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Research Article / Araştırma Makalesi

Effects of tryptophan, a precursor for melatonin, on IVF outcomes and Doppler parameters

Melatoninin kaynağı triptofanın IVF hastalarının sonuçlarına ve Doppler parametrelerine etkisi

Fatma Devran Bıldırcın[1], Handan Çelik[1], Ayşe Zehra Özdemir[1], Pervin Karlı[2], Bahattin Avcı[3], Sertaç Batıoğlu[1]

[1] Ondokuz Mayıs University IVF Center, School of Medicine, Ondokuz Mayıs University, Samsun, Turkey

[2] Amasya University, School of Medicine, Department of Obstetrics and Gynecology, Amasya, Turkey

[3] Ondokuz Mayıs University, School of Medicine, Department of Biochemistry, Samsun, Turkey

ABSTRACT

Aims: Melatonin is the most powerful antioxidant and protects sperm, oocyte, and embryo against oxidative stress. The effect of tryptophan, which is the building block of melatonin, on follicular melatonin levels and IVF outcomes is unknown. The objective of this study was to investigate the effect of tryptophan administration, a precursor for melatonin, on the levels of intrafollicular melatonin with the aim to reveal the correlation between tryptophan and the total number and quality of oocytes as well as clinical pregnancy rates. In addition, we aimed to examine the effect of melatonin increased by tryptophan on uterine and ovarian blood flow.

Material and Method: Out of 103 patients who applied to Ondokuz Mayıs University Hospital, IVF clinic for IVF treatment, 51 patients were administered a 100 mg dose of tryptophan orally (Group A) and 50 control patients who were randomly selected did not receive tryptophan (Group B). Firstly, follicular melatonin levels were compared between Group A receiving tryptophan and Group B without tryptophan. Both groups were also compared according to the oocyte count, oocyte count, fertilized oocyte count, embryo count, and pregnancy rates, ultimately. In addition, all patients were measured for uterine and ovarian artery blood flow by vaginal ultrasound on the day of OPU.

Results: There were no differences in age (32.16±3.82 years vs 33.06±4.44 years) (p=0.276), BMI (28.45±2.82 kg/m)^2 vs 28.15±3.03kg/m2 (p=0.602) and peak estradiol levels (2451.69±469.75 pg/ml vs 2420.26±443.71 pg/ml) (p=0.73) between the groups. Group A exhibited high levels of melatonin in the follicular fluid with a mean value of 259.8 pg/ml, whereas Group B had 91.3 pg/ml (p<0.001). There were found significant differences in the oocyte count (9.08±3.22 vs 7.66±1.89) (p=0,008), mature oocyte count (7.2±2.8 vs 6.1±1.8) (p=0,021) and fertilized oocyte count (6.35±2.44 vs 5.28±1.69) between group A and group B. Pregnancy rates were higher in group A (35.3%). The pregnancy rate (30%) was lower in Group B, which did not receive tryptophan and had low melatonin levels in follicular fluid. However, there was no statistically significant difference. Uterine, ovarian artery systolic and diastolic blood flows of Group A were significantly lower than Group B (p<0.001).

Conclusions: Administration of tryptophan to IVF patients significantly increases the level of melatonin in follicular fluid. The results demonstrate that high levels of melatonin in follicular fluid may increase oocyte count and quality although they do not significantly improve clinical pregnancy rates.

Keywords: Tryptophan, melatonin, pregnancy, IVF-ICSI

ÖZ

Amaç: Melatonin bilinen güçlü bir antioksidandır ve sperm, oosit ve embrioyu oksidatif strese karşı korur. Melatoninin yapı taşı olan triptofanın foliküler melatonin seviyesine ve IVF sonuçlarına etkisini bilinmemektedir. Bu çalışmada IVF tedavisinde melatonin prekürsörü olan triptofan desteğinin intrafolliküler melatonin seviyesine etkisini incelemek ve bunun oosit sayısı, oosit kalitesi nihayetinde klinik gebelik oranlarında yaptığı farklılığı araştırmak amaçlandı. Ayrıca triptofan desteği ile artan melatoninin uterin ve overiyan kan akımına etkisi amaçlandı.

