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THE EFFECTS OF VITAMIN D LEVELS ON PREGNANCY OUTCOMES IN PATIENTS RECEIVING FROZEN EMBRYO TRANSFER

DONMUŞ EMBRİYO TRANSFERİ YAPILAN HASTALARDA D VİTAMİNİ DÜZEYLERİNİN GEBELİK SONUÇLARINA ETKİSİ

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ABSTRACT

Objective: The aim of this study is to evaluate the effects of 25-OH vitamin D on pregnancy outcomes in infertile patients undergoing a frozen embryo transfer.

Methods: In this prospective, single-blind study conducted at Kocaeli University Medical Faculty Hospital, Center for Assisted Reproductive Techniques, baseline serum levels of 25-OH vitamin D were measured at the start of treatment in 276 infertile patients who were scheduled to undergo frozen embryo transfer (FET). Cases with 25-OH-D vitamin levels lower than the level of deficiency (<20 ng/ml, group A, n=48) and higher than the level of deficiency (≥20 ng/ml, group B, n=44) were compared in terms of the rates of pregnancy as an outcome of the FET cycle, clinical pregnancy, ongoing pregnancy, live birth, implantation, pregnancy loss, and multiple pregnancy.

Results: Cases in groups 1 and 2 had similar demographic characteristics, and the serum AMH levels, one of the cycle follow-up parameters, were statistically significantly higher in group 1 compared to group 2 (p=0.014). Pregnancy (41.6% vs. 31.8%), clinical pregnancy (35.4% vs. 25%), ongoing pregnancy (25% vs. 18.2%), live birth (20.8% vs. 18.2%), pregnancy loss (18.8% vs. 13.6%) and twin pregnancy (4.2% vs. 9.1%) were similar between the groups (p=0.328, p=0.278, p=0.428, p=0.749, p=0.507, p=0.421, respectively).

Conclusion: There was no correlation between pregnancy outcomes from frozen embryo transfer and baseline serum 25-OH vitamin D levels obtained at the start of treatment. There is no indirect evidence showing that vitamin D level exerts its effects on fertility through endometrial receptivity and the implantation process.

Keywords: 25-OH Vitamin D, Frozen embryo transfer, pregnancy outcome

ÖZ

Amaç: Donmuş embriyo transferi yapılan infertil hastalarda 25-OH vitamin D'nin gebelik sonuçlarına etkisini değerlendirmek.

Yöntem: Kocaeli Üniversitesi Tıp Fakültesi Hastanesi, Üremeye Yardımcı Teknikler Merkezinde prospektif -tek kör olarak yürütülen bu çalışmada donmuş embriyo transferi (DET) yapılması planlanan 276 infertil olgunun serum 25-OH vitamin D düzeyleri tedavi başlangıcında elde edildi. 25-OH-D vitamini seviyelerinin yetmezlik seviyesinde düşük olduğu olgular (<20 ng/ml, grup A, n=48) ve yetmezlik seviyesinin üstünde olduğu (≥20 ng/ml, grup B, n=44) olgular DET siklusu sonucundaki gebelik, klinik gebelik, devam eden gebelik, canlı doğum, implantasyon, gebelik kaybı ve çoğul gebelik oranları karşılaştırıldı.

Bulgular: Grup 1 ve grup 2 olguların demografik özellikleri benzerdi ve siklus takip parametrelerinden serum AMH düzeyleri grup 1 de grup 2 'ye göre istatiksel olarak anlamlı yüksek izlenmiştir (p=0,014). Gruplar arasında gebelik (% 41,6 vs %31,8), klinik gebelik (%35,4 vs %25), devam eden gebelik (%25 vs %18,2), canlı doğum (%20,8 vs %18,2), gebelik kaybı (%18,8 vs %13,6) ve ikiz gebelik (% 4,2 vs % 9,1) benzerdi (sırasıyla; p= 0,328 p= 0.278, p= 0,428, p= 0,749, p= 0,507, p= 0,421).

Sonuç: Donmuş embriyo transferinden elde edilen gebelik sonuçları ile tedavi başlangıcında elde edilen serum 25-OH vitamin D seviyeleri arasında ilişki tespit edilmemiştir. Vitamin D seviyesinin fertilite üzerindeki etkilerini endometriyal reseptivite ve implantasyon süreci üzerinden gösterdiğine dair dolaylı kanıt elde edilmemiştir.

