

An investigation of the effects on follicular-embryonal development and pregnancy outcomes of serum and follicular fluid ischemia-modified albumin in cases of unexplained infertility receiving *in vitro* fertilization treatment

A. Yaylalı¹, M. Bakacak^{2,*}, Z. Bakacak³, F. Tolun⁴

¹Department of Histology and Embryology and IVF Center, Kahramanmaraş Sutcu Imam University, School of Medicine, Kahramanmaraş (Turkey)

²Department of Obstetrics and Gynecology and IVF Center, Kahramanmaraş Sutcu Imam University, School of Medicine, Kahramanmaraş (Turkey)

³Private Clinic, Kahramanmaraş (Turkey)

⁴Department of Biochemistry, Kahramanmaraş Sutcu Imam University, School of Medicine, Kahramanmaraş (Turkey)

Summary

Objective: To show the effect of the oxidative stress index (OSI) of serum and follicular fluid and ischemia-modified albumin (IMA) levels on embryonic development and pregnancy in cases of unexplained infertility applied with in-vitro fertilization (IVF). **Materials and Methods:** A total of 80 patients were treated with antagonist protocol in the first cycle of IVF for a diagnosis of unexplained infertility. We recorded demographic data, blood and follicular fluid levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), anti-Müllerian hormone (AMH), OSI and IMA. Using ROC analysis for follicular fluid OSI and IMA, patients were grouped according to the cutoff values determined (11.15 and 0.509, respectively). **Results:** No correlation was detected between serum OSI and IMA values and the implantation rate. In the follicular fluid OSI ≤ 11.15 group (n = 32), beta HCG positivity was seen in 65.6% of patients and live births in 50%. In the IMA ≤ 0.509 group (n = 24), beta HCG positivity was seen in 66.6% and live births in 50%. With respect to follicular fluid OSI and IMA values, the difference in terms of both beta HCG positivity and live birth rates was statistically significant ($p = 0.003$, $p = 0.007$, and $p = 0.044$, $p = 0.028$, respectively). The BMI value was significantly high in the IMA > 0.509 group ($p < 0.05$). **Conclusions:** In cases of unexplained infertility with high BMI, increased IMA but not OSI in follicular fluid during IVF treatment could be related to decreased implantation and live birth rates. Follicular fluid IMA levels below the cutoff value may predict pregnancy success.

Key words: Embryogenesis; Implantation; *In vitro* fertilization; Ischemia-modified albumin; Oxidative stress index; Unexplained infertility.

Introduction

Infertility affects approximately 13%-15% of couples. The term *unexplained infertility* describes cases where no abnormality is detected in sperm analysis, ovulation tests and pathological evaluation of the fallopian tubes [1]. The prevalence of unexplained infertility varies between 22% and 28%. Tests used in the investigation of routine fertility are insufficient in the evaluation of the reproductive system. The etiology of unexplained infertility has primarily been ascribed to immunological, genetic and endocrinological factors [2]. According to recent literature, oxidative stress is associated with conditions affecting the female reproductive system, including unexplained infertility, endometriosis, tubal infertility, Polycystic Ovary Syndrome (PCOS), hydatidiform molar pregnancy and embryopathies [3].

Oxidative stress causes the emergence of reactive oxygen species (ROS), substances that occur naturally within cells during any aerobic process. An increase of these substances in the body has been reported to cause DNA damage, lipid peroxidation and protein damage [4].

Oxygen used in aerobic metabolism plays an important role in the energy requirements of human gametes and in the physiological processes of free radicals in the ovaries. Several studies have shown that ROS found in the follicular fluid environment could affect the folliculogenesis and steroidogenesis cycle. Oocytes obtained from weakly vascularized follicles with reduced development potential have been shown to have low intrafollicular oxygen; lipid peroxidation is elevated in these conditions [5].

Oxidative stress contributes to the development of impaired embryos, halting embryo growth in assistive reproductive treatment that in turn increases apoptosis and DNA

damage related to induced cell membrane damage. As a result of apoptosis, fragmented embryos, which are a reason for limited implantation, decrease live birth rates [6, 7].