Gereç ve Yöntem: IVF tedavisi için Ondokuz Mayıs Üniversitesi Hastanesi IVF Kliniği'ne başvuran 103 hastadan 51 hastaya triptofan oral 100 mg verilirken (Grup A); randomize seçilen 50 kontrol hastasına verilmedi (Grup B). Öncelikle triptofan alan Grup A ve almayan Grup B arasında foliküler melatonin seviyesi kıyaslandı. Yine her iki grup oosit sayısı, matür oosit sayısı, fertilize oosit ve embrio sayıları nihayetinde gebelik oranları açısından karşılaştırıldı. Ayrıca tüm hastaların OPU yapıldığı gün uterin ve overiyan arter kan akımları vajinal ultrasonografi ile ölçülüp kayıt edildi.

Bulgular: Gruplar arasında yaş (32,16±3,82 yıl vs 33,06±4,44 yıl) (p=0,276), BMI (28,45±2,82 kg/m^2 vs. 28,15±3,03 kg/m^2) (p=0,602), pik östradiol (2451,69±469,75 pg/ml vs 2420,26±443,71 pg/ml) (p=0,73) değerleri için fark yoktu. Grup A'da folikül sıvısındaki melatonin düzeyi yüksek olup ortalama değeri 259,8 pg/ml; Grup B'de 91,3 pg/ml olarak elde edilmişti (p<0,001). Oosit sayısı(9,08±3,22 vs 7,66±1,89) (p=0,008), matür oosit sayısı (7,2±2,8 vs 6,1±1,8) (p=0,021), fertilize oosit sayısı (6,35±2,44 vs 5,28±1,69) (p=0,012) için grup A ve B arasında anlamlı farklılık görüldü. Yine Grup A'da gebelik oranları (%35,3) daha fazlaydı. Triptofan kullanmayan ve folikül sıvısında melatonin düzeyi düşük olan Grup B'de ise daha az gebelik(%30) elde edilmişti. Ancak bu fark istatistiksel olarak anlamlı değildi (p=0,723). Grup A'nın uterin, ovariyan arter sistolik ve diyastolik kan akımları Grup B'ye göre anlamlı derecede düşük izlendi (p<0,001).

Sonuç: IVF hastalarına triptofan verilmesi follikül sıvısında melatonin seviyelerini önemli ölçüde arttırmaktadır. Bu sonuçlara göre foliküler sıvıdaki yüksek melatonin düzeyleri klinik gebelik oranlarını anlamlı ölçüde arttırmasa da oosit sayısı ve kalitesinin gelişmine olumlu destek sağlayabilir.

Anahtar Kelimeler: Triptofan, melatonin, gebelik, IVF-ICSI

Corresponding Author: Pervin Karlı, Amasya Üniversitesi Sabuncuoğlu Şerafeddin Eğitim ve Araştırma Hastanesi, Kadın Sağlığı ve Hastalıkları Kliniği, Amasya, Türkiye

E-mail: parpi2300@hotmail.com

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INTRODUCTION

Many couples who are unable to conceive spontaneously resort to assisted reproductive techniques such as in-vitro fertilization (IVF). Although these techniques help infertile couples, the rate of pregnancy remains around 29% per cycle (1). Research and studies on infertility prioritize to improve IVF outcomes. There are ongoing research and studies regarding novel adjuvant therapies to meet expectations and reduce health costs. Recently, there has been an increasing interest in the effects of oxidative stress on IVF outcomes.

Standard IVF procedures such as superovulation, oocyte cryopreservation, and embryo freezing lead to the accumulation of reactive oxygen species (ROS). Thus, oocytes and embryos are exposed to high concentrations of ROS. Excess ROS in follicular fluid is considered to impair oocyte quality by inducing apoptosis of oocyte and granulosa cells (2). It has been suggested that anti-oxidant therapy reduces the harmful effects of ROS and therefore improves success rates (3).

Melatonin is a potent free radical scavenger and a broad-spectrum antioxidant. Melatonin alleviates oxidative stress by neutralizing ROS. It modulates the physiology and molecular biology of the cells through different mechanisms. Many human and animal studies support the use of melatonin in the treatment of infertility due to its antioxidant properties (4).

