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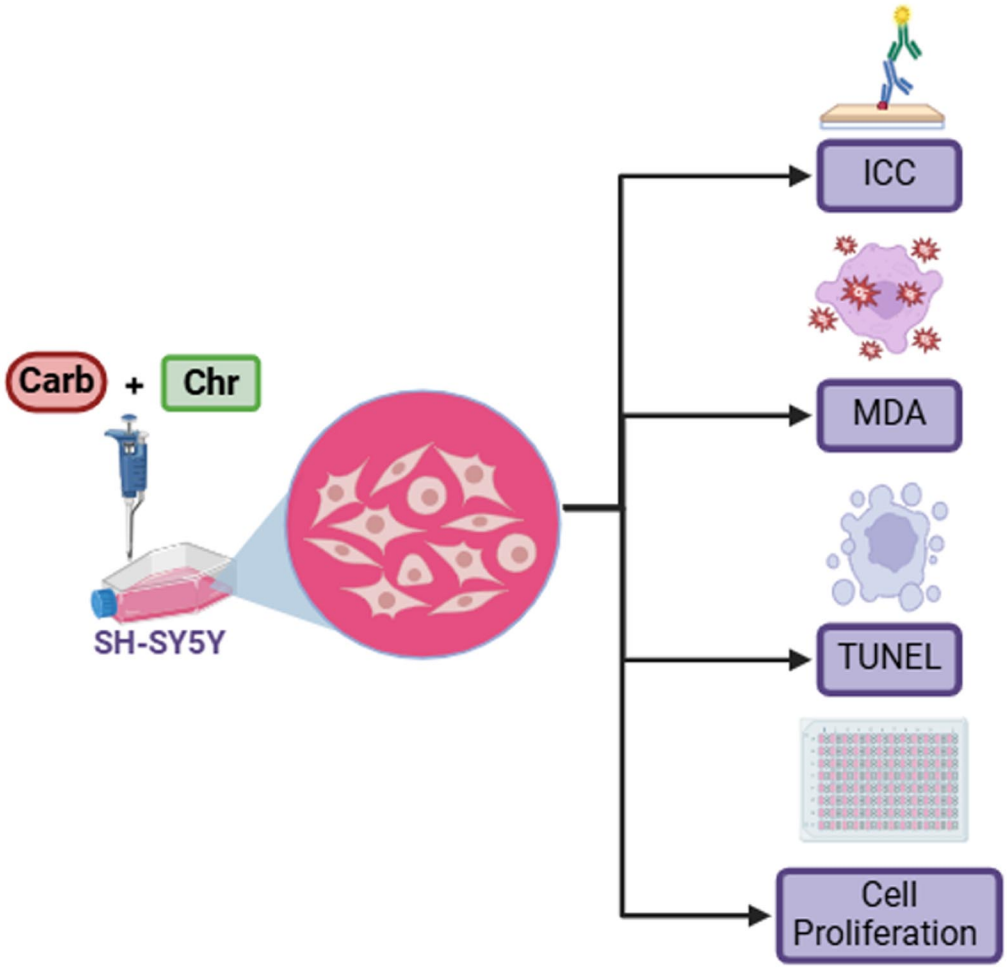

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[CONTENTS]

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AIM AND SCOPES
Journal of Cellular Neuroscience and Oxidative Stress is an online journal that publishes original research articles, reviews and short reviews on the molecular basis of biophysical, physiological and pharmacological processes that regulate cellular function, and the control or alteration of these processes by the action of receptors, neurotransmitters, second messengers, cation, anions, drugs or disease.

Areas of particular interest are four topics. They are;

A-Ion Channels (Na^+- K^+ Channels, Cl^- channels, Ca^{2+} channels, ADP-Ribose and metabolism of NAD^+, Patch-Clamp applications)

B-Oxidative Stress (Antioxidant vitamins, antioxidant enzymes, metabolism of nitric oxide, oxidative stress, biophysics, biochemistry and physiology of free oxygen radicals)

C-Interaction Between Oxidative Stress and Ion Channels in Neuroscience
(Effects of the oxidative stress on the activation of the voltage sensitive cation channels, effect of ADP-Ribose and NAD^+ on activation of the cation channels which are sensitive to voltage, effect of the oxidative stress on activation of the TRP channels in neurodegenerative diseases such Parkinson's and Alzheimer's diseases)

