



# High serum progesterone levels on the day of embryo transfer in patients undergoing artificial frozen-thawed blastocyst transfer: Is there a ceiling effect?

## Yapay dondurulmuş-çözdürülmüş blastokist transferi yapılan hastalarda embriyo transferi günündeki yüksek serum progesteron düzeyleri: Tavan etkisi var mı?

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### Abstract

**Objective:** To evaluate the potential ceiling effect of high serum progesterone levels on the day of embryo transfer for pregnancy outcomes in patients undergoing artificial frozen-thawed blastocyst transfer (FET) cycles.

**Materials and Methods:** This retrospective cohort study included 595 patients who underwent artificial FET cycles. We evaluated progesterone levels and found that 40.6 ng/mL corresponded to the 90<sup>th</sup> percentile and 23.9 ng/mL corresponded to the 50<sup>th</sup> percentile. Based on these findings, we categorized progesterone levels as <20 ng/mL (n=220, 37.0%), 20-40 ng/mL (n=312, 52.4%), and ≥40 ng/mL (n=63, 10.6%). The primary outcome measures were the clinical pregnancy rate (CPR) and live birth rate (LBR).

**Results:** Blastocyst morphology grades, including expansion, trophectoderm, and inner cell mass grades, were significantly associated with clinical pregnancy (p<0.001 for all). Progesterone levels between 20 and 40 ng/mL were associated with higher CPR (p=0.043). In the multivariate analysis, only blastocyst expansion and inner cell mass grades were independently and significantly associated with CPR [p=0.011, odds ratio (OR)=1.6, (confidence interval) CI 95%=1.13-2.39, and p=0.007, OR=1.65, CI 95%=1.14-2.39, respectively]. The progesterone level and trophectoderm grade were not statistically significant. Regarding LBR, only blastocyst expansion grades 4 and trophectoderm grades A or B were significantly associated.

**Conclusion:** Based on these data, we speculate that if serum progesterone levels exceed 40 ng/mL on the day of embryo transfer in patients undergoing artificial FET cycles, there is no need to reduce the progesterone dose.

**Keywords:** Progesterone, frozen-thawed blastocyst transfer, ceiling effect

### Öz

**Amaç:** Yapay dondurulmuş-çözünmüş blastosist transferi (FET) siklusu uygulanan hastalarda embriyo transfer gününde yüksek serum progesteron düzeylerinin gebelik sonuçları üzerindeki tavan etkisi olup olmadığını değerlendirmeyi amaçladık.

**Gereç ve Yöntemler:** Bu çalışma yapay FET döngüsü uygulanan 595 hastayı içeren retrospektif bir kohort çalışmasıydı. Progesteron düzeylerine göre yüzdelik dilimleri değerlendirdiğimizde 40,6 ng/mL 90. yüzdeliğe, 23,9 ng/mL ise 50. yüzdeliğe karşılık geliyordu. Bu bulguya dayanarak progesteron düzeyi kesme noktasını <20 ng/mL, n=220 (%37,0); 20-40 ng/mL, n=312 (%52,4) ve ≥40 ng/mL, n=63 (%10,6) olarak belirledik. Birincil sonuç ölçüsü, klinik gebelik (CPR) ve canlı doğum oranı (LBR) olarak belirlendi.

**PRECIS:** There is no ceiling effect of high serum progesterone levels on day of embryo transfer (>40 ng/mL) for pregnancy outcomes in patients undergoing artificial frozen-thawed blastocyst transfer.