Increased ROS during ischemia decreases the cobalt binding capacity of albumin; this new chemically changed albumin is known as ischemia-modified albumin (IMA). IMA measurement has recently been proposed as a sensitive marker for the diagnosis of myocardial ischemia and acute coronary syndrome [8]. In addition, IMA is used in the diagnosis of pulmonary thromboembolism, lower limb ischemia, and cerebrovascular ischemic diseases [9]. IMA is currently regarded as a marker of oxidative stress related to ischemia reperfusion in any organ because it is elevated in various clinical entities associated with oxidative stress, such as systemic sclerosis, type-2 diabetes, and PCOS [10-13].

To the best of our knowledge, there are no studies focusing on the relationship of IMA levels and unexplained infertility, and the effect on IVF results. Accordingly, we set out to measure the oxidative stress index (OSI) and IMA levels in both serum and follicular fluid in patients applied with IVF in order to study the effect on embryo development and pregnancy.

Materials and Methods

Patients included in the study received IVF treatment following a diagnosis of unexplained infertility at the Kahramanmaraş Sutcu Imam University IVF Centre between May 2018 and May 2019. Informed consent was obtained from all the study participants. Patients ranged 23-38 years old, showing no uterine or tubal pathology, nor diagnosis of male factor and ovulation disorder. All patients were undergoing treatment with antagonist protocol in the first cycle of IVF after not achieving pregnancy with at least two intrauterine inseminations. OSI and IMA values in the blood and follicular fluid were analyzed. Age, duration of infertility, and gravida were recorded. Follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), and anti-Müllerian hormone (AMH) hormone profiles were recorded at the initial visit. Patients outside the age range, unwilling to participate, or with any chronic disease, history of cancer, endocrine disorder, as well as those from whom eggs had not been taken or transferred, were excluded from the study.

The antagonist protocol (rFSH, Gonal F, Merck Sereno, Modugno, Italy) was applied for follicle stimulation starting on the second or third day of each patient's menstrual cycle. To prevent early ovulation, 250 mcg Cetorelix (Cetrotide, Pharm Int., Idron, France) was started when the dominant follicle size reached 13 mm. Data were recorded of the total stimulation days, total FSH dose administered, human chorionic gonadotropin (HCG) day E2 and endometrium thickness. When follicles exceeded 17 mm, 250 mcg/0.5 mL HCG (Ovitrelle, Merck Sereno, Modugno, Italy) was administered, followed by oocyte pick-up (OPU) after 36 hours.

Immediately prior to OPU, blood was measured for IMA level and OSI. Serum was separated from blood samples and stored at -80 °C until assay. The follicular fluid collected during OPU was stored in an incubator until application of intracytoplasmic sperm injection (ICSI). Although follicular fluid collected with oocytes is ordinarily destroyed, it was retained and stored at -80 °C in order to measure IMA levels. Upon conclusion of the study, samples were treated and the IMA levels were calculated using the rapid, calorimetric method as described in detail by Bar-Or D *et al.* [14]. A Spekol® 1300 spectrophotometer was used for the measurements.

Total Oxidant Status (TOS) and Total Antioxidant Status (TAS) levels were measured using commercially available kits (Rel Assay Kit Diagnostics, Turkey), with their ratio accepted as the OSI. In the calculation, the resulting unit of TAS was converted to $\mu\text{mol/L}$, and the OSI value was calculated according to the following formula: $\text{OSI (arbitrary unit)} = \text{TOS } (\mu\text{mol H}_2\text{O}_2 \text{ equivalent/L}) / \text{TAS } (\mu\text{mol Trolox equivalent/L})$, as described in detail by Kosecic M *et al.* [15].

For each patient, a record was made of the following: total number of oocytes collected in OPU; mature oocytes (MII); metaphase I (MI); oocytes with germinal vesicle (GV); degenerated oocytes (DG) as well as MII oocytes applied with the micro-injection process; fertilization rates at 12-16 hours after micro-injection; embryo quality (grades) on the second, third and fifth days; transfer day; pregnancy rates; live births; and abortus. For luteal support following transfer, patients were administered 90 mg vaginal progesterone (Crinone®; Merck Serono, Istanbul, Turkey) and subcutaneous progesterone 25 mg (Prolutex, IBSA, Lamon, Switzerland) for two weeks.

Statistical analysis

Data obtained in the study were analyzed statistically using IBM SPSS for Windows, version 22.0 software (IBM statistics for Windows version 22, IBM Corporation, Armonk, NY, USA). The results were stated as mean \pm standard deviation (SD). Repeated measurements were evaluated with variance analysis (Repeated Measures ANOVA with Bonferroni correction). When comparing paired groups, the Tukey HSD method was applied. A value of $p < 0.05$ was accepted as statistically significant.