D-Gene and Oxidative Stress
(Gene abnormalities. Interaction between gene and free radicals. Gene anomalies and iron. Role of radiation and cancer on gene polymorphism)

READERSHIP

Biophysics	Biochemistry
Biology	Biomedical Engineering
Pharmacology	PhysiologyGenetics
Cardiology	Neurology
Oncology	Psychiatry
Neuroscience	Neuropharmacology

Keywords
Ion channels, cell biochemistry, biophysics, calcium signaling, cellular function, cellular physiology, metabolism, apoptosis, lipid peroxidation, nitric oxide, ageing, antioxidants, neuropathy, traumatic brain injury, pain, spinal cord injury, Alzheimer's Disease, Parkinson's Disease.

RESEARCH ARTICLE

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The oocyte quality and follicular fluid lipid peroxidation and antioxidant levels in the patients undergoing in vitro fertilization treatment

Dilek Ulusoy KARATOPUK

Department of Histology and Embryology, Medicine School, University of Suleyman Demirel, Isparta, Türkiye

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***Address for correspondence:**

Assist. Prof. Dr. Dilek Ulusoy KARATOPUK,
Department of Histology and Embryology, Medicine School,
University of Suleyman Demirel, Isparta, Türkiye
E-mail: *dilekkaratopuk@sdu.edu.tr*

List of Abbreviations;

FF, *Follicular Fluid;* ***GSH,*** *Glutathione;* ***GSH-Px,*** *Glutathione Peroxidase;* ***ROS,*** *Reactive Oxygen Species;* ***IVF,*** *In Vitro Fertilization;* ***MII,*** *Metaphase II;* ***MDA,*** *Malondialdehyde;* ***BMI,*** *Body Mass Index;* ***OS,*** *Oxidative Stress;* ***SOD,*** *Superoxide Dismutase;* ***UI,*** *Unexplained Infertility;* ***MF,*** *Male Factor;* ***ASRM,*** *American Society for Reproductive Medicine;* ***ml,*** *Mililiter;* ***SPSS,*** *Statistical Package for the Social Sciences.*

Abstract

This study aimed to compare demographic, embryological, and biochemical parameters between male factor infertility and unexplained infertility groups undergoing IVF treatment. Demographic parameters such as age and (BMI, along with embryological factors including the number of oocytes retrieved, MII oocytes, fertilization rate, and Grade II embryo quality, were evaluated. Biochemical markers, including MDA, GSH, GSH-Px, vitamin A, vitamin E, and ß-carotene, were also analyzed. No significant differences were observed between the two groups regarding demographic or embryological outcomes ($p > 0.05$), although Grade I embryo quality showed a significant difference ($p = 0.047$), underscoring the importance of embryo quality in IVF success. The clinical pregnancy and live birth rates were higher in the control group, but the differences were not statistically significant.

Further biochemical analysis revealed significantly higher MDA levels in the patient group, indicating increased oxidative stress ($p < 0.001$). Conversely, antioxidant levels, including GSH, GSH-Px, vitamin A, and vitamin E, were found to be lower in the patient group, suggesting a weakened defense against oxidative stress.

In conclusion, this study emphasizes the importance of managing oxidative stress through antioxidant levels to potentially improve IVF success rates.

Keywords: Follicular fluid; In vitro fertilization; Lipid peroxidation; Oocyte quality; Embryo quality; Vitamin E.

Introduction

Follicular fluid (FF) is a natural environment that supports the final stage of oocyte maturation, containing components such as steroid hormones, growth factors, and both enzymatic and non-enzymatic molecules, including antioxidants (Fonseca et al., 2023). In addition to endocrine factors, it is widely accepted today that nutrition can affect reproductive performance through modulation of the endocrine environment (Schweigert et al., 2003). Although the roles of gonadotropins and steroids in follicular development have been extensively studied in various

species, there is limited information on the importance of micronutrients, such as minerals, trace elements, and vitamins. Certain micronutrients can modulate endocrine mechanisms by mimicking or acting similarly to steroid hormones (Schweigert et al., 2003). Moreover, changes in their composition may influence the in vitro fertilization (IVF) process and outcomes (Fonseca et al., 2023).

