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The duration of progesterone administration determines pregnancy outcome in frozen-thawed embryo transfer

Progesterone in FET cycles

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Abstract
Aim: In this study, we aimed to compare the effects of different progesterone treatment durations on pregnancy outcomes in patients undergoing frozen-thawed embryo transfer (FET).
Material and Methods: A total of 1468 patients who were scheduled for frozen-thawed embryo transfer were included in the study. The patients were divided into four groups according to the duration of progesterone administration. The patients in the first group received progesterone treatment for 3 days, the patients in the second group for 4 days, the patients in the third group for 5 days and the patients in the fourth group for 6 days, followed by FET. The primary outcome measure of our study was to evaluate clinical pregnancy rate, ongoing pregnancy rate, live birth rate and miscarriage rate per pregnancy.
Results: The frequencies of gestational sac, fetal heartbeat, ongoing pregnancy, live birth and miscarriage rates were significantly higher in the patients who were given progesterone for 6 days than in other groups ($p < 0.001$, for each). In a multivariate analysis, we found that 5-day embryos have 1.6-fold higher live birth rates than day 3 embryos (OR: 1.677, 95%CI: 1.328-2.117, $p<0.001$). In addition, we found that lower numbers of transfer ($p=0.008$), lower 3rd day P4 values ($p=0.001$), higher CZP day P4 values ($p<0.001$) and higher cycle days during transfer ($p<0.001$) are associated with higher live birth rates
Discussion: In FET cycles, giving progesterone for six days before transfer significantly increases the fertility outcome compared to 3,4 or 5 days of progesterone treatment.

Keywords
Frozen-Thawed, IVF, Pregnancy Rate, Live Birth, Progesterone

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Introduction

While planning in vitro fertilization (IVF), the embryo may need to be frozen due to various causes. Collected oocytes can be frozen and stored under appropriate conditions until embryo transfer is performed with thawed embryos in later cycles given that required characteristics or conditions are met [1,2]. Finding the best time for implantation in frozen embryo transfer is crucial for successful clinical outcomes. There are various approaches that enable synchronization of the frozen embryo day and endometrium development. The transfer process can be performed by ensuring that the most suitable time of the endometrium for implantation is synchronized with the frozen embryo day by closely monitoring the endometrium, or by utilizing interventions that prepare the endometrium from the first day of menstruation [3]. However, to date, there has been no consensus on the optimal duration of progesterone use for endometrium preparation in frozen embryo transfer [4,5]. This study was planned to compare the effects of different progesterone treatment durations on pregnancy outcomes in patients undergoing frozen-thawed embryo transfer (FET).

Material and Methods

This retrospective cohort study was performed among patients admitted to the Memorial Kayseri and Istanbul IVF centers between 01.01.2017 and 31.12.2018. The data of 35,670 patients who received IVF treatment were scanned. A total of 1468 women with frozen embryos aged between 18-42 years were included in the study. Women older than 42 years of age and those who had undergone fresh cycle transfers were excluded. Ethical approval was obtained from the Ethical Committee of Erciyes University. The patients were divided into four groups according to the duration of progesterone administration. The patients in the first group received progesterone treatment for 3 days, the patients in the second group for 4 days, the patients in the third group for 5 days and the patients in the fourth group for 6 days, followed by FET.

The primary outcome measure of our study was to evaluate clinical pregnancy rate, ongoing pregnancy rate, live birth rate and miscarriage rate per pregnancy. Clinical pregnancy rate was defined as evidence of a gestational sac, confirmed by ultrasound examination. The ongoing pregnancy rate was defined as evidence of a gestational sac with fetal heart motion at 12 weeks, confirmed with ultrasound examination. The live birth rate was 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.

