact that the embyos have reached the blastocyst stage rather than P.

Conclusions

Traditionally high levels of serum P were accepted to be better for the FET cycles. On the other hand, recent studies reported that higher serum levels of P does not mean increased receptivity [13,14]. They also showed that high P levels on the day of embroy transfer was associated with decreased clinical pregnancy and live birth rates [13-15]. In line with this, it has been reported that endometrial dating is delayed for at least 2 days in one of four cases in whom artificial endometrial preparation for FET is performed [11,12]. Due to all these reasons, the use of E and P for endometrial preparation in FET cycles should be done according to very stricture rules.

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