Oxidative stress (OS) in FF has been associated with oocyte growth quality and reduced fertilization rates. While OS is considered one of the factors affecting oocyte and follicle development, further research and data are needed to clarify its effects. Reactive oxygen species (ROS), produced during physiological processes in healthy follicles, play a role in oocyte quality, maturation, and subsequent stages of development. An excess of ROS can adversely affect the follicle, oocyte, and consequently embryo quality, thus impacting IVF success rates (Alasmari et al., 2018; von Mengden et al., 2020; Tural et al., 2021). ROS include several radicals such as superoxide and singlet oxygen.

Antioxidants are responsible for scavenging ROS. Among the enzymatic scavengers of ROS are glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD), while non-enzymatic scavengers include reduced glutathione (GSH), vitamin A (retinol), vitamin E (α-tocopherol), and β-carotene (Agarwal et al., 2005; Jamro et al., 2019; Vašková et al., 2023; Afrough et al., 2024). The primary scavenger of ROS in the lipid phase of oocyte membranes and cells is tocopherol, and GSH of antioxidant action recovers its oxidized state. Anovulation is linked to low levels of vitamin A and carotenoids, which shield cells from superoxide radicals (Gode et al., 2019). One prevalent characteristic of IVF is an imbalance between the oxidant and antioxidant systems (Bahadori et al., 2017).

IVF is considered a critical treatment for infertility, yet success rates remain suboptimal. Patients undergoing IVF experience numerous social, psychological, and physiological challenges. Infertility prevalence among reproductive-aged couples is approximately 15%, with etiologies including male factors (30%) and unexplained infertility (25%). In unexplained infertility (UI), factors such as oocyte quality or tubal issues may play a role, but underlying causes remain unclear, and success rates have yet to reach the desired levels (Şentürk et al., 2022).

The potential of OS products as biomarkers for IVF success is still debated. Therefore, the aim of this study is to investigate OS biomarkers, including malondialdehyde (MDA), thiol group antioxidants such as GSH and GSH-Px, as well as levels of antioxidant vitamins (vitamin A, vitamin E, and β-carotene). By using an unexplained infertility and male factor (MF) IVF cycle approach, we aim to evaluate the relationship between OS homeostasis in FF and serum and the number of collected oocytes, MII (metaphase II) oocyte count, fertilization, embryo quality, pregnancy, and birth outcomes. Additionally, this study will explore the correlations between OS biomarkers and antioxidant enzymes (Campos Petean et al., 2008; Jeremic et al., 2025).

Material and Methods

Controls and Patients

This study was conducted on UI and MF infertile patients admitted to Suleyman Demirel University IVF unit. The study was approved by the Institutional Ethics Committee of Suleyman Demirel University (92/2).

The diagnosis of unexplained infertility was made after performing recommended tests based on the guidelines of the ASRM PC (Practice Committee of the American Society for Reproductive Medicine, 2006). If the results of all these tests were normal, the couples were accepted as UI. The control group comprised of 20 primary infertile women undergoing an intracytoplasmic sperm injection (ICSI)- embryo transfer success cycle due to MF. Severe male infertility including azoospermia and severe oligoasthenospermia were excluded. The patients had not received any drug which contained vitamin and minerals. Comparisons across the two groups of $n = 20$ for each.

Sample collection

FF samples were aspirated from mature follicles and collected, then centrifuged at 2500 rpm for 10 minutes. Blood samples were obtained immediately prior to oocyte retrieval, and both serum and FF samples were refrigerated and stored at −80°C until analysis. Oocytes were assessed under light microscopy (Olympus CX-21, Tokyo, Japan) to determine the number of MII oocytes (Bhardwaj et al., 2021). All MII oocytes underwent ICSI, and fertilization was defined by the presence of two pronuclei, which were recorded. Embryos were transferred on days 3, 4, or 5. The number of transferred embryos based on the patient's age and embryo quality was noted. Clinical pregnancy was defined by the detection of a gestational sac with fetal heart pulsations at 5-7 weeks post-embryo transfer, while the live birth rate referred to the delivery of a viable fetus.

Embryo quality was assessed and classified based on morphological appearance under an inverted microscope (Olympus IX-70, Tokyo, Japan) on days 3, 4, and 5 (Machtinger & Racowsky, 2013; Şentürk et al., 2022).

The MDA analyses

Using the thiobarbituric acid reaction using Placer et al.'s technique (1966), MDA levels in plasma and FF were determined. Lowry's method (1951) was used to measure the protein contents of serum and FF. The MDA levels in the FF and serum were quantified as μmol/g protein.