Statistical Analysis

All analyses were performed on SPSS v21 (SPSS Inc., Chicago, IL, USA). Q-Q and histogram plots were used to determine whether variables are normally distributed. Data are given as mean±standard deviation or median (1st quartile - 3rd quartile) for continuous variables according to normality of distribution and as frequency (percentage) for categorical variables. Normally distributed variables were analyzed with one-way analysis of variances (ANOVA). Non-normally distributed variables were analyzed with the Kruskal-Wallis test. Multiple logistic regression analysis (forward conditional method) was performed to determine significant factors of the live birth. P-value of less than 0.05 was considered statistically significant.

Results

A summary of embryo transfer and duration of progesterone use are shown in Table-1. The age of the patients who received progesterone for 4 days was higher than in other groups ($p<0.001$). The prevalence of gestational sac, fetal heartbeat, ongoing/missed pregnancy and live birth were significantly higher in the patients who were given progesterone for 6 days than in other groups ($p<0.001$). Additionally, the gestational sac, fetal heartbeat, ongoing pregnancy, live birth rates of the patients who received progesterone for 5 days were statistically significantly higher than the patients who received progesterone for 4 days ($p<0.001$) (Figure-1). Male factor ($p=0.016$), progesterone levels ($p<0.001$), endometrial thickness ($p=0.035$), embryo day ($p<0.001$) and cycle day during transfer ($p <0.001$) were found to be significantly associated with live birth. We performed multiple logistic regression analysis to determine significant factors of the live birth. We found that 5th embryo day ($p<0.001$), lower numbers of transfer ($p=0.008$), lower 3rd day P4 values ($p=0.001$), higher CZP day P4 values ($p<0.001$) and higher cycle days during transfer ($p<0.001$) are associated with higher live birth rates. Number of days P4 used ($p=0.273$), age ($p=0.550$), presence of male factor ($p=0.605$), endometrial thickness on the CZP day ($p=0.151$), endometrial

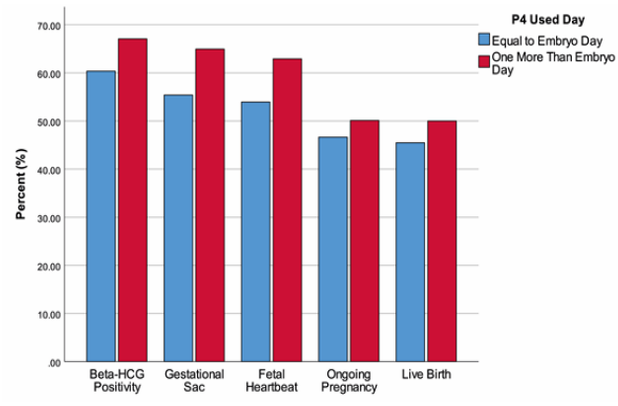

Figure 1. Pregnancy and live birth rates with regard to day of using P4

Table 1. Summary of embryo and P4 used days

Embryo Day	
3rd	529 (36.04%)
5th	939 (63.96%)
Number of Days P4 Used	
3 days	186 (12.67%)
4 days	343 (23.37%)
5 days	296 (20.16%)
6 days	643 (43.8%)
Number of days of using P4	
Equal to Embryo Day	482 (32.83%)
One More Than Embryo Day	986 (67.17%)

Data are given as frequency (percentage)

Table 2. Significant factors of the live birth, multiple logistic regression analysis

	β coefficient	Standard Error	Wald	p	Exp(β)	95.0% Confidence Interval for Exp(β)	
Number of Transfer	-0.117	0.044	7.044	0.008	0.889	0.815	0.970
P4 (3rd Day)	-1.693	0.480	12.465	<0.001	0.184	0.072	0.471
P4 (CZP Day)	1.151	0.247	21.699	<0.001	3.162	1.948	5.133
Embryo Day (5th)	0.517	0.119	18.918	<0.001	1.677	1.328	2.117
Cycle Day During Transfer	0.108	0.026	17.809	<0.001	1.114	1.060	1.172
Constant	-2.090	0.471	19.703	<0.001	0.124		

Dependent Variable: Live birth; Nagelkerke R2=0.096

thickness on the ET day (p=0.273) and embryo count (p=0.134) were found to be non-significant (Table 2).