The biosynthesis of melatonin is derived from tryptophan via the pineal gland within the brain. Tryptophan, a precursor for melatonin synthesis, is an essential amino acid and needs to be obtained through the foods that naturally contain it. Tryptophan is removed from plasma via the pineal gland and then hydroxylated with tryptophan hydroxylase in pinealocytes into 5-hydroxytryptophan (5-HTP) and then 5-HTP is converted into serotonin. Serotonin is then converted into N-acetyl 5-methoxy tryptamine, i.e. melatonin, via the enzyme NAT (N-acetyltransferase). All substances involved in the biosynthesis of melatonin by tryptophan exhibit a certain level of antioxidant activity. 5-HTP has been reported to be a more potent radical scavenger compared to melatonin and vitamin C (5).

In this study, the melatonin level in the follicular fluid was measured upon the administration of tryptophan, a melatonin precursor, in IVF patients with the aim to investigate the effects on IVF outcomes. At the same time, we investigated the correlation between the levels of melatonin in the follicular fluid on the day of oocyte pickup (OPU) and oocyte count, embryo quality and clinical pregnancy. In addition, we measured and compared uterine, ovarian artery systolic and diastolic blood flows on OPU day.

MATERIAL AND METHOD

A total of 103 patients who applied to Medical Faculty of Ondokuz Mayıs University, IVF Center were included in the study. The study was designed as a randomized controlled single-blind study.

Ethical Declaration

All authors and the study protocol have complied with the World Medical Association Declaration of Helsinki regarding ethical issues and principles in research involving human subjects. Local ethics committee approval was obtained for the study (OMU TAEK 20117-339) and written informed consent was obtained from the subjects who participated in the study.

The study included a total of 103 women undergoing IVF cycles. 2 patients discontinued treatment and the study was carried out with the remaining 101 patients. A total of 51 patients included in the study were given a dose of 100 mg tryptophan daily (group A), whereas the remaining 50 patients (group B) did not receive tryptophan. The two groups were randomized using a 1: 1 randomization ratio. Embryologists were "blind" as they did not know which group received tryptophan (**Figure 1**).

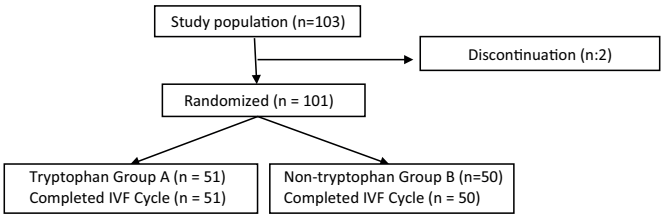

Figure 1. Distribution of patients

Inclusion Criteria

Our study included patients with a history of at least 1-year infertility, regular ovulation and menstruation in addition to normal spermiogram results and tubal patency confirmed by HSG. The patients were selected based on the criteria of the American College of Obstetricians and Gynecologists (ACOG) guidelines. Unremarkable spermiogram results according to ACOG criteria, presence of ovulation, normal tubal patency and uterine cavity by hysterosalpingogram, normal ovarian reserve and diagnosis of infertility by diagnostic laparoscopy were among the inclusion criteria.

Exclusion Criteria

Patients with male factor infertility, poor ovarian reserve, tubal factor infertility and those with any other causes of infertility were excluded. In addition, patients with systemic chronic diseases, uterine fibroids or polyps, endometriosis, polycystic ovary syndrome, endocrine disorders, patients using melatonin-interacting drugs (hypnotics, antidepressants, antiepileptics), continuous medication use, patients undergoing IVF-ICSI due to low ovarian reserve, male factor and tubal factor infertility, patients with BMI under 25, anovulation and those working night shifts were excluded from the study.

Primary outcome: Clinical pregnancy rate.

Secondary outcome: Oocyte count, mature oocyte count, fertilized oocyte count and uterine, ovarian artery systolic and diastolic blood flows.

Tryptophan Administration

The study group (group A) receiving Tryptophan (Lifetime Q-5-Hydroxy Tryptophan) was administered 100 mg tablets orally every day at 22:00 from the day of ovarian stimulation injections (cycle day 2-3). The last capsule was collected at 22:00 the night before oocyte retrieval.

Ovulation Induction

All patients underwent ovulation induction with the standard antagonist protocol. Oocyte pick-up (OPU) was performed 36 hours following the HCG treatment and intracytoplasmic sperm injection (ICSI) was performed 4-6 hours after that. Embryo transfer was performed 3 days after oocyte retrieval. Starting from the day of OPU, progesterone was administered intramuscularly (progestin 50 mg; Koçak, Turkey) and estrogen (estrofem 2 mg; NovoNordisk, Denmark) was administered orally as luteal support. Pregnancy was diagnosed in patients with positive bHCG 14 days after transfer. The diagnosis of clinical pregnancy was confirmed upon the presence of fetal heartbeat.