GSH and GSH-Px analyses

Using Sedlak and Lindsay's (1968) method, the GSH content of the serum and FF samples was determined at 412 nm. GSH result was expressed as μmol/g protein. The spectrophotometric method of Lawrence and Burk (1976) was used to assess the GSH-Px activity of FF samples at 37 °C and 412 nm using a UV-1800 (Shimadzu, Kyoto, Japan). As IU/g protein, the GSH-Px activity data were shown.

Antioxidant vitamin analyses

Samples of serum and FF 0.25 milliliter (ml) were saponified by adding 1 ml of ethanol and then heating for 30 minutes at 70 °C. The samples were allowed to chill on ice before being combined with 2 ml of water and 1 ml of n-hexane. They were then allowed to rest for 10 minutes in order to allow for phase separation. The concentration of vitamin A at 325 nm was determined using an aliquot of 0.5 ml of n-hexane extract (Suzuki and Katoh, 1990). After reactants were introduced, hexane's absorbance value was determined at 535 nm using a spectrophotometer (UV-1800) (Desai et al., 1980). Using a spectrophotometer, the amount of β-carotene in hexane was determined at 453 nm (Suzuki and Katoh, 1990). The results of vitamin A, E, and β-carotene were shown as μmol/l.

Statistical Analyses

The data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 24.0 for Windows (SPSS Inc, Chicago, IL, USA). All results are expressed as means ± standard deviation. A normality test (Shapiro-Wilk test) was applied to determine whether the data followed a normal distribution. When the normality assumption was met, independent t-tests or paired t-tests were used to assess differences between groups. To evaluate the treatment effect, one-way repeated measures ANOVA was performed to examine significant differences between groups. Following the ANOVA results, post-hoc analysis was conducted using the LSD (Least Significant Difference) test. Additionally, effect size (Cohen's d) was calculated to assess the practical significance of the treatment intervention. Statistical significance was set at $p < 0.05$.

Results

In this study, data on age, body mass index (BMI), the number of retrieved oocytes, MII, fertilization, total embryo count, grade I, grade II, pregnancy, clinical

Table 1. Demographic information and IVF results by groups (n=20).

	Controls (MF) n=20		**Patients (UI) n=20**		
	Mean	Std. Deviation	Mean	Std. Deviation	*p*-value
Age	27.40	5.7	29.10	4.68	0.24
BMI (kg)	26.84	4.71	27.36	4.29	0.40
Retrieved oocyte	13.80	10.23	13.60	5.80	0.48
Number of MII oocyte	11.30	8.08	9.20	5.07	0.25
Fertilization	7.40	3.81	5.70	2.50	0.13
Total embryo	7.20	3.39	5.70	2.50	0.14
Number of grade I	4.60	2.07	3.30	1.06	0.05*
Number of grade II	1.90	1.20	1.80	1.14	0.43

pregnancy, and birth outcomes were recorded for 20 volunteers in each group diagnosed with male factor infertility and unexplained infertility who underwent IVF treatment, totaling 40 participants. To determine OS and antioxidant levels, MDA, GSH, GSH-P-x, OS parameters, as well as levels of vitamin A, vitamin E, and β-carotene were measured in FF and serum samples.

Age, BMI, the number of collected oocytes, MII, fertilization, total embryo count, and grade I and grade II findings for the control and patient groups (Table 1) were analyzed using the independent samples t-test. According to the results, no significant differences were found between the groups in terms of age, BMI, the number of collected oocytes, MII, fertilization, total embryo count, and grade II variables (p> 0.05). However, the grade I variable showed a significant difference between the groups (p = 0.047).

pregnancy, clinical pregnancy, and birth rates between the control and patient groups were not statistically significant (p> 0.05).

The biochemical parameters such as MDA, GSH, GSH-Px, vitamin A, vitamin E, and β-carotene were compared in FF samples obtained from control and patient groups (Table 3). Accordingly, MDA levels were found to be significantly higher in the patient group (p < 0.001). This indicates an increase in OS, with a large effect size (Cohen's d = 0.83).

In the control group, GSH, GSH-Px, vitamin A, and vitamin E levels were significantly higher compared to the patient group. However, when examining the effect size of these parameters, GSH-Px and vitamin E exhibited a high effect size (Cohen's d = 0.79-0.77), while GSH had a moderate effect size (Cohen's d = 0.52), and vitamin A showed a small effect size (Cohen's d = 0.13).

