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Do High Progesterone Levels Affect Clinical Pregnancy Rates in Freeze-All Cycles?

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ABSTRACT Objective: To evaluate the effect of early progesterone elevation on clinical pregnancy and abortion rates in freeze-all cycles. **Material and Methods:** Assessments were carried out on patients who underwent frozen embryo transfer (FET) between 2015 and 2018 after tertiary referral at the Ondokuz Mayıs University In-Vitro Fertilization (IVF) Center, Samsun, Turkey. Serum progesterone levels were measured at the beginning (p1) and on the 11th day (p2) of the HRT cycle, and two subgroups were identified as <1.5 ng/L and ≥1.5 ng/L. **Results:** FET was indicated for 204 women with symptoms of unexplained infertility (n=130), male factor (n=54), or poor ovarian reserve (n=20). No significant correlation was found between clinical pregnancy rates and progesterone levels on the 2nd to 3rd day (p=0.389) nor on the 11th day of the HRT cycle (p=0.407). Similar results were also obtained for the correlation between the abortion rates and progesterone levels on the second, third, or 11th days of the cycle (p=1.000, not significant). **Conclusion:** Although progesterone elevation is believed to cause early maturation of the endometrium, the present findings indicate clinically nonsignificant effects of progesterone levels on pregnancy and abortion rates in frozen cycles.

Keywords: Embryo transfer; progesterone; infertility

In patients undergoing controlled ovarian hyperstimulation for in-vitro fertilization (IVF), elevated progesterone levels may cause early maturation of the endometrium, negatively affecting pregnancy rates. The effects of progesterone hypersecretion on frozen embryo transfers (FET), however, remain unclear. Nowadays, FETs are often preferred in IVF, although scant data are available on the effects of elevated progesterone in these cycles. A decrease in pregnancy rates correlated with elevated progesterone during the late follicular phase has been reported.[1,2] Altered gene expression and cytokine profiles in the endometrium lead to its early maturation, which are triggered by elevated progesterone.[1,3,4] This results in unsuccessful embryo implantation due to asynchronization.[5] Premature progesterone elevation may be caused by high estrogen at induction, high doses of follicle-stimulating hormone (FSH) during stimulation, and increased numbers of pre-ovulatory follicles.[6-8]

In FET, the embryo is transferred after preparing the endometrium without inducing ovulation, and therefore, there is no supraphysiological hormonal environment. However, reports suggest that progesterone elevation in FETs is possible, thus progesterone levels are monitored so that embryo transfer could be postponed in case of high progesterone levels.[9] The endometrium may be prepared for FET by different methods. The patient's endogenous

cycle may either be allowed to continue and embryo transfer could be decided accordingly (natural cycles). In an alternate method, hormone replacement therapy (HRT) with estrogen and progesterone may be administered externally to suppress the patient's native cycle. However, decreased pregnancy rates have been reported in both methods, which are correlated with late follicular phase progesterone elevations.[9,10]

The present study was aimed to investigate the effect of progesterone elevation on pregnancy outcomes in frozen embryo cycles with HRT.

MATERIAL AND METHODS

This study was conducted in accordance with the revised Helsinki Declaration of 2013 and this article approved by the Ethics Committee of the Faculty of Medicine of Ondokuz Mayıs University, Decision No. 2018/5. Patients (N=204) undergoing first-time FET procedure at the University Hospital IVF Center, aged 18 to 40 years. Frozen embryos for all study subjects were from previous IVF cycles. The exclusion criteria included patients with endometrial thickness less than 7 mm on the day of progesterone administration and prior use of GnRH agonist cycles. All study participants provided informed consent prior to enrollment.

Oral estradiol hemihydrate (Estrofem® 2 mg; Novo Nordisk, Denmark) was used for endometrial preparation; initiated on Day 2-3 of the cycle following a transvaginal ultrasound at increasing dosages of 4 mg/day on Days 1-4, 6 mg/day on Days 5-8, and 8 mg/day from Day 9 onward, post which, a second transvaginal ultrasound was performed. A minimal endometrial thickness cutoff of 7 mm was kept for embryo transfer. Progesterone (Progestan 50 mg; Koçak, Turkey) was dosed intramuscularly at 100 mg for five days prior to embryo transfer. One or two Day 5 embryos were transferred depending on the patient's age and the quality and number of embryos.