Sample Collection

Serum samples were taken from the patients to determine the level of melatonin on the second day of menstruation. Transvaginal ultrasound-guided ovum pick up (OPU) was performed under general anesthesia. The levels of melatonin in serum (MSOpu) and Follicular (MFolOpu) fluid were measured on OPU day. The follicular fluid was aspirated from the first dominant follicle without adding any diluent or contaminating with blood. All aspiration procedures were performed between 9-11 am. After oocyte isolation, the follicular fluid samples were centrifuged at 3000 rpm for 10 minutes to separate the supernatants from the tubes containing the follicle fluid and all samples were stored at -80 ° C until the day of OPU. In addition, uterine and ovarian artery Doppler blood flows were measured and recorded by vaginal ultrasound by the same specialist (FDB) on the day of OPU. Doppler ultrasonography was performed using GE LOGIQ P5 3.75 mHz convex probe ultrasound device while the patient was on the supine and lithotomy positions.

Laboratory Analysis

Melatonin levels in serum and follicular fluid samples were examined by Enzyme-linked Immunosorbent Assay (ELISA) method using commercial kits of Melatonin ELISA kit (USCN Life Science Inc., Wuhan, China, Lot. No.E90908Hu) in Research Laboratories of Faculty of Medicine, Ondokuz Mayıs University, Department of Biochemistry Research Laboratories. The samples with high concentration were repeated.

Statistical Method

The data were analyzed using IBM SPSS V23. Kolmogorov-Smirnov test was used to examine the normal distribution. The independent t-test was used for comparing normally distributed data, whereas the Mann Whitney U test was used for comparing non-normally distributed data. The Chi-square test was used for the comparison of categorical data. Normally distributed data were presented as mean±-standard deviation while non-normally distributed data were presented as median (min-max). The categorical data were presented as frequency (percentage). The significance level was accepted as $p<0.05$.

RESULTS

There was no significant difference between the mean age of the patients (32.16-33.06 years) ($p = 0.276$). The mean BMI was (28.45 kg/m^2) in the tryptophan group, which was the same in the non-tryptophan group (28.15 kg/m^2) ($p=0.602$). There was no difference in the mean estradiol values according to the administration of tryptophan ($p=0.73$). The mean value of Group A was 2451,69 pg/ml, whereas the mean value of non-tryptophan group was 2420,26 pg/ml.

The mean values of serum melatonin levels (MSD2) on cycle day 2 did not show significant difference according to the administration of tryptophan ($p=0.429$). The mean value of tryptophan group was 29.28, whereas it was 28.04 in non-tryptophan group. There was no difference in serum melatonin levels (MSOPU) on OPU day according to the administration of tryptophan ($p=0.3307$). The mean value of patients receiving tryptophan was 52.41 while it was 44.98 in the non-tryptophan group, however, there was no significant difference.

The level of melatonin (MFolOPU) in aspirated follicular fluid during OPU on the day of OPU revealed significant differences according to the administration of tryptophan ($p<0.001$). The mean value of Mfol OPU was 259.8 in patients receiving tryptophan, whereas the level of Mfol OPU was 91.3 in those who did not receive tryptophan. It was noted that the administration of tryptophan significantly increased the melatonin level in the follicular fluid.

On the day of OPU, uterine artery blood flow was measured by Doppler. There was found a significant difference between the subjects who received tryptophan and had low follicular melatonin levels and the subjects who did not receive tryptophan and had low follicular melatonin levels ($p<0.001$).

The mean value of uterine Arterial Doppler Pulsatile Index (DoppUa PI) was 1.78 in Group A and 1.83 in Group B.

The mean value of Uterine Arterial Doppler Resistance Index (DoppUa RI) was 0.83 in the tryptophan group and 0.88 in the non-tryptophan group ($p <0.001$).

There was a highly significant difference in the evaluation of ovarian artery blood flow by Doppler between those with low melatonin levels in both the tryptophan and non-tryptophan groups ($p<0.001$).

