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ORIGINAL RESEARCH

Additional Daily Intramuscular Progesterone for Luteal Phase Support does not Improve Live Birth Rates of Programmed Frozen-Thawed Embryo Transfer Cycles*

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ABSTRACT

This retrospective cohort study aims to investigate whether additional daily intramuscular progesterone (IMP) for luteal phase support improves live birth rates of programmed frozen-thawed embryo transfer (FET) cycles. The study was conducted at a tertiary level university hospital assisted reproductive technology (ART) center between January 2014 and Jan 2021. Six hundred four infertile patients with single-day 5-6 frozen-thawed blastocyst embryo transfer were enrolled in the study. All patients received either 8% micronized vaginal gel or vaginal progesterone capsules for luteal phase support. Intramuscular progesterone was added to vaginal progesterone depending on the in vitro fertilization (IVF) specialist's choice. Luteal phase support (LPS) was started 6 days before transfer in frozen-thawed cycles and continued until the end of the first trimester. Cycles were compared depending on vaginal progesterone types (8% gel vs. capsule) and the presence of intramuscular progesterone. The primary outcome was the live birth rate. A total of 604 FET cycles were enrolled. Using 8% micronized progesterone or progesterone capsules did not change the live birth rates (24% vs. 25.9%). As the main result, intramuscular progesterone support with vaginal progesterone compared with only vaginal progesterone did not improve the live birth results (22% vs. 24%). In conclusion, this study demonstrated that routine IMP progesterone given in combination with vaginal progesterone does not improve ART outcomes. This combination may be beneficial in a selective population with a monitored luteal phase. Using any form of vaginal progesterone alone is adequate for LPS.

Keywords: Luteal Phase Support. Intramuscular progesterone. Infertility. Vaginal progesterone. Intra-cytoplasmic sperm injection.

Luteal Faz Desteğinde Günlük Intramuskuler Progesteron İlavesi Donma-Çözme Embriyo Transferi Sikluslarında Canlı Doğum Oranlarını Artırmaz

ÖZET

Bu retrospektif kohort çalışması, programlanmış dondurulmuş-çözünmüş embriyo transfer (FET) döngülerinde luteal faz desteğinde intramuskuler progesteron eklenmesinin canlı doğum oranlarını arttırıp arttırmadığını araştırmayı amaçlar. Çalışma, Ocak 2014 ile Ocak 2021 arasında üçüncü basamak bir üniversite hastanesinde yardımcı üreme teknolojisi (ART) merkezinde yapıldı. Çalışmaya, teki 5.-6. gün dondurulmuş-çözünmüş blastokist embriyo transferi yapılan 604 infertil hasta dahil edildi. Tüm hastalara luteal faz desteği için ya %8 mikronize vajinal jel ya da vajinal progesteron kapsülleri verildi. İntramusküler progesteron, tüp bebek uzmanının tercihine bağlı olarak vajinal progesterona eklendi. Luteal faz desteği (LPS), dondurulmuş-çözünmüş döngülerde transferden 6 gün önce başlatıldı ve birinci trimesterin sonuna kadar devam etti. Sikluslar, vajinal progesteron tiplerine (8% jel vs. kapsül) ve intramusküler progesteronun varlığına bağlı olarak karşılaştırıldı. Birincil sonuç canlı doğum oranıydı. %8 mikronize progesteron veya progesteron kapsülleri kullanımı canlı doğum oranlarını değiştirmedi (sırasıyla %24 ve %25,9). Ana sonuç olarak, sadece vajinal progesteron yerine vajinal progesteronla birlikte intramusküler progesteron desteği, canlı doğum sonuçlarını iyileştirmedi (%22'ye karşılık %24). Sonuç olarak, bu çalışma rutin olarak verilen intra musküler progesteronun, vajinal progesteronla birlikte verilmesinin ART sonuçlarını iyileştirmediğini göstermektedir. Bu kombinasyon, luteal fazı monitorize edilmiş olan seçili bir popülasyonda faydalı olabilir. Herhangi bir vajinal progesteron formunun tek başına LPS için yeterli olduğu sonucuna varılmıştır.

Anahtar Kelimeler: Luteal Faz Desteği. İnfertilite. İntramuskuler Progesteron. Vajinal Progesteron. İntrasitoplazmik Sperm İnjeksiyonu.