The serum samples were collected to analyze progesterone levels at the beginning (p1) and on the 11th day (p2) of the cycle. Progesterone levels ≥1.5 ng/L were considered elevated (1, 2). Increased luteinizing hormone (LH) levels (≥10 IU/L) were used as an exclusion criterion for patients in this analysis since the aim was to assess only the effects of increased progesterone. An LH level of 10 IU/l or more was accepted as LH elevation.[11] Patients with elevated LH levels were not included in this study in order to specifically analysis the effects of the isolated elevation of progesterone. Progesterone levels at beginning of the cycle (p1) and progesterone levels on the 11th day of cycle (p2) variables were divided into two groups separately as 1.5 ng/l and ≥1.5 ng/l.

Clinical pregnancy was confirmed with the appearance of a gestational sac with fetal heartbeat seen in ultrasonography. Abortion was defined as diagnosed pregnancy loss (confirmed gestational sac) before 20 weeks. A period of 20 weeks of gestation was defined as ongoing pregnancy. Statistical analysis was performed with SPSS 21 software (Chicago, US).

RESULTS

Among the 204 women enrolled in the study, 130 presented with unexplained infertility, 54 with male factor, and 20 with poor ovarian reserve. Successful pregnancies were recorded in 44% (58/130) women in the unexplained infertility group, 50% (27/54) in the male factor group, and 20% (4/20) in the poor ovarian reserve group. No significant differences were found (p=in the women divided into two subgroups as pregnant and non-pregnant with respect to the following characteristics: age (30.6±4 vs. 31.2±4.6, respectively; p=0.5680); Day 2-3 FSH level (8.3±2 mU/mL vs. 7.1±2.9 mU/mL, respectively; p=0.0809); antral follicle count (16.1±3 vs. 14.3±5, respectively; p=0.2793); and transfer day endometrium thickness (10.2±1.5 vs. 9.6±1.3, respectively; p=0.1606). The demographic characteristics of the subgroups (pregnant and nonpregnant women) are presented in Table 1.

Further, p1 progesterone elevations were seen in five (2.4%) women, while p2 elevations were seen in six (2.9%). Pregnancy rates were higher (44.2%) in subjects with p1 values <1.5 ng/L, and lower (20%) in subjects with p1 values ≥1.5 ng/L. Progesterone levels between p1 and p2 periods did not significantly affect clinical pregnancy rates

TABLE 1: Comparison of the demographic details of the pregnant and non-pregnant woman.

		Pregnant (n)	Non-pregnant (n)	p
Patient groups	Unexplained Infertility	58	72	Chi-square 0.5453.
	Poor ovarian reserve	4	16	p=0.761341.
	Male factor	27	27	
Day 2-3 FSH levels (mU/mL)		8.348 ±2.080	7.179±2.919	0.0809
Antral follicle count	16.09±3.988	14.36±5.144	0.2793	
Patients age	30.65±4.996	31.21±4.654	0.5680	
Endometrium thickness (mm)	10.22±1.536	9.643±1.311	0.1606	

(CPR) (p=0.389). For the p2 group, pregnancy rates were 42.9% in low (<1.5 ng/L) and 66.7% in high (≥1.5 ng/L) progesterone levels, respectively. Progesterone levels did not affect CPR for the p2 elevations (p=0.407). The correlation between CPR and p1-p2 elevations is shown in Table 2.

There was 82 and 79 ongoing pregnancies for the p1/p2 low progesterone (<1.5 ng/L) groups, respectively, while six abortions each were recorded for this group. For the high progesterone p1/p2 groups (≥1.5 ng/L), there were one and four ongoing pregnancies and zero abortion cases, respectively. The association between abortion and p1 and p2 levels is shown in Table 3.

TABLE 2: Relationship between clinical pregnancy rate and p1 and p2 levels.

	CPR		
	Negative (n,%)	Positive (n,%)	p
P1			
<1.5 ng/L	111 (55.8%)	88 (44.2%)	0.389
≥1.5 ng/L	4 (80%)	1 (20%)	
P2			
<1.5 ng/L	113 (57.1%)	85 (42.9%)	0.407
≥1.5 ng/L	2 (33.3%)	4 (66.7%)	

*P1: Day 2 progesterone level; **P2: Day 11 progesterone level; *** CPR: Clinical pregnancy rate.

TABLE 3: Association between abortion and p1 and p2 levels.

	Ongoing pregnancy	Abortion	p
P1			
<1.5 ng/L	82	6	1.000
≥1.5 ng/L	1	0	
P2			
<1.5 ng/L	79	6	1.000
≥1.5 ng/L	4	0	

*P1: Day 2 progesteron level.
**P2: Day 11 progesteron level.
Abortus is the diagnosed pregnancy loss before 20 weeks after observing of a gestational sac. Ongoing pregnancy is defined as pregnancy completed 20 weeks of gestation.