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**Bulgular:** Blastokist morfolojisi olarak genişleme derecesi, trofoektoderm ve iç hücre kütle derecesi klinik gebelik ile istatistiksel olarak anlamlı düzeyde ilişkili olduğunu bulduk (hepsi için  $p < 0,001$ ) ve 20-40 ng/mL arasındaki progesteron düzeyinin ise daha yüksek CPR ile ilişkili olduğunu bulduk ( $p = 0,043$ ). Çok değişkenli analizde; CPR ile ilişkili faktörler yalnızca blastosist genişlemesi ve iç hücre kütle derecesi bağımsız ve anlamlı faktörlerdi [ $p = 0,011$ , (odds oranı)  $OO = 1,6$ , güven aralığı (GA)  $95\% = 1,13-2,39$  ve  $p = 0,007$ ,  $OO = 1,65$ , GA  $95\% = 1,14-2,39$ , sırasıyla]. Progesteron düzeyi ve trofoektoderm derecesi istatistiksel olarak anlamlı bulunmadı. Faktörler ile LBR arasındaki ilişkinin değerlendirilmesinde sadece blastokist genişleme derecesi 4'e eşit veya üzerinde ve trofoektoderm derecesi A veya B istatistiksel olarak anlamlı LBR ile ilişkiliydi.

**Sonuç:** Bu verilere göre yapay FET siklusu yapılan hastalarda embriyo transferi gününde serum P4 düzeyi 40 ng/mL'nin üzerinde ise kullanılan progesteron dozunun azaltılmasına gerek olmadığını düşündük.

**Anahtar Kelimeler:** Progesteron, dondurulmuş-çözülmüş blastokist transferi, tavan etkisi

## Introduction

Progesterone increases during the luteal phase of the menstrual cycle with the occurrence of ovulation and prepares the endometrium for embryo implantation<sup>(1-3)</sup>. In natural conception, progesterone is synthesized by the corpus luteum, whereas exogenous progesterone is obtained in artificial frozen embryo transfer (FET) cycles in which there is no corpus luteum<sup>(4)</sup>. This exogenous progesterone is required for synchronization between the embryo and endometrium<sup>(5)</sup>. However, an important question arises in artificial FET cycles: What should be the lower and upper thresholds for serum progesterone levels on the day of embryo transfer for synchronization?

In recent studies, values in the range of 8.8-9.2 ng/mL were usually used as lower threshold values for serum progesterone (P4) levels on the day of embryo transfer in patients undergoing artificial FET cycles, and pregnancy outcomes were compared between patients with P4 levels above and below those thresholds<sup>(6-8)</sup>. The main reason for choosing these thresholds in such studies is that they reflect the minimal mid-luteal progesterone level that a healthy corpus luteum should secrete to prevent luteal phase defects<sup>(2)</sup>. Most previous studies have evaluated optimal serum progesterone concentrations following vaginal administration. Serum progesterone concentrations and pregnancy outcomes have been evaluated in a limited number of studies<sup>(9,10)</sup>.

Although many studies have investigated lower threshold values, there is a paucity of data in the literature on higher threshold values, indicating the possible ceiling effect of P4. Therefore, in this study, we examined whether high serum progesterone levels on the day of embryo transfer have a ceiling effect on pregnancy outcomes in patients undergoing artificial FET cycles.

## Materials and Methods

This retrospective cohort study included 595 patients who underwent artificial FET cycles at our fertility center between 2017 and 2021. The study was conducted in accordance with the ethical standards established in the 1964 Declaration of Helsinki.

Patients younger than 35 years of age for whom the transfer of one top-quality embryo was performed and, to eliminate age-related bias, patients older than 35 years of age for whom

euploid embryo transfer was performed were included in the study. In our clinic, blastocyst morphology was evaluated using the Gardner-Schoolcraft classification system<sup>(11)</sup>.

Patients with uterine diseases and changes in the progesterone dose or route according to serum progesterone levels on the day of embryo transfer were excluded from the study.