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Progesterone plays a vital role in the implantation of the embryo and the maintenance of early pregnancy. It is well established that while optimal progesterone supply is easily provided by the corpus luteum in natural pregnancies, this physiological process becomes a complex problem to solve in ART (assisted reproductive technologies) pregnancies[1]. Using excessive dosages of gonadotropins and high estradiol levels, pituitary suppression in fresh embryo transfer cycles, and artificial endometrial preparation in frozen-thawed embryo transfer (FET) cycles negatively affect the functions of the corpus luteum[2,3]. Consequently, dysfunctional corpus luteum may result in inadequate progesterone production, negatively impacting embryo implantation. Advances in cryopreservation techniques, genetic screening capabilities, and the avoidance of ovarian hyperstimulation and multifetal pregnancies, along with the detrimental effects of supraphysiologic gonadotropin levels on the endometrium, have led to an increase in FET cycles[4,5]. Therefore, the management of luteal phase progesterone support has become a popular research topic, especially in FET cycles.

With the increasing number of FET cycles, various luteal phase progesterone support routes have been developed. Progesterone support can be administered orally, intramuscularly, vaginally, or rectally[6-9]. Each route has different bioavailability, metabolism, and specific adverse effects. Oral progesterone has low bioavailability and is rarely used alone by IVF specialists[10]. Most recent studies on oral progesterone have investigated dydrogesterone due to its agonistic effect on progesterone receptors[11-14]. There are few studies on the rectal form, and it has not been well investigated[15-17]. The vaginal and intramuscular routes are the most commonly used for LPS. Intramuscular progesterone (IMP) may cause local pain, patient discomfort, inflammatory response, and local abscesses[6,18], while the vaginal route can cause local irritation and vaginal discharge[19]. Despite these drawbacks, the vaginal route is the most preferred due to its ease of application, direct uterine effect without bioelimination, and similar implantation rates compared with IMP[20-21].

Numerous studies and meta-analyses have been published on the use of these progesterone forms for LPS. There are conflicting results on which form of progesterone yields the best pregnancy outcomes in frozen-thawed ET cycles. It remains unclear which route, form, and dosage are optimal. This retrospective study aimed to determine the optimum luteal phase progesterone support model by analyzing our ART center database, considering the different LPS strategies employed by various ART specialists.

Material and Method

Study Protocol, Ethical Approval

This retrospective cohort study was conducted at the ART Center of a tertiary level university hospitalr between January 2014 and January 2021. The Clinical Trials Ethical Committee of the University approved the study protocol with the number 2021-10/15. Patients were selected from the electronic database of the ART Center.

Patient Selection

Patients who underwent frozen-thawed embryo transfer with any infertility etiology were screened from the electronic database. Women who underwent single-day 5-6 good-quality (Gardner A-B) frozen-thawed blastocyst transfer were selected. Women aged over 40 years, body mass index (BMI) >40 kg/m^2, and with uterine pathology were excluded from the study. Each woman was included only once to avoid bias.

Infertility Examination and COH Protocols

Couples that were admitted to the ART unit were enrolled in a standard initial infertility workup. Medical history, demographic parameters, ovarian reserve testing, hysterosalpingography, sperm analysis, and transvaginal ultrasound were routinely performed. Patients who completed routine assessments for infertility and had an indication for ICSI treatment were involved in the ICSI program.

All women underwent a baseline scan on the second/third day of menstruation. Following exclusion of endometrial pathology and the presence of ovarian cysts, controlled ovarian stimulation (COH) was started through daily gonadotropin injections. The daily gonadotropin dosage varied according to patient age, BMI, and ovarian reserve. Recombinant follicle-stimulating hormone (FSH) or human menopausal gonadotropin (hMG) was given, and microdose flare-up, antagonist or long protocol was started depending on the physician's choice. The trigger was administered as a gonadotropin-releasing hormone (GnRH) analog or human chorionic gonadotropin (hCG) depending on the etiology or cycle characteristics. Oocyte pick up was performed after 34-36 hours of trigger. ART etiology, number of collected oocytes, estradiol levels on the trigger day, and the patient or laboratory schedule were factors that determined the fresh or frozen-thawed embryo transfer. Day 5 or 6, good-quality (Gardner classification Grade A or B) blastocysts and single embryos were transferred to all patients.