DISCUSSION

During a normal menstrual cycle, progesterone peaks after 14 days and plays a critical role in preparing the endometrium for implantation and continuation of pregnancy. An isolated increase in progesterone without LH elevation in stimulated cycles prior to human chorionic gonadotropin (HCG) trigger, in the late follicular phase, is defined as premature progesterone elevation, and is seen in 35-38% of stimulated cycles.[1,12,13] High doses of FSH used in stimulation and the presence of a large number of preovulatory follicles are likely causes for this elevation.[2,13,14] The FSH-induced hyperactivity of the enzyme 3β-hydroxysteroid dehydrogenase in granulosa cells increases the biosynthesis of progesterone from pregenolone.[15] Although an early progesterone elevation in fresh cycles is linked with decreased pregnancy rates, the pregnancy rates are unaffected by high progesterone levels during FETs.[1,2]

It is unclear how elevated progesterone decreases pregnancy rates. The alterations in the gene expression profile may possibly be a factor.[4,5] The dysregulation of the implantation window and asynchronization with the precociously matured endometrium are caused by early progesterone elevation. Endometrial thickness measured in the present study had no significant correlation with the progesterone levels. The premature proges-

terone elevations have also been reported in frozen cycles, causing the implantation window to shift, although few studies regarding these effects have been published.[10,16]

Early progesterone elevation has been reported in both natural cycles and HRT-induced protocols of frozen cycles. Premature progesterone elevations driven by the patient's own unsuppressed cycle has been reported, although, whether it does affect pregnancy outcomes is still debatable.[9,16] In the current study, all FET cycles were HRT cycles.

In HRT cycles, externally administered estrogen and progesterone suppress the patient's natural cycle, therefore, less progesterone elevations are expected. Kofinas et al., in patients undergoing HRT-assisted frozen cycles, investigated progesterone levels on the 19th day of frozen cycles performed with HRT. They showed that progesterone levels above 20 ng/dL on Day 19 of the cycle were associated with low live birth rates and high rates of abortion.[10] In the current study, progesterone levels were on the 11th day of frozen cycles, where six patients had early progesterone elevation (cutoff value=1.5 ng/dL), and 4 had confirmed pregnancies. Unlike the Kofinas study, early progesterone elevations did not affect pregnancy outcomes in this patient group.

This being a retrospective study has certain limitations. Different centers used different cutoff values for identifying early progesterone elevation, with each clinic determining their own cutoff values for evaluation. Additionally, early progesterone elevation was more frequent with increasing numbers of oocytes in stimulated cycles. Another study has reported that progesterone elevation did not decrease pregnancy rates in high-responder patients, suggesting that different progesterone cutoff values may be required in high responder, normoresponder, and poor responder patients.[14]

CONCLUSION

Even with the increasing popularity of frozen cycles due to elective concerns and medical indications, there are no concise set of protocols to be followed for monitoring hormones for maximal implantation probability in these cycles. Although the cases of early progesterone elevation have been linked to decreased pregnancy rates due to early endometrium maturation and asynchrony between the embryo and endometrium, the results of this study show no clinically meaningful reductions in pregnancy rates linked to progesterone elevations in frozen cycles. Further studies are required to effectively bridge the paucity of data regarding this problem in the literature, and better elucidate the effects of early progesterone elevation on FET.

Declarations

-Availability of data and material: *The data analyzed in the current study are available from AZÖ on request.*

-Competing interests: *The authors declare that they have no competing interests.*

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Informing

Due to the presence of the name of the journal editor's among the authors, the assessment process of the study was conducted by the guest editor.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Ayşe Z. Özdemir, Çağrı Gülümser;* ***Design:*** *Ayşe Z. Özdemir;* ***Control/Supervision:*** *Çağrı Gülümser;* ***Data Collection and/or Processing:*** *Ayşe Z. Özdemir, Pervin Karlı;* ***Analysis and/or Interpretation:*** *Çağrı Gülümser, Aysın Türkmen;* ***Literature Review:*** *Ayşe Z. Özdemir, Pervin Karlı;* ***Writing the Article:*** *Ayşe Z. Özdemir, Pervin Karlı;* ***Critical Review:*** *Aysın Türkmen, Davut Güven;* ***References and Fundings:*** *Davut Güven;* ***Materials:*** *Ayşe Z. Özdemir, Pervin Karlı.*