Table 2. Pregnancy, clinical pregnancy and birth rates between groups (n=20).

	Pregnancy		**Clinical Pregnancy**		**Birth**	
	Positive	Negative	Positive	Negative	Yes	No
Controls (MF) (n=20)	14	6	14	6	12	8
Patients (UI) (n=20)	12	8	12	8	11	9

Table 3. Antioxidant and malondialdehyde (MDA) levels in follicular fluid of control and patients (n=20, Mean ± Std. Deviation).

	Controls (MF) (n=20)	**Patients (UI) (n=20)**	***p*-value**
MDA*	7.46 ± 0.90	9.18 ± 0.82	< 0.001
GSH*	4.01 ± 0.60	3.56 ± 0.42	0.036
GSH-Px$^{\Delta}$	9.63 ± 0.86	4.74 ± 0.71	< 0.001
Vitamin A$^{\#}$	5.32 ± 0.14	4.86 ± 0.13	< 0.001
Vitamin E$^{\#}$	18.45 ± 0.62	16.62 ± 0.89	< 0.001
ß-Carotene$^{\#}$	1.37 ± 0.15	1.32 ± 0.14	0.217

*µmol/g protein. $^{\Delta}$IU/g protein. $^{\#}$µmol/l.

The positive and negative rates for pregnancy and clinical pregnancy, as well as whether or not labor occurred, were recorded between the groups (Table 2). When examining pregnancy outcomes, no significant difference was found, although the number of negative pregnancies was slightly higher in the patient group. The clinical pregnancy and birth rates were found to be slightly higher in the control group. However, the differences in

No significant difference was observed for β-carotene between the groups (p = 0.217), and therefore, no clinical difference was noted.

The biochemical parameters such as MDA, GSH, vitamin A, vitamin E, and ß-carotene were compared in serum samples obtained from control and patient groups (Table 4). Accordingly, MDA levels were found to be significantly higher in the patient group compared to the

control group, while GSH, vitamin A, vitamin E, and ß-carotene levels were significantly lower ($p < 0.001$). ß-carotene levels were also found to be lower in the patient group compared to the control group, with a significant difference (p=0.001).

When evaluating the effect sizes, vitamin E exhibited a high effect size (Cohen's d = 1.27), MDA showed a moderate effect size (Cohen's d = 0.43), while GSH (Cohen's d = 0.27), vitamin A (Cohen's d = 0.17), and ß-carotene (Cohen's d = 0.18) showed small effect sizes.

Table 4. Antioxidant and malondialdehyde (MDA) levels in the serum of control and IVF patients (n=20, Mean ± Std. Deviation).

	Controls (MF) (n=20)	Patients (UI) (n=20)	*p*-value
MDA*	5.66 ± 0.50	7.40 ± 0.35	< 0.001
GSH*	4.13 ± 0.26	3.46 ± 0,28	< 0.001
Vitamin A#	4.94 ± 0.15	4.48 ± 0.19	< 0.001
Vitamin E#	29.93 ± 1.12	25.66 ± 1.41	< 0.001
β-Carotene#	1.80 ± 0.13	1.52 ± 0.21	= 0.001

*µmol/g protein. #µmol/l

Discussion

This study aimed to compare the demographic, embryological, and biochemical parameters between groups diagnosed with male factor infertility and unexplained infertility who underwent IVF treatment. The comparison included demographic parameters such as age and BMI, as well as embryological parameters like the number of oocytes retrieved, the number of MII oocytes, fertilization rate, and Grade II embryo quality. Additionally, biochemical parameters such as MDA, GSH, GSH-Px, vitamin A, vitamin E, and ß-carotene were also compared.

In the statistical analysis of demographic and IVF treatment outcomes, no significant differences were found between the control and patient groups regarding age, BMI, number of oocytes retrieved, number of MII oocytes, fertilization rate, and Grade II embryo quality ($p > 0.05$). These findings suggest that demographic characteristics and treatment response were similar between the groups, and consequently, the differences between the groups did not have a direct impact on treatment outcomes. Moreover, the absence of a difference in Grade II embryo quality further emphasizes the relationship between embryo quality and IVF outcomes (Browne et al., 2009; Choi et al., 2015; Bahadori et al., 2017; Alasmari et al., 2018; Şentürk et al., 2022).