Endometrial Preparation and Luteal Phase Support Modalities

Patients were examined on the second or third day of the menstruation, and oral 2 mg Estradiol valerate

(Estrofem, NovoNordisk, Denmark) three times a day (6 mg) was started in the absence of intrauterine pathology or a>10-mm follicle in transvaginal ultrasound. A second ultrasound examination was performed one week later. After 12-13 days of Estrogen replacement, if the endometrium had a trilaminar pattern with at least 6-7 mm thickness, the embryo transfer was scheduled, and progesterone treatment was commenced. and the blastocysts were warmed and transferred on the 6th day of progesterone administration.

Luteal phase support was programmed by two senior IVF specialists. The clinical approaches of the two seniors were different. I.K. routinely prescribed intramuscular progesterone to vaginal progesterone for LPS, whereas G.U. gave only vaginal progesterone. The preferred vaginal progesterone types were the 8% micronized progesterone gel and progesterone 200 mg vaginal capsules. The vaginal progesterone dosage was twice a day for the gel and three times a day for the capsule, starting six days before the frozen-thawed transfers. Vaginal support was continued until a negative pregnancy test or the detection of fetal cardiac activity. IMP was prescribed 25 mg once a day, starting on embryo transfer day, and continuing until the pregnancy test. The serum progesterone levels were not monitored.

A positive pregnancy test was defined as serum beta-hCG levels of 10 IU/L on the 9-10th day after embryo transfer. A decline in serum b-hCG levels before ultrasound verification of a gestational sac was termed as biochemical abortus. Miscarriage was termed as the spontaneous loss of a pregnancy before the 20th week. Birth after the 24th week of pregnancy was defined as live birth.

Patients were divided into groups depending on the LPS strategy used (vaginal progesterone type, presence of IMP use). Demographic parameters, cycle parameters, and pregnancy outcomes were compared between the groups. The live birth rate was the primary outcome. Each patient was enrolled in the groups only once to avoid bias.

Statistical Analysis

Depending on the distribution, continuous variables are defined as mean ± standard deviation (SD) or median (25th-75th percentile). Categorical variables are defined as percentages. Continuous variables were compared between the groups using the independent samples t-test or the Mann-Whitney U test, as appropriate. Categorical variables were compared using the Chi-square test and its derivatives. A two-sided p-value of 0.05 was considered statistically significant. Due to the retrospective nature of the study, a power analysis could not be performed to calculate the required sample size.

Results

A total of 604 cycles with single day 5 or 6 good quality frozen-thawed blast transfers were enrolled into the study.

The first analysis was regarding the type of vaginal progesterone. The vaginal progesterone type was micronized 8% gel in 527 cycles and micronized 200 mg capsule in 77 cycles. All demographic parameters (age, BMI, etiology, infertility duration, previous cycle number), ovarian reserve tests [anti-mullerian hormone (AMH), antral follicle count (AFC)], stimulation protocols, number of picked up oocytes, metaphase-2 oocytes, and two-pronuclei embryos were comparable (Table I).

Table I. Patient Characteristics and Cycle Parameters, Depending on Vaginal Progesterone Type

Frozen Embryo Transferred Cycles N=604			
	Vaginal Gel N=527	**Vaginal Capsule N=77**	p
Demographics			
Age	31.2 ± 4.4	31.2 ± 4.4	0.97
BMI	25.6 ± 5.1	26.7 ± 5.7	0.1
Infertility Duration	6 (4-8)	6 (4-8)	0.9
Etiology			0.78
Unexplained	23%	31.2%	
Male	23.2%	20.8%	
Tubal	6.1%	4%	
PCOS	14%	11%	
DOR	18.3%	23.4%	
Both(F&M)	6.7%	4%	
Other	8.7%	5.6%	
Previous Cycle No.	2 (2-4)	2 (2-3)	0.64
Ovarian Reserve			
AMH	3.08 (1.3-5.6)	2.3 (1-4.7)	0.216
AFC	12 (8-17)	12 (8-18)	0.85
COH Parameters			
Stimulation Protocol			0.5
Antagonist	86%	88%	
Long	3%	2%	
Micro-Dose	6%	3%	
Other	5%	7%	
Daily Dosage	277 (206-300)	300 (225-318)	0.05
Estradiol on the day of trigger	2260 (1285-3663)	2195 (1296-3211)	0.45
OPU &Embryology			
Follicle Count on the day of trigger	12 (8-17)	11 (9-16)	0.7
No. of Oocyte	15 (9-21)	16 (8-24)	0.9
No. of MII	11 (7-17)	11 (6-17)	0.5
No. of 2PN	7 (4-11)	7 (4-11)	0.6
I.M Progesteron	%87.3	93.5%	0.12