Oral estrogen (Estrofem, Novo Nordisk, Istanbul, Turkey) was administered on day 2-3 of the menstrual period with an ascending protocol: 4 mg/day for the first 4 days, 6 mg/day for the next 4 days, and 8 mg/day for the last 4 days. The day after the procedure, endometrial thickness was evaluated by transvaginal ultrasonography. Endometrial preparation was considered sufficient if the endometrial thickness was 7 mm. After endometrial preparation, 50 mg of progesterone (Koçak Farma İlaç ve Kimya Sanayi A.Ş., Istanbul, Turkey) was administered intramuscularly for 5 days. The serum progesterone levels were measured 2 h before the embryo transfer and approximately 16 h after the last dose of progesterone was administered.

In our evaluation of progesterone levels according to percentiles, 40.6 ng/mL corresponded to the 90<sup>th</sup> percentile and 23.9 ng/mL corresponded to the 50<sup>th</sup> percentile. Based on these findings and rounding up the values, we established progesterone level thresholds as  $< 20$  ng/mL ( $n = 220$ , 37.0%), 20-40 ng/mL ( $n = 312$ , 52.4%), and  $\geq 40$  ng/mL ( $n = 63$ , 10.6%). The primary outcome measures were clinical pregnancy and live birth rates. The blastocyst grading was based on the assessment of inner cell mass and trophoctoderm appearance, as described by Gardner and Schoolcraft<sup>(11)</sup>.

We compared pregnancy outcomes according to age, body mass index (BMI), blastocyst expansion, trophoctoderm and inner cell mass grades, and progesterone threshold levels. Data were analyzed using IBM SPSS Statistics 25 for Windows (IBM Corp., Armonk, NY, USA). All procedures performed in the 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. Since our study was designed retrospectively, IRB, informed consent and ethical approval has not been obtained.

## Results

The median age of the patients was 31 years (range: 20-46), and the mean BMI was  $24.5 \pm 4.0$  kg/m<sup>2</sup>. The overall clinical pregnancy rate was 61.8% (368/595), and the live birth rate was 52.9% (315/595). Patients were subdivided into two groups based on

clinical pregnancy and live birth outcomes. The ages and BMIs of the patients were comparable between the two groups (Table 1). Blastocyst morphology, as indicated by the expansion grade and the grades of the trophoctoderm and inner cell mass, was significantly associated with clinical pregnancy ( $p < 0.001$  for all). Progesterone levels of 20-40 ng/mL were associated with higher clinical pregnancy rates ( $p = 0.043$ ). In the multivariate analysis, only blastocyst expansion and inner cell mass grades were found to be independent significant factors associated with the clinical pregnancy rate [ $p = 0.011$ , odds ratio (OR): 1.6, 95% confidence interval (CI): 1.13-2.39, and  $p = 0.007$ , OR: 1.65, 95% CI: 1.14-2.39, respectively]. The progesterone level and trophoctoderm grade were not statistically significant ( $p = 0.310$  and  $p = 0.489$ , respectively) (Table 2). Regarding the live birth rate, only a blastocyst expansion grade of  $\geq 4$  and trophoctoderm grades A and B were significantly associated with a difference (Table 3).

**Discussion**

In the present study, we demonstrated that high serum progesterone levels on the day of embryo transfer may not have a ceiling effect on pregnancy outcomes in patients undergoing artificial FET cycles. Our findings are important because there is a paucity of data on the possible ceiling effect of P4 in the literature. Thus, our data offer guidance for future prospective studies on this topic.

The receptive endometrium for embryo implantation is mainly coordinated by estrogen and progesterone, and increased estrogen levels during the implantation period can disrupt implantation<sup>(12,13)</sup>. However, limited data are available on

the association between increased progesterone levels and embryo implantation. In an experimental study by Liang et al.<sup>(14)</sup>, increased progesterone levels during the implantation period had a deleterious effect on endometrial receptivity and decidualization. However, their study was conducted in rats, and we cannot extrapolate the extent to which progesterone levels have similar negative effects on endometrial receptivity in humans or whether we could reach the theoretical progesterone levels with the doses of exogenous progesterone applied during FET cycles.