The mean value of Doppler ovarian artery pulsatile index (DoppOa PI) was as low as 1.07 in patients receiving tryptophan, whereas it was 1.32 in the non-tryptophan group.

The mean values of Doppler overian artery resistance index (DoppOa RI) were also significantly different according to tryptophan administration (p<0.001). The mean value was 0.83 in the tryptophan group, whereas it was 1.01 in the non-tryptophan group (**Table 1**).

Table 1. Comparison of parameters according to the administration of tryptophan

	Group A (n = 51)	Group B (n = 50)	P
Age	32.16 ± 3.82	33.06 ± 4.44	0.276
BMI	28.45 ± 2.82	28.15 ± 3.03	0.602
Peak Oestradiol Level	2451.69 ± 469.75	2420.26 ± 443.71	0.730
MS D2 (serum on day 2)	29.28 ± 7.58	28.04 ± 8.07	0.429
MS Opu (serum on OPU day)	52.41 ± 50.56	44.98 ± 10.48	0.307
M FolOpu	259.8 ± 64.31	91.3 ± 19.83	<0.001
DoppUa PI	1.78 ± 0.03	1.83 ± 0.04	<0.001
DoppUa RI	0.83 ± 0.03	0.88 ± 0.05	<0.001
DoppOa PI	1.07 ± 0.19	1.32 ± 0.14	<0.001
DoppOa RI	0.83 ± 0.21	1.01 ± 0.15	<0.001
Oocyte Count	9.08 ± 3.22	7.66 ± 1.89	0.008
Mature Oocyte Count	7.2 ± 2.8	6.1 ± 1.8	0.021
Fertilized Oocyte Count	6.35 ± 2.44	5.28 ± 1.69	0.012
G1 Embryo	5.41 ± 2.22	4.04 ± 1.67	0.001

The mean values of total oocytes retrieved were significantly different with respect to the levels of melatonin in the follicular fluid according to the administration of tryptophan (p= 0.008). The mean value was 9.08 in Group A and 7.66 in Group B, respectively. Similarly, Mature Oocyte Count (p=0.021), Fertilized Oocyte Count (p=0.012) and G1 embryo count (p=0.001) were also significantly higher in Group A (**Table 2, Figure 2-3**)

Table 2. Comparison of pregnancy rates between the groups

Pregnancy Rate	Positive	Negative
Group A	18 (35.3)	33 (64.7)
(Group B)	15 (30)	35 (70)

p = 0.723 (for positive)

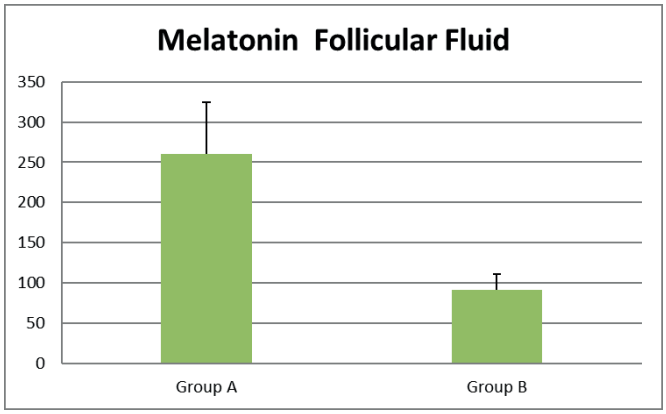

Figure 2. Comparison of melatonin in follicular fluid between the groups

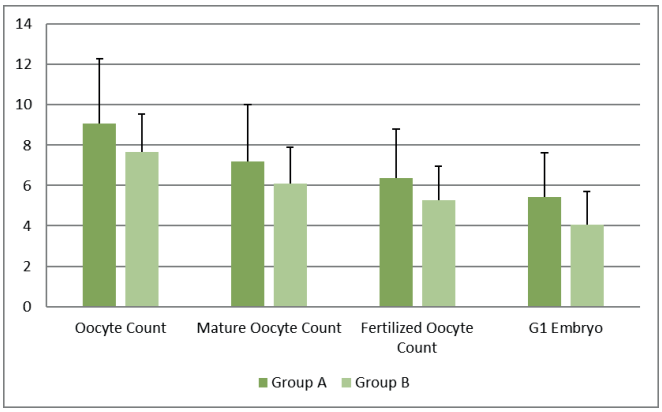

Figure 3. Schematic representation of the oocyte count, mature oocyte count, fertilized oocyte count and G1 embryo count between the groups

Pregnancy rates were higher in Group A (35.3%). In Group B, lower rates of pregnancy (30%) were achieved. However, there was no statistically significant difference in pregnancy rates between the groups (p=0.723).