•Values with median (25-75 Percentiles) or mean (Standard Deviation)

When we analyzed the pregnancy outcomes, we found that there was no difference between the groups in terms of both positive b-hCG rates, miscarriage rates, and live birth rates according to the use of vaginal gel.

The pregnancy test was positive in 36% of the vaginal gel group and 38.9% of the vaginal capsule group (p=0.78). Live birth rates were 24% in the vaginal gel group and 25.9% in the vaginal capsule group (p=0.82). The median fetal birth weight, gestational age at birth, and delivery type were similar in both groups (Table II).

Table II. Pregnancy and Neonatal Outcomes, Depending on Vaginal Progesterone Type

Frozen Embryo Transferred Cycles N=604			
	Vaginal Gel N=527	**Vaginal Capsule N=77**	p
Pregnancy Results			
Positive b-hCG	36% (190/527)	38.9% (30/77)	0.78
Biochemical Abort.	5.1% (27/527)	5% (4/77)	0.9
Ectopic Pregnancy	0.3% (2/527)	1.2% (1/77)	0.3
Miscarriage	6.6% (35/527)	6.5% (5/77)	0.9
Livebirth	**24% (126/527)**	**25.9% (20/77)**	0.82
Gest. Age at birth	38 (37-39)	37 (36-38)	0.52
Fetal Weight	3300 (2855-3635)	3110 (2800-3500)	0.45
Delivery type			0.7
C/S	81%	86%	
Vaginal	19%	14%	

•Values with median (25-75 Percentiles)

Table III. Patient Characteristics and Cycle Parameters, Depending on IMP Usage

Frozen Embryo Transferred Cycles N=604			
	Intramuscular (-) 72	**Intramuscular (+) 532**	p
Demographics			
Age	31.3 ± 4.4	31.2 ± 4.4	0.7
BMI	25.2 ± 4.2	25.8 ± 5.2	0.6
Infertility Duration	6 (3-8)	6 (4-8)	0.3
Etiology			0.5
Unexplained	25.4%	23.9%	
Male	22.5%	22.9%	
Tubal	5.6%	5.8%	
PCOS	9%	15%	
DOR	23.9%	18.2%	
Both(F&M)	5%	7%	
Other	8.6%	7.2%	
Previous Cycle No.	2 (1-3)	2 (1-4)	0.8
Ovarian Reserve			
AMH	4.4 (1.9-7.8)	2.9 (1.3-5.5)	0.13
AFC	10 (8-20)	12 (8-17)	0.9
COH Parameters			
Stimulation Protocol			0.06
Antagonist	82%	85%	
Long	2.8%	2.8%	
Micro-Dose	5.6%	5.5%	
Other	10%	6%	
Daily Dosage	225 (200-300)	300 (225-300)	**0.04**
Estradiol on the day of trigger	2279 (999-4014)	2251 (1316-3400)	0.8
OPU &Embryology			
Follicle Count on the day of trigger	11 (8-13)	12 (8-17)	0.55
No. of Oocyte	16 (10-22)	15 (9-21)	0.59
No. of MII	12 (7-16)	11 (7-17)	0.59
No. of 2PN	8 (5-12)	7 (4-11)	0.12

* Values with median (25-75 Percentiles) or mean (Standard Deviation)

The second analysis was regarding IMP use. The number of frozen-thawed cycles with or without IMP was 532 vs. 72. Groups were comparable for all parameters (Table III). The positive b-hCG rates, miscarriage rates, and live birth rates were similar except for ectopic pregnancy rates (ectopic pregnancy rates: 2.7% (2/72) IMP (-) group and 0.2% (1/532) in IMP (+) group, p<0.01). Live birth rates were 22% in IMP (-) group and 24% in IMP (+) group, p=0.61 (Table IV).