According to the literature, excessive progesterone may accelerate endometrial development and cause the implantation window to open and close earlier. As a result, it is speculated that this may cause asynchrony between the embryo and the endometrium<sup>(15-17)</sup>. Based on this information, previous studies have been conducted with the aim of determining the

**Table 2.** Association between clinical and laboratory markers and clinical pregnancy

	Clinical pregnancy				p-value
	Positive		Negative + biochemical		
	n	%	n	%	
<b>Age</b>					
<35	299	63.5	172	36.5	<b>0.110</b>
$\geq 35$	69	55.6	55	44.4	
<b>BMI</b>					
<25	199	60.7	129	39.3	<b>0.864</b>
$\geq 25$	121	61.4	76	38.6	
<b>Progesterone level</b>					
<20.0	130	59.1	90	40.9	<b>0.43</b>
20.0-40.0	206	66.0	106	34.0	
$\geq 40.0$	32	50.8	31	49.2	
<b>Blastocyst expansion grade</b>					
3	26	43.3	34	56.7	<b>&lt;0.001</b>
4	229	70.7	95	29.3	
5 and 6	113	53.6	98	46.4	
<b>Trophoctoderm grade</b>					
A	159	74.3	55	25.7	<b>&lt;0.001</b>
B	197	55.8	156	44.2	
C	12	42.9	16	57.1	
<b>Inner cell mass grade</b>					
A	87	77.0	26	23.0	<b>&lt;0.001</b>
B	216	64.5	119	35.5	
C	65	44.2	82	55.8	

BMI: Body mass index

**Table 1.** General characteristics

	Mean $\pm$ standard deviation	Median (range)
<b>Age</b>	31.3 $\pm$ 4.7	31 (20-46)
<b>BMI</b>	24.5 $\pm$ 4.0	23.7 (17.2-41.0)
	<b>n</b>	<b>%</b>
<b>Age</b>		
<35	471	79.2
$\geq 35$	124	20.8
<b>BMI</b>		
<25	328	55.1
$\geq 25$	197	33.1
Missing	70	11.8
<b>Clinical pregnancy</b>		
Biochemical	57	9.6
Positive	368	61.8
Negative	170	28.6

BMI: Body mass index

**Table 3.** Evaluation of the relationship between factors and the live birth rate

	Pregnancy				p-value
	Live birth		Abortus		
	N	%	N	%	
<b>Age</b>					
<35	254	88.5	33	11.5	<b>0.752</b>
≥35	61	87.1	9	12.9	
<b>BMI</b>					
<25	171	88.1	23	11.9	<b>0.389</b>
≥25	100	84.7	18	15.3	
<b>Progesterone level</b>					
<20.0	111	87.4	16	12.6	<b>0.686</b>
20.0-40.0	177	88.1	24	11.9	
≥40.0	27	93.1	2	6.9	
<b>Blastocyst expansion grade</b>					
3	19	70.4	8	29.6	<b>0.9</b>
4	200	90.5	21	9.5	
5 and 6	96	88.1	13	11.9	
<b>Trophectoderm grade</b>					
A	138	90.8	14	9.2	<b>0.13</b>
B	171	87.7	24	12.3	
C	6	60.0	4	40.0	
<b>Inner cell mass grade</b>					
A	76	90.5	8	9.5	<b>0.337</b>
B	185	88.9	23	11.1	
C	54	83.1	11	16.9	
BMI: Body mass index					

upper level of progesterone at which pregnancy rates begin to decrease during FET cycles<sup>(9,15,18,19)</sup>. However, the first point of concern regarding these studies is that the effects of different progesterone administration routes on serum progesterone levels will vary, and thus, the cutoff values may also vary according to whether the administration route was vaginal, intramuscular, or subcutaneous. Yovich et al.<sup>(15)</sup> used only the vaginal route for luteal support and reported a cutoff point of >31.45 ng/mL, whereas Kofinas et al.<sup>(9)</sup> used only the intramuscular route and reported ≥20 ng/mL. Interestingly, the cutoff value was thus found to be higher in the study using the vaginal route. On the other hand, Alyasin et al.<sup>(18)</sup> used both vaginal and intramuscular routes and found that serum progesterone levels >32.5 ng/mL on the day of embryo transfer were associated with lower live birth rates. The differences in the administration routes used in these studies make it difficult to compare the results. In contrast, our study showed that