A significant difference was observed in Grade I embryo quality ($p = 0.047$). Higher embryo quality is known to improve pregnancy rates and increase treatment success (Browne et al., 2009; Choi et al., 2015; Bahadori et al., 2017; Alasmari et al., 2018; Şentürk et al., 2022). The clinical impact of this difference will be clarified through further research involving larger sample sizes.

The efficacy of IVF treatment provides important insights into the success of treatment across different infertility causes. Clinical pregnancy and live birth rates were higher in the control group compared to the patient group. While this difference was not statistically significant, future research with advanced diagnostic tests and larger sample sizes could contribute to improving IVF success rates by targeting specific infertility types (Abdallah et al., 2020).

In the FF samples, the MDA levels were significantly higher in the patient group, and the difference was statistically significant ($p < 0.001$). Pekel et al. (2015) highlighted increased MDA levels in a similar study. Elevated MDA levels indicate increased OS, which can negatively affect infertility treatment outcomes. The high effect size of MDA (Cohen's d = 0.83) suggests that OS is a crucial biochemical parameter for patients undergoing IVF treatment (Pekel et al., 2015). Furthermore, MDA levels have been shown to change with age and significantly impact IVF outcomes (Chen et al., 2023). In contrast, antioxidant levels such as GSH, GSH-Px, vitamin A, and vitamin E were significantly higher in the control group, indicating stronger antioxidant defence mechanisms and more effective protection against OS. Several studies have correlated these antioxidants with oocyte quality, fertilization rates, embryo quality, pregnancy, and birth

outcomes (Paszkowski et al., 1995; Melo et al., 2016; Bahadori et al., 2017).

The difference in GSH levels demonstrated a moderate effect size (Cohen's d = 0.52), aligning with findings that high GSH levels are correlated with improved embryo quality, fertilization rates, and pregnancy success (Choi et al., 2015; Nishihara et al., 2018; Zal et al., 2020).

While MDA levels were high in infertile women (Mehendale et al., 2009), vitamin E concentrations were lower in studies on female infertility (Prieto et al., 2012; Bahadori et al., 2017; Skowrońska et al., 2020). Our study's results, showing a high effect size for vitamin E (Cohen's d = 0.77), support the positive impact of vitamin E on IVF outcomes, as reported in other studies.

GSH-Px levels demonstrated a high effect size (Cohen's d = 0.79), indicating the critical role of this parameter in fertilization (Paszkowski et al., 1995). Vitamin A levels showed a small effect size (Cohen's d = 0.13), and its role in the process, along with other antioxidants, was found to be less significant (Bhardwaj et al., 2021). Skowrońska et al. (2020) stated that combinations of vitamins A and E could play a critical role in the development of effects depending on the day.

Although no significant difference in ß-carotene levels was found between groups (p = 0.217), its effect size was small (Cohen's d = 0.18), indicating variability in its impact (Tiboni et al., 2004; Bhardwaj et al., 2021). Schweigert et al. (2003) stated that the amount of transition into follicular fluid could affect fertilization success.

In serum samples, MDA levels were also higher in the patient group compared to the control group ($p < 0.001$). This finding indicates increased OS, which can negatively influence IVF treatment outcomes. Additionally, GSH, vitamin A, vitamin E, and ß-carotene levels were significantly lower in the patient group ($p < 0.001$), suggesting that the patient group had a weakened ability to cope with OS (Schweigert et al., 2003; Campos Petean et al., 2008; Browne et al., 2009; Pekel et al., 2015; Melo et al., 2016; Jamro et al., 2019; Skowrońska et al., 2020). Similar results were observed in the FF, emphasizing the importance of OS and antioxidants in IVF treatment outcomes.

In conclusion, this study reveals that, while there are similarities in treatment response between MF and UI groups, certain factors such as embryo quality show significant differences. Understanding the impact of OS and antioxidant levels on IVF treatment success could be a crucial step in modifying treatment strategies. Furthermore, potential improvements in treatment could contribute to better IVF outcomes in the future.

Author contributions DUK prepared the tables and conceptualised and designed the study. DUK carried out antioxidant and lipid peroxidation investigations.

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Ethical Approve The local human ethics committee of Suleyman Demirel University approved the project. (Protocol Number: 92/2).

ORCID

D Ulusoy Karatopuk 0000-0002-9984-294X

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