DISCUSSION

Melatonin is a very powerful antioxidant produced via the pineal gland. Unlike other antioxidants, melatonin can exert its antioxidant effects both directly or through MT1 and MT2 receptors (6-8). Melatonin has an amphiphilic molecular character, which allows it to easily pass through the cell membrane and dispense to the nucleus (7,9,10). Most importantly, the metabolites of melatonin also show antioxidant effects without leading to oxidative stress (8). This leads to the formation of a cascade. Melatonin increases the effect of other antioxidants such as glutathione peroxidase and superoxide dismutase (11). Melatonin produced in the reproductive organs is thought to play a role in the regulation of many reproductive processes. Granulosa cells contain melatonin receptors. (12). In addition, large follicles have more melatonin than small ones (13). Again, it was observed that melatonin supplementation lead to a higher increase in serum levels compared to follicular fluid levels (14). These results suggest that melatonin may be effective in oocyte maturation. Free oxygen radicals are formed in each stage of IVF treatment. Melatonin has therefore been investigated in many studies due to its antioxidant effects in adjuvant therapy. In their study, Tamura et al. (15) found that the level of melatonin decreased in parallel with increasing levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG), which is a marker of oxidative stress in follicular fluid. Rizzo et al. (16) carried out a study and compared two groups receiving myo-inositol plus folic acid plus melatonin and myo-inositol plus folic acid revealing that the number of mature oocytes was higher in the melatonin group. Tamura et al. (17) studied patients undergoing unsuccessful IVF, divided the patients into two groups and treated the first group with orally administered melatonin. It was found that fertilization rates were higher in the group receiving melatonin. However, Fernando et al. (18) divided the patients into four groups in the pilot double blind study. They found that there were no differences

in pregnancy rates or the oocyte and embryo parameters other than the level of melatonin in follicular fluid between the patients who received placebo or 2mg, 4mg and 8mg melatonin replacement twice daily. Tong et al. (19) showed that the level of melatonin in follicular fluid was a marker of oocyte count and quality. In addition, it was revealed that there was a correlation between melatonin levels in follicular fluid and AMH levels as a marker of ovarian reserve. Again, it was found that the level of melatonin in follicular fluid was correlated with IVF outcomes, which was consistent with our study. The patients with high melatonin levels exhibited a higher number of oocytes, retrieved, more fertilized oocytes and higher rates of blastocyst. In this study, we investigated the effect of tryptophan, which is a precursor for melatonin and an essential amino acid, on IVF outcomes and found that the group treated with tryptophan exhibited a higher number of oocytes, mature oocytes and fertilized oocytes although there was no statistical difference in clinical pregnancy rates. The study also examined the effect of tryptophan on Doppler parameters in IVF patients. Although melatonin altered blood flow in many vascular beds, Fernando et al. (20) demonstrated that administration of melatonin in IVF patients did not alter uterine and ovarian Dopplers. We found higher levels of uterine and ovarian Doppler flow in the tryptophan group. Our study was significant in respect to including patients only with unexplained infertility as a homogenous group, besides, the patients were examined for both serum and follicular fluid. A greater number of patients should be examined in order to present more accurate results. Melatonin is a highly safe preparation when used as an antioxidant. No serious side effects were observed in any of the studies (21). No teratogenic effects were observed in the pregnant or infertile patient population (22). However, there are several authors who argue that it can aggravate diseases such as rheumatoid arthritis or multiple sclerosis as it causes immunostimulation. There can be found some authors reporting autoimmune hepatitis in the literature (23-25). In this study, we did not encounter any side effects associated with the administration of tryptophan.

In conclusion, tryptophan, a melatonin precursor, did not lead to a statistically significant difference in pregnancy rates but it increased the number and quality of oocytes in IVF patients. Prospective studies with greater number of patients are needed to obtain more accurate results.

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No

CONFLICT OF INTEREST DISCLOSURE

The authors have stated explicitly that there are no conflicts of interest in connection with this article

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