Table IV. Pregnancy and Neonatal Outcomes, Depending on IMP Usage

Frozen Embryo Transferred Cycles N=604			
	Intramuscular (-) 72	**Intramuscular (+) 532**	p
Pregnancy Results			
Positive b-hCG	40% (29/72)	36% (191/532)	0.56
Biochemical Abort.	5.5% (4/72)	5% (27/532)	0.8
Ectopic Pregnancy	2.7% (2/72)	0.2% (1/532)	**0.01**
Miscarriage	9.7% (7/72)	6.2% (33/532)	0.28
Livebirth	22% (16/72)	24% (130/532)	0.61
Gest. Age at birth	37 (35-39)	38 (37-39)	0.6
Fetal Weight	3300 (2915-3762)	3250 (2840-3600)	0.8
Delivery type			0.6
C/S	75%	82%	
Vaginal	25%	18%	

•Values with median (25-75 Percentiles)

Discussion and Conclusion

In our study, we found that the use of progesterone vaginal gel or capsules, and the addition of extra progesterone supplementation beyond standard luteal phase progesterone treatment without determining serum progesterone levels, did not increase the live birth rates in frozen-thawed embryo transfer cycles.

Prior studies have provided mixed results on this topic. Until recently, the luteal phase was not evaluated or discussed in detail regarding the success of IVF cycles. However, its importance has become better understood. Progesterone supply is essential, and there is ongoing debate on whether vaginal progesterone alone provides sufficient progesterone to achieve implantation due to the lack of corpus luteum function in hormone-replacement frozen embryo transfer cycles. Today, the necessity of strict monitoring of the luteal phase, akin to the stimulation phase, is under discussion, with serum and tissue concentrations of progesterone being the main focus.

Intramuscular progesterone administration, used since the early years of IVF, is being replaced by oral, vaginal, rectal, and even subcutaneous routes due to the inability for self-administration and adverse effects such as local pain and sterile abscess formation. The high progesterone concentration achieved in uterine

tissue through vaginal use is leading the way as a preferred option[20-24]. A survey study of 21 ART centers in Europe showed that physicians mostly preferred vaginal progesterone for LPS[25]. A recent meta-analysis comparing vaginal versus intramuscular forms in fresh and frozen cycles, encompassing 15 randomized controlled trials (RCTs) and 5656 patients, indicated no significant differences between vaginal progesterone and intramuscular progesterone regarding ongoing pregnancies (RR=0.90, 95% CI: [0.76-1.06]; p=0.21). Moreover, vaginal progesterone was significantly associated with greater satisfaction compared with intramuscular progesterone[26]. Van der Linden published a meta-analysis in 2015 comparing all routes of progesterone administration[27]. This analysis included 45 RCTs with over thirteen thousand patients, finding no conclusive evidence between all routes (intramuscular, vaginal, oral, subcutaneous, rectal). However, none of the comparisons had high-quality evidence. The vaginal form was again the most preferred route in all RCTs.

One of the secondary results of our study was the comparable pregnancy outcomes between different types of vaginal progesterone, either gel or capsule. Numerous studies in the literature have shown similar results. It is known that optimal dosage vaginal progesterone does not change pregnancy outcomes. A systematic review and meta-analysis of 18 RCTs compared different vaginal progesterone types and concluded that all types were equally safe and effective for LPS in ART cycles[28].

Recent literature has suggested that low or very high supraphysiologic progesterone levels in the luteal phase may contribute to IVF failure. Labarta et al. recently showed that nearly one-third of patients receiving micronized vaginal progesterone had inadequate serum progesterone levels, with levels lower than 8.8 ng/dL negatively impacting ART results[29]. They advised monitoring progesterone levels in the mid-luteal phase when using vaginal progesterone and adjusting doses accordingly. They also showed similar results in their previous study with oocyte donation cycles, finding the threshold to be 9.2 ng/dL[30]. A retrospective analysis dividing patients into five groups based on mid-luteal progesterone levels suggested that additional luteal support might improve IVF outcomes in patients with low serum P4 levels (<10 ng/dL) in the mid-luteal phase[31].