Anahtar Kelimeler: 25-OH Vitamin D, Donmuş Embriyo Transferi, Gebelik Sonucu.

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Introduction

The current data of the World Health Organization show that 10-15% of married couples are affected by infertility. Assisted reproductive techniques are the only way to achieve pregnancy for most infertile couples.

In-vitro fertilization (IVF) is the ideal treatment method for couples who are unable to conceive naturally or by in-utero insemination, or for infertile patients who are not suitable for these methods. Pregnancy success in freeze-thaw cycles is still low in cases when embryos cannot be transferred in a fresh state, and novel strategies are being tested to improve success. One of these evaluations is whether the serum 25-OH-D vitamin levels of a female patient have an effect on pregnancy outcome in frozen embryo transfer (FET) cases.[1]

Vitamin D, produced mainly in the skin in the body, is a steroid hormone that is fat-soluble. Ergocalciferol, which is contained in plants, and cholecalciferol, which is prevalent in animal foods, are the main sources of exogenous vitamin D intake.[2] The effects of vitamin D on many systems in the body have been shown in numerous studies.[3,4] Studies both with humans and animals are conducted to clarify the potential role of vitamin D in female fertility.[5]

The purpose of this study is to compare the success of pregnancy outcomes between the cases where the baseline serum 25-OH-vitamin D level obtained from female patients at the beginning of the frozen embryo cycle is above (≥20 ng/mL) and below (<20 ng/mL) the insufficiency. Accordingly, it is aimed to reveal whether the serum vitamin D level affects endometrial receptivity and implantation success.

Methods

This study was carried out with 276 infertile women who were scheduled to undergo frozen embryo transfer (FET) at Kocaeli University Medical Faculty Hospital, Center for Assisted Reproductive Techniques. All participants were included in the study after obtaining informed consent.

Patients aged between 24-42 years who were scheduled to undergo FET and had at least one good quality frozen embryo with the diagnosis of single or combined unexplained infertility, male factor infertility, anovulation, low ovarian reserve, bilateral tubal factor and endometriosis were included in the study. Cases who did not want to participate in the study, who had endometrial polyps, submucous myomas, uncorrected uterine anomalies, hydrosalpinx, or uncontrolled systemic diseases, were excluded from the study.

Following the confirmation of ovulation in the luteal phase of the previous cycle, a suppressed FET cycle was performed in all cases by starting leuprolide acetate (Lucrin 5mg/ml/2.8ml vial, 14 Syringe Kit SC /Abbot) with a daily dose of subcutaneous 10 IU for pituitary suppression. On the third day of the menstrual cycle, the patients were then called for an ultrasound and blood tests. The patients were called for the measurement of serum 25-OH vitamin D levels, TSH, AMH, estradiol, and progesterone by drawing 3-5 cc blood daily on the third day of the menstrual cycle. The study continued using the blinded method by preventing the researcher and patient from knowing the 25-OH vitamin D levels. Cases whose TSH levels were not in the 0.5-4.5 mIU/L range were excluded from the study. Patients with an estradiol level of > 50 and a progesterone level of > 1 ng/mL continued to take Lucrin at a dose of 10 IU/day until complete suppression was achieved. Estrogen therapy was not started in these cases until it was determined by drawing blood every three days that suppression had been achieved. Estrogen treatment was not initiated in cases who had endometrial thickness of >5 mm or had a follicle cyst larger than >14mm in the adnexal area in the ultrasound examination which was performed concurrently. Estrogen therapy was started in these cases when the endometrial thickness was ≤ 5 mm and no cyst was detected in the adnexal area in the ultrasonographic follow-up performed every three days.