values >40 ng/mL did not have a negative effect on pregnancy outcomes. Therefore, we speculate that excessive progesterone use may have a ceiling effect on the endometrium; however, the dosages or routes of progesterone that we use for luteal support may not be able to cause this ceiling effect.

A retrospective cohort study conducted by González-Foruria et al.<sup>(19)</sup>, which included 3,183 FET cycles, supports our data. The authors showed that high serum progesterone levels before embryo transfer did not impair reproductive outcomes in patients undergoing artificial FET cycles. Based on these data, authors speculated that when adequate endometrial progesterone impregnation is achieved, serum progesterone levels are not related to reproductive outcomes.

It should be kept in mind that vaginal and intramuscular progesterone administration significantly differ regarding tissue and serum concentrations. Although we do not know the exact adverse effects of high tissue concentrations, there may be possible relaxin inhibition in the tissue, which may cause adverse effects on both implantation and perinatal outcomes<sup>(20)</sup>. In addition, systemic progesterone concentrations are critical for the immunological mechanisms during implantation and pregnancy.

### Study Limitations

There are two limitations to this study. First, this was a retrospective study, and the number of patients with serum progesterone levels ≥40 ng/mL. Despite these limitations, a key strength of this study was that it was conducted at a single center using the same luteal-phase support protocol for all patients.

### Conclusion

In conclusion, based on the current study data, we suggest that if serum P4 level is >40 ng/mL on the day of embryo transfer in patients undergoing artificial frozen-thawed blastocyst transfers, there is no need to reduce the dose of progesterone used.

### Ethics

**Ethics Committee Approval:** All procedures performed in the 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. Since our study was designed retrospectively, IRB, informed consent and ethical approval has not been obtained.

**Informed Consent:** Informed consent was obtained from all participants included in the study.

### Authorship Contributions

Concept: Y.A.T., B.D., M.B., G.B., Design: Y.A.T., B.D., B.Dü., M.B., G.B., Data Collection or Processing: Y.A.T., F.K.B., N.F., Analysis or Interpretation: Y.A.T., B.Dü., F.K.B., N.F., M.B., G.B., Literature Search: Y.A.T., B.D., B.Dü., N.F., M.B., Writing: Y.A.T., B.D., F.K.B., G.B.