REFERENCES

1. Bosch E, Labarta E, Crespo J, Simón C, Remohi J, Jenkins J, et al. Circulating progesterone levels and ongoing pregnancy rates in controlled ovarian stimulation cycles for in vitro fertilization: analysis of over 4000 cycles. Hum Reprod. 2010;25(8):2092-100. [Crossref] [PubMed]
2. Venetis CA, Kolibianakis EM, Bosdou JK, Lainas GT, Sfontouris IA, Tarlatzis, BC, et al. Estimating the net effect of progesterone elevation on the day of hCG on live birth rates after IVF: a cohort analysis of 3296 IVF cycles. Hum Reprod. 2015;30(3):684-91. [Crossref] [PubMed]
3. Boomsma CM, Kavelaars A, Eijkemans MJ, Fauser BC, Heijnen CJ, Macklon NS. Ovarian stimulation for in vitro fertilization alters the intrauterine cytokine, chemokine, and growth factor milieu encountered by the embryo. Fertil Steril. 2010;94(5):1764-8. [Crossref] [PubMed]
4. Labarta E, Martínez-Conejero JA, Alamá P, Horcajadas JA, Pellicer A, Simón C, et al. Endometrial receptivity is affected in women with high circulating progesterone levels at the end of the follicular phase: a functional genomics analysis. Hum Reprod. 2011;26(7):1813-25. [Crossref] [PubMed]
5. Van Vaerenbergh I, Fatemi HM, Bourgain C. Premature progesterone rise and gene expression. Human Reprod. 2011;26(10):2913. [Crossref] [PubMed]
6. Farhi J, Ben-Haroush A, Andrawus N, Pinkas H, Sapir O, Fisch B, et al. High serum oestradiol concentrations in IVF cycles increase the risk of pregnancy complications related to abnormal placentation. Reprod Biomed Online. 2010;21(3):331-7. [Crossref] [PubMed]
7. Joo BS, Park SH, An BM, Kim KS, Moon SE, Moon HS. Serum estradiol levels during controlled ovarian hyperstimulation influence the pregnancy outcome of in vitro fertilization in a concentration-dependent manner. Fertil Steril. 2010;93(2):442-6. [Crossref] [PubMed]
8. Al-Azemi M, Kyrou D, Kolibianakis EM, Humaidan P, Van Vaerenbergh I, Devroey P, et al. Elevated progesterone during ovarian stimulation for IVF. Reprod Biomed Online. 2012;24(4):381-8. [Crossref] [PubMed]
9. Weissman A, Levin D, Ravhon A, Eran H, Golan A, Levran D. What is the preferred method for timing natural cycle frozen-thawed embryo transfer? Reprod Biomed Online. 2009;19(1):66-71. [Crossref] [PubMed]
10. Kofinas JD, Blakemore J, McCulloh DH, Grifo J. Serum progesterone levels greater than 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(9):1395-9. [Crossref] [PubMed] [PMC]
11. Griesinger G, Weig M, Schroer A, Diedrich K, Kolibianakis EM. Mid-cycle serum levels of endogenous LH are not associated with the likelihood of pregnancy in artificial frozen-thawed embryo transfer cycles without pituitary suppression. Hum Reprod. 2007;22(10):2589-93. [Crossref] [PubMed]
12. Bosch E, Valencia I, Escudero E, Crespo J, Simón C, Remohí J, et al. Premature luteinization during gonadotropin-releasing hormone antagonist cycles and its relationship with in vitro fertilization outcome. Fertil Steril. 2003;80(6):1444-9. [Crossref] [PubMed]
13. Venetis CA, Kolibianakis EM, Bosdou JK, Tarlatzis BC. Progesterone elevation and probability of pregnancy after IVF: a systematic review and meta-analysis of over 60 000 cycles. Hum Reprod Update. 2013;19(5):433-57. [Crossref] [PubMed]
14. Griesinger G, Mannaerts B, Andersen CY, Witjes H, Kolibianakis EM, Gordon K. Progesterone elevation does not compromise pregnancy rates in high responders: a pooled analysis of in vitro fertilization patients treated with recombinant follicle-stimulating hormone/gonadotropin-releasing hormone antagonist in six trials. Fertil Steril. 2013;100(6):1622-8. [Crossref] [PubMed]
15. Oktem O, Akin N, Bildik G, Yakin K, Alper E, Balaban B, et al. FSH stimulation promotes progesterone synthesis and output from human granulosa cells without luteinization. Hum Reprod. 2017;32(3):643-52. [Crossref] [PubMed]
16. Groenewoud ER, Macklon NS, Cohlen BJ, Al-Oraiby A, Brinkhuis EA, Broekmans FJ, et al. The effect of elevated progesterone levels before HCG triggering in modified natural cycle frozen-thawed embryo transfer cycles. Reprod Biomed Online. 2017;34(5):546-54. [Crossref] [PubMed]