In cases who met the criteria (estradiol <50 ng/mL, progesterone < 1 ng/mL, endometrial thickness ≤ 5mm, no cysts in the adnexal area) in the blood tests and ultrasound examinations performed, 6 mg oestradiol ng/mL (Estrofem tablet 2 mg 28 tablets /Novo Nordisk) was started orally divided into three equal doses daily while Leuprolid acetate was continued with a daily dose of 5 IU. The patients were called for the first ultrasonographic evaluation at the earliest on the 10th day of the menstrual cycle and on day 7 of the estrogen therapy. In this evaluation, while the estrogen therapy was continued with the same dose in cases with an endometrial thickness of ≥ 8 mm and a blood progesterone level of <1 ng/mL, leuprolide acetate therapy was discontinued, and twice-a-day vaginal progesterone therapy was initiated (Crinone 90 mg gel 8% /Merck). Cases with an endometrial thickness of less than 8 mm were examined every other day to monitor for an increase in the endometrial thickness. Estrogen therapy was administered at a dose of 8 mg/day to the cases whose endometrial thickness did not increase sufficiently in 2 consecutive follow-ups. If available, two thawed embryos were transferred, and if not, one thawed embryo was transferred on the 4th day of vaginal progesterone in cases with a third-day embryo, on the 6th day of vaginal progesterone in cases with a 5th-day embryo, and on the 7th day of vaginal progesterone in cases with a 6th-day embryo.

The embryo transfer was performed under the supervision of transabdominal ultrasonography with a full bladder. After the visualization of the cervix with a speculum, the cervix was purified from drug residues with saline and cleared of mucus by aspiration with a mucus-attracting catheter. First, a mock transfer was performed to determine the cervical canal and the uterine position. Then, with a full echo soft catheter (Prodimed) the cervix was passed by using a stylet only in patients that necessitated it, and the embryo transfer was completed by applying the lowest pressure possible on the Hamilton syringe without approaching the middle

portion of the uterine cavity by more than 15 mm and without a fundal contact. No teneculum was used or no cervical dilatation was performed in any patient. The position of the air balloon was clearly observed in all cases. The embryo transfer catheter was slowly removed, and no bed rest was recommended for the patients after the transfer. Daily doses of 6 mg oral estrogen and 180 mg vaginal progesterone were continued after the embryo transfer. No vitamin treatments were recommended. On the 12th day after the embryo transfer, blood hCG levels were measured to confirm pregnancy.

The main outcomes that were aimed to achieve in this study were the pregnancy rate revealed by hCG positivity, the clinical pregnancy rate obtained by ultrasonographic confirmation of the embryonic heartbeat, and the ongoing pregnancy rate confirmed by exceeding the 10th week of pregnancy. Additionally, the secondary aim of the study was to obtain the rates of multiple pregnancy and rates of abortion. After achieving the primary aims of the study, the study was unblinded, and the pregnancy success as a result of the FET cycle was compared between the cases with blood 25-OH-D vitamin levels above the deficiency level (≥20 ng/ml) and the cases at the insufficiency level (20 ng/ml).

The data analysis was performed with SPSS for Windows 20.0 package program. A Kolmogorov-Smirnov test was completed to check if the continuous variables were normally distributed. Descriptive statistics were presented as mean ± standard deviation or median (minimum-maximum) for continuous variables, while categorical variables were presented as number and percentage (%) of cases. The student's t-test was used to determine the significance of the difference between the groups in terms of means. The nonparametric Mann-Whitney U Test was used for the data whose means could be calculated as the groups did not fit the normal distribution. The Pearson's Chi-Square Test was used for the data whose means could not be calculated. The results were considered statistically significant for a p value of <0.05.

Results

A comparison of the demographics of study groups are presented in Table 1. Both groups showed similar demographics and infertility diagnoses (Table 1).

While the comparison of the characteristics of the FET cycle between the groups is presented in Table 2, the comparison of the rates of pregnancy, clinical pregnancy, ongoing pregnancy, live birth, abortion, and twin pregnancies is presented in Table 3.

The serum AMH value of the group with a serum 25-(OH) Vitamin D level higher than 20 was statistically significantly lower than the group with a low serum 25-OH vitamin D level. No significant difference was found between the groups in terms of both the characteristics of the FET cycle and the rates of pregnancy, clinical pregnancy, ongoing pregnancy, live birth, pregnancy loss, and twin pregnancy.

The comparison of the rates of pregnancy, clinical pregnancy, ongoing pregnancy, live birth, abortion, and twin pregnancy between cases with 25-OH vitamin D levels of <20 ng/mL and cases with 25-OH vitamin D levels of ≥20 ng/mL is presented in Table 3. There was no significant difference in pregnancy achievement and pregnancy outcomes between the two groups (p>0.05).

The retrospective analysis of the means of 25-OH vitamin D levels of cases who got pregnant, achieved clinical pregnancy, had an ongoing pregnancy, and gave live birth revealed no significant differences in the means of vitamin D levels between the groups, and the results are presented in Table 4.