Discussion

In this study the effect of the day of progesterone treatment on fertility outcome was examined and we found that the duration of progesterone administration improves live birth rates. When the studies conducted with the inclusion of various progesterone durations and embryo days were examined, the results seemed confusing. For instance, in a study that examined 4-day embryo transfer with progesterone administration for 3 days and embryo transfer 5-day embryo transfer with progesterone administration for 4 days, Sharma and Majumdar reported that the frequency of pregnancy was 41% in the group who received progesterone for 3 days, and 18.5% in the group who received progesterone for 4 days. They reported that both pregnancy and implantation rates were significantly better in the 3-day progesterone group [6]. However, it is possible that their findings were confounded by the day of transfer and the embryo quality. In a similarly designed study, Prapas et al. showed the exact opposite, indicating that 4 day- progesterone administration was more effective in terms of successful clinical results [7].

In a study examining the results of frozen-thawed embryo transfer after 6 or 7 days of progesterone administration, Ding et al [8] reported that there was no significant difference between the groups, however, implantation rate was higher in women under the age of 39 as a result of 6-day embryo transfers, although the difference was not statistically significant. They concluded that a less developed endometrium may have increased the chance of successful implantation by ensuring embryo presence within the critical window of implantation [8]. In two different studies, it has been shown that there is no significant difference in terms of clinical results between 2 or 3 embryo day transfers after different durations of progesterone administration [7,9]. Therefore, even though there are a considerable number of combinations to consider on this subject it is apparent that different progesterone administration times either do not have superiority to each other or the results are contradictory to other studies. Although our univariate analyzes showed that the day of progesterone administration affects live birth rates, multivariate analyzes revealed that the progesterone day administration did not affect live birth rates. Lu and colleagues examined the results of a total of 4 different progesterone administration groups [10]. They reported that there were no significant differences in terms of any important parameters when comparisons were performed with regard to progesterone administration. We also determined two other studies, both conducted by Vijver et al., that seemed to be somewhat comparable to our study. In the first of these studies, they examined the results of 5 and 7 days of progesterone administration, and reported that frequency of pregnancy was 28% after 7 days of progesterone administration and 33% after 5 days of progesterone administration; however, there was no statistically significant difference between the groups [11]. In the second study comparing the results of 5 and 3 days of progesterone administration, the authors reported a pregnancy rate of 27% with 5 days of progesterone administration and 19% with 3 days of progesterone administration. Again, there was no significant difference between these two groups. In addition, they suggested that early pregnancy losses were statistically significantly higher in the 3-day progesterone administration group, concluding that 5 days of progesterone administration could be preferred [12]. Similar to our study, none of the other studies comparing the results of similar groups found significant differences between the groups in terms of live birth rate.

It was determined that, the number of embryos transferred, embryo day, 3rd day and progesterone day progesterone levels, and cycle day during transfer independently influenced the live birth rate. It was seen that our results were in agreement with the majority of the literature. Various studies have shown that transfers with day 5 & 6 embryos had better results compared to earlier embryo transfers [13,14]. Furthermore, day 5 transfers are also reported to be superior to day 6 transfers [15], even though some studies have found similar success with 5 and 6 days of development [16].

Conclusion

It is evident that the cycle day at transfer is affected by embryo day; however, multivariate analysis revealed that higher cycle day during transfer positively affected live birth rate, whereas embryo day was not significant. Another factor that is frequently associated with IVF success, namely the number of transfers, was also found to be effective on live birth rate; however, the relationship in this study was in direct contrast to the majority of studies, which report that the probability of live

birth increases in parallel with the number of transfers [17]. The effect of serum progesterone level on live birth rate, similar to prior publications, was also determined to be significant on live birth rate in our study [18]. In conclusion, our results show that gestational sac, fetal heartbeat, ongoing pregnancy, and live birth rates increased with the prolonged day of progesterone treatment, and there was no significant effect on live birth rate in multivariate analysis.

Scientific Responsibility Statement
The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement
All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest
None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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