In contrast, Alyasin et al. found that serum progesterone levels higher than 32.5 ng/mL on the day of embryo transfer were associated with lower live birth rates in frozen-thawed cycles. Thomsen et al. reported that mid-luteal progesterone levels above 250 nmol/L (approximately 78 ng/mL) consistently reduced pregnancy chances[32,33]. Boynukalin et al. investigated the threshold progesterone level predicting ongoing pregnancy success in frozen-thawed euploid embryo transfer, finding an optimal cut-off value of 20.6 ng/mL[34].

A recent systematic review and meta-analysis by Melo et al. investigated the association between luteal serum progesterone levels and FET outcomes[35]. This meta-analysis of 21 cohort studies showed that for thresholds <10 ng/mL, participants with higher progesterone levels experienced more ongoing pregnancies or live births, more clinical pregnancies, and fewer miscarriages than those with lower serum progesterone levels. However, there was uncertainty about whether supraphysiologic progesterone levels were associated with better treatment outcomes. As discussed, progesterone levels lower than 10 ng/mL are linked with lower live birth rates, and higher levels from uncontrolled progesterone administration may negatively impact pregnancy outcomes. Although these studies provide important thresholds for progesterone, the significant heterogeneity in publications, different measurement methods, and days limit the generalizability of these values. Additionally, serum progesterone levels may not correlate with uterine tissue levels. While local endometrial progesterone measurement is optimal, it is not routinely practical. Therefore, plasma progesterone levels may not accurately reflect uterine progesterone effects. Progesterone receptor activity may also vary among women. Moreover, plasma progesterone fluctuations and variability in monitoring days (early or mid-luteal phase) are confounding factors. These issues limit the use of a standard cut-off value. Consequently, we retrospectively analyzed our data to examine the results of empiric extra progesterone support without luteal phase monitoring.

There is limited data on the effect of empirically adding extra progesterone supplementation to standard vaginal treatment on IVF success. Devine et al. published an interim analysis of a three-arm RCT comparing vaginal progesterone, daily intramuscular progesterone (IMP), and vaginal progesterone plus every third day IMP. The interim analysis concluded that using only vaginal progesterone resulted in decreased ongoing pregnancies due to increased miscarriage, leading to the discontinuation of the vaginal progesterone arm[36]. Contrary to Devine's findings, miscarriage rates did not increase in the vaginal progesterone-only group in our study. The final version of Devine's research reported that the live birth rate was significantly lower in women receiving only vaginal progesterone (27%) compared to those receiving IMP (44%) or combination treatment (46%)[37]. The authors recommended using vaginal progesterone supplemented with IMP every third day.

Polat et al. conducted a retrospective study on the same topic, investigating every third day IMP supplementation to vaginal progesterone in frozen-

thawed cycles and found that IMP did not enhance ongoing pregnancy rates compared to vaginal progesterone alone[38].

Our retrospective analysis of cycles with different luteal phase supplementation approaches by two senior IVF specialists—vaginal progesterone alone and vaginal plus IM progesterone—revealed no differences in IVF success. This could be due to insufficient progesterone levels with vaginal supplementation and supraphysiologic levels in IM progesterone-added cycles.

Our study has strengths and limitations. We compared different LPS approaches used by two senior IVF specialists at the same ART center. G.U. never used IMP, while I.K. routinely used IMP in all FET cycles. Vaginal progesterone was administered based on pharmacy availability, providing spontaneous randomization of patients in groups. We also reported miscarriage rates, live birth rates, and neonatal outcomes along with positive pregnancy rates. Despite the retrospective design, these factors strengthen our results. However, the lack of mid-luteal progesterone levels complicates result interpretation. Transferring all embryos, including day 5 and day 6 embryos, on the same day (6th day of progesterone) might introduce a bias. However, it has been shown that transferring embryos on the same day does not significantly alter pregnancy outcomes[39].

In conclusion, routine IMP progesterone combined with vaginal progesterone does not improve ART outcomes. This combination may benefit a selective population with monitored luteal phases. More RCTs are needed to clarify luteal phase progesterone monitoring and management in ART cycles.

Ethics Committee Approval Information:
Approving Committee: Bursa Uludag University Faculty of Medicine Clinical Research Ethics Committee
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Researcher Contribution Statement:
Idea and design: G.U., I.K., B.A.; Data collection and processing: T.A., C.Ç; Analysis and interpretation of data: K.A., I.K; Writing of significant parts of the article: K.A.
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