In the study, we did not find any relationship between the levels of serum vitamin D and achieving pregnancy in the FET cycle.

Discussion

There is no agreement on the ideal vitamin D levels for female reproductive health and fertility at the moment. Although the possible effect of vitamin D on the outcomes of assisted reproductive therapy (clinical pregnancy and live birth) has been evaluated in a limited number of studies, the data are inconsistent.[6-10]

Studies examining the relationship between serum vitamin D levels and the effectiveness of IVF cycles reported that the clinical pregnancy rate is associated with vitamin D deficiency. According to a study measuring the 25-OH vitamin D levels in follicular fluid instead of serum, high vitamin D levels are associated with significantly higher clinical pregnancy and implantation rates, and follicular fluid vitamin D levels are an independent predictor of IVF cycle success.[11] Similarly, research done over various IVF cycles has suggested a relationship between vitamin D levels and pregnancy outcome.[12-13]

In contrast to these studies, which discovered a significant correlation between vitamin D levels and the success of IVF cycles using fresh embryos, Anifandis et al. reported a negative correlation between follicular fluid 25-OH vitamin D level, embryo quality, and clinical pregnancy rate.[14]

We aimed to evaluate the data obtained from FET cycles of patients with good and very good quality embryos frozen in the previous cycle to rule out ovarian factors and reveal whether vitamin D has an effect on endometrial receptivity and the implantation process. As good quality embryos are already frozen, standardizing FET is easier. As a result, many concomitant variables associated with the patient and her partner in new cycles are eliminated, and the true effect on receptivity can be assessed. In this prospectively designed, single-blind study, no statistically significant relationship was found between the serum 25-OH vitamin D levels measured at the start of the FET cycle and pregnancy success. In this study, we evaluated case groups with vitamin D levels both below and above 20 ng/mL. Furthermore, unlike many other studies, all samples were examined

Table 1. Comparison of important characteristics of groups.

	Group 1 Vitamin D level <20 ng / mL. (n= 48)	**Group 2 Vitamin D level ≥ 20ng / mL (n= 44)**	**p value**
Age (year)*	30.48 ± 4.36	32.00 ± 4.30	0.144
Partner age (year)*	34.27 ± 4.16	35.10 ± 6.47	0.939
Marriage duration (year)*	7.62 ± 4.55 (n=24)	5.95 ± 4.39 (n=20)	0.210
Gravida (n)*	0.52 ± 1.24	0.55 ± 0.82	0.307
Parity (n)*	0.10 ± 0.31	0.14 ± 0.38	0.636
Abortion (n)*	0.35 ± 1.06	0.41 ± 0.76	0.243
BMI (kg/size^2) *	25.94 ± 4,94 (n=24)	24,99 ± 3,99 (n=33)	0.518
Smoking (n, %)	3 (6.3%)	5 (11.4%)	0.473 *
Chronic medical disease (n, %)	4 (8.3%)	9 (20.5%)	0.095
Previous uterine surgery (n, %)	9 (18.75%)	11 (25.0%)	0.468
Number of previous fresh IVF	1.08 ± 1.13	1.14 ± 0.98	0.443
Number of previous FET	0.75 ± 0.81	0.91 ± 0.74	0.728
Genetic (n, %)	1 (2.1%)	0 (0.0%)	0.522 *
Advanced age (n, %)	0 (0%)	2 (4.5%)	0.226 *
Endometriosis (n, %)	1 (2.1%)	2 (4.5%)	0.467 *
Bilateral Tubal factor (n, %)	2 (4.2%)	4 (9.1%)	0.298 *
Low ovarian reserve (n, %)	4 (8.3%)	7 (15.9%)	0.263
Azosperm (n, %)	5 (10.4%)	2 (4.5%)	0.255 *
Anovulation (n, %)	11 (22.9%)	6 (13.6%)	0.252
Unexplained infertility (n, %)	4 (8.3%)	5 (11.4%)	0.444 *

Values are given as mean ± standard deviation. *p value was calculated by Fischer Chi Square Test.**Abbreviations; BMI: Body mass index, ICSI: Intracytoplasmic sperm injection, FET: Frozen embryo transfer, PCOS: Polycystic ovary syndrome