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## References

- Mesen TB, Young SL. Progesterone and the luteal phase: a requisite to reproduction. *Obstet Gynecol Clin North Am.* 2015;42:135-51.
- Jordan J, Craig K, Clifton DK, Soules MR. Luteal phase defects: sensitivity and specificity of diagnostic methods for common clinical use. *Fertil Steril.* 1994;62:54-62.
- Daya S. Luteal support: progestogens for pregnancy protection. *Maturity.* 2009;65(Suppl 1):S29-34.
- Veleva Z, Orava M, Nuojua-Huttunen S, Tapanainen JS, Martikainen H. Factors affecting the outcome of frozen-thawed embryo transfer. *Hum Reprod.* 2013;28:2425-31.
- Haiyan L, Gang Y, Yu L, Lin L, Xiaoli C, Qingxue Z. Does serum progesterone level impact ongoing pregnancy rate in frozen embryo transfer using artificial preparations with vaginal progesterone? Study protocol for a randomized controlled trial. *Trials.* 2022;23:3.
- Labarta E, Mariani G, Holtmann N, Celada P, Remohi J, Bosch E. Low serum progesterone levels on the day of embryo transfer are associated with decreased ongoing pregnancy rates in oocyte donation cycles after artificial endometrial preparation: a prospective study. *Hum Reprod.* 2017;32:2437-42.
- Labarta E, Mariani G, Paoletti S, Rodriguez-Varela C, Vidal C, Giles J, et al. Impact of low serum progesterone levels on the day of embryo transfer on pregnancy outcome: a prospective cohort study of artificial cycles with vaginal progesterone. *Hum Reprod.* 2021;36:683-92.
- Melo P, Chung Y, Pickering O, Price MJ, Fishel S, Khairy M, et al. Serum luteal-phase progesterone levels in assisted conception women undergoing frozen embryo transfer: a systematic review and meta-analysis. *Fertil Steril.* 2021;116:1534-56.
- Kofinas JD, Blakemore J, McCulloh DH, Grifo J. Serum progesterone levels >20 ng/dl on day of embryo transfer are associated with lower live birth and higher pregnancy loss rates. *J Assist Reprod Genet.* 2015;32:1395-9.
- Boynukalin FK, Gultomruk M, Turgut E, Demir B, Findikli N, Serdarogullari M, et al. Measuring serum progesterone levels on the day of transfer can be an additional tool for maximizing ongoing pregnancies in single euploid-frozen blastocyst transfers. *Reprod Biol Endocrinol.* 2019;17:102.
- Gardner DK, Schoolcraft WB. Culture and transfer of human blastocysts. *Curr Opin Obstet Gynecol.* 1999;11:307-11.
- Ma WG, Song H, Das SK, Paria BC, Dey SK. Estrogen is a critical determinant that specifies the duration of the window of uterine receptivity for implantation. *Proc Natl Acad Sci U S A.* 2003;100:2963-8.
- Chang KT, Su YT, Tsai YR, Lan KC, Hsuuw YD, Kang HY, et al. High estradiol levels directly affect blastocyst implantation and post-implantation development directly in mice. *Biomed J.* 2022;45:179-89.
- Liang YX, Liu L, Jin ZY, Liang XH, Fu YS, Gu XW, et al. High progesterone concentrations are harmful to endometrial receptivity and decidualization. *Sci Rep.* 2018;8:712.
- Yovich JL, Conceicao JL, Stanger JD, Hinchliffe PM, Keane KN. Mid-luteal serum progesterone concentrations govern the rates of cryopreserved embryo transfers under hormone replacement. *Reprod Biomed Online.* 2015;31:180-91.
- Kalakota NR, George LC, Morelli SS, Douglas NC, Babwah AV. Toward an Improved Understanding of the Effects of Elevated Progesterone Levels on Human Endometrial Receptivity and Oocyte/Embryo Quality during Assisted Reproductive Technologies. *Cells.* 2022;11:1405.
- Zhao J, Hao J, Xu B, Wang Y, Li Y. Effect of slightly elevated progesterone levels on hCG trigger day on clinical pregnancy rate in GnRH-ant IVF/ICSI cycles. *Reprod Health.* 2022;19:66.
- Alyasin A, Agha-Hosseini M, Kabirinasab M, Saeidi H, Nashtaei M. Serum progesterone levels >32.5 ng/ml on the day of embryo transfer are associated with a lower live birth rate after artificial endometrial preparation: a prospective study. *Reprod Biol Endocrinol.* 2021;19:24.
- González-Foruria I, Garcia S, Alvarez M, Racca A, Hernandez M, Polyzos NP, et al. Elevated serum progesterone levels before frozen embryo transfer do not have a negative impact on reproductive outcomes: a large retrospective cohort study. *Fertil Steril.* 2023;120:597-604.
- De Ziegler D, Pirtea P, Ayoubi JM. Implantation Failures and Miscarriages in Frozen Embryo Transfers Timed during Hormone Replacement Cycles (HRT): A Narrative Review. *Life (Basel).* 2021;11:1357.