Table 2. Comparison of FET cycle characteristics between groups

	Group 1 Vitamin D Level <20 Ng / Ml (n= 48)	**Group 2 Vitamin D Level ≥20 Ng / Ml (n= 44)**	**P Value**
AMH Level (Ng/Ml) *	9.73 ± 7.79 (n=26)	4.67 ± 4.21 (n=23)	**0.014**
TSH Level (Miu/L) *	1.96 ± 1.24 (n=41)	1.84 ± 0.77 (n=38)	0.702
AFC*	23.46 ± 16.09 (n=12)	14.38 ± 9.36 (n=16)	0.113
Estrogen Used Time (Day)*	9.79 ± 2.93	9.98 ± 2.42	0.379
Endometrial Thickness (mm)*	9.94 ± 1.86 (n=47)	10.06 ± 1.69 (n=43)	0.487
Progesterone Level at the end of Proliferation Phase (ng/ml) *	0.53 ± 0.3 (n=20)	0.43 ± 0.27 (n=24)	0.094
Number Of Embryos Transferred (n)*			
Day 3 Embryos (n, %)	18 (37.5%)	13 (29.5%)	0.420
Day 5 Embryos (n, %)	21 (43.8%)	22 (50.0%)	0.548
Day 6 Embryos (n, %)	9 (18.8%)	9 (20.5%)	0.837

*Values are given as mean ± standard deviation. ** Abbreviations; AMH: Anti-mullerian Hormone, AFC: Antral Follicle Count, TSH: Thyroid Stimulant Hormone

Table 3. Comparison of pregnancy, clinical pregnancy, ongoing pregnancy, live birth, abortion, and twin pregnancy rates between groups

	Group 1 Vitamin D level <20 ng/ml (n= 48)	Group 2 Vitamin D level ≥20 ng / ml (n= 44)	p value
Pregnancy Rate	20 (41.6%)	14 (31.8%)	0.328
Clinical Pregnancy Rate	17 (35.4%)	11 (25.0%)	0.278
Ongoing Pregnancy Rate	12 (25.0%)	8 (18.2%)	0.428
Live Birth Rate	10 (20.8%)	8 (18.2%)	0.749
Abortion Rate	9 (18.8%)	6 (13.6%)	0.507
Twin Pregnancy Rate	2 (4.2%)	4 (9.1%)	0.421 *

*p value was calculated by Fischer Chi-Square Test.

immediately without being frozen, but neither the researchers nor the patients were aware of their vitamin D levels. This reflects the strength of our work in minimizing bias. Similar to our study, Van de Vijyer et al. in their prospective cohort studies, evaluated 280 infertile cases whose FET cycle was planned, in two separate groups as cases with 25-OH vitamin D levels below and above 20 ng/mL on the day of embryo transfer, the pregnancy rate in the vitamin D deficient group was found to be similar when compared to the vitamin D sufficient group (respectively; 40.9% vs. 48.3%, p=0.2).[15] Similarly, no difference was found between the clinical pregnancy rates (32.2% vs 37.9%, respectively, *p*=0.3). Clinical pregnancy rates in this study were similar in cases of insufficiency, deficiency, and normal levels of vitamin D, and the multivariate logistic regression analysis revealed that vitamin D status was not associated with pregnancy outcomes. Moreover, in their study, which involved randomizing 114 infertile cases with 25-OH vitamin D levels of <30 ng/L into two groups with and without vitamin D replacement, Aflatoonian et al. reported that the results of the two groups had similar results in terms of ongoing FET cycle pregnancy and clinical pregnancy.[1] When these findings are considered together with the findings of our study, we are of the opinion that there is no data showing that vitamin D exerts its effects on reproductive functions through endometrial receptivity and implantation.

The fact that the study was not designed to reveal the effects of vitamin D levels at the tissue level is a limitation. However, the study focused on the clinical outcomes needed in current practices and provided explanatory information on this subject. Nevertheless, when the limitations of our study are considered, it is clear that randomized controlled trials with high quality large samples are needed to determine the optimal 25(OH) vitamin D levels and the effects of vitamin D supplementation on fertility.

Compliance with Ethical Standards

This study was approved by Kocaeli University Non-interventional Clinical Research Ethics Committee (Decision number: 2017/176, Date: 07/06/2022)

Conflict of Interest

The authors have no conflicts of interest relevant to this article.

Author Contribution

Authors have contributed equally to this work.

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