Results

The data of 80 patients were analyzed. When the correlation between beta HCG positivity and live birth rates was examined with the serum and follicular fluid OSI values, no significant correlation was determined between HCG positivity and serum OSI and live birth rates ($p = 0.775$, $r = 0.033$ and $r = -0.053$, $p = 0.639$). A statistically significant negative correlation was determined between follicular fluid OSI values and beta HCG positivity and live birth rates ($r = -0.407$, $p < 0.001$, and $r = -0.312$, $p = 0.005$). Comparable analysis for IMA values likewise found no significant correlation between HCG positivity and serum IMA

Table 1. — The demographic and endocrine characteristics of patients in groups formed according to the specified OSI and IMA cutoff values.

	Follicle Fluid OSI		<i>p</i>	Follicle Fluid IMA		<i>p</i>
	OSI < 11.15 (n = 32)	OSI > 11.15 (n = 48)		IMA < 0.509 (n = 24)	IMA > 0.509 (n = 56)	
Age (years)	28.46 ± 5.8	27.77 ± 5.4	0.58	27.20 ± 5.54	28.41 ± 5.60	0.38
Infertility duration (years)	5.84 ± 3.16	6.04 ± 2.76	0.76	5.91 ± 3.07	5.98 ± 2.86	0.92
Gravida	0.25 ± 0.5	0.35 ± 0.63	0.44	0.33 ± 0.63	0.30 ± 0.56	0.83
BMI (kg/m ²)	25.84 ± 2.51	27.08 ± 2.42	0.70	24.37 ± 2.39	26.96 ± 2.42	< 0.01
Basal FSH (U/L)	5.9 ± 1.3	6.08 ± 1.33	0.55	6.20 ± 1.38	5.92 ± 1.29	0.38
Basal LH (U/L)	6.3 ± 1.8	6.76 ± 1.79	0.61	5.98 ± 1.80	6.23 ± 1.55	0.73
Basal E2 (ng/L)	50.21 ± 4.91	49.02 ± 7.71	0.87	48.37 ± 6.09	50.83 ± 4.91	0.16
AMH (ng/mL)	2.64 ± 0.58	2.74 ± 0.60	0.47	2.52 ± 0.56	2.78 ± 0.60	0.07
Duration of stimulations (days)	9.71 ± 1.27	9.14 ± 1.22	0.40	9.29 ± 1.16	9.41 ± 1.31	0.70
Total FSH dose (IU)	2107 ± 447	2168 ± 511	0.58	2202 ± 468	2119 ± 494	0.49
hCG day Endometrium thickness (mm)	9.93 ± 1.29	10.02 ± 1.27	0.77	10.50 ± 1.14	9.76 ± 1.27	0.12
HCG day E2	1799 ± 454	1691 ± 410	0.27	1777 ± 417	1716 ± 436	0.56

OSI: Oxidative stress index, IMA: Ischemia-modified albumin, BMI: Body mass index, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, E2: Estradiol, AMH: Anti-Müllerian hormone, HCG: Human chorionic gonadotropin.

and live birth rates ($r = -0.030$, $p = 0.790$, and $r = -0.045$, $p = 0.695$), along with statistically significant negative correlation between follicular fluid IMA values and beta HCG positivity and live birth rates ($r = -0.615$, $p < 0.001$, and $r = -0.412$, $p = 0.005$).

In the correlation analysis applied, a significant relationship was determined between beta HCG positivity and live birth rates; through ROC analysis of follicular fluid OSI and IMA values, a follicular fluid IMA value < 0.509 predicted live birth with 78.6% sensitivity and 50.8% specificity. When ROC analysis was applied to the relationship between live births and the follicular fluid OSI value, we found that a cutoff value of < 11.15 predicted live births at 61.5% sensitivity and 70.4% specificity. In the ROC analyses of the IMA and OSI values in the follicular fluid, the same cutoff value of 0.509 for IMA in the follicular fluid was found to predict beta HCG positivity with sensitivity of 76.5% and specificity of 45.3%, while a cutoff value of 11.15 for follicular fluid OSI predicted beta HCG positivity with sensitivity of 58.8% and specificity of 76.1%.

Patients were grouped above and below the specified cutoff values for follicular fluid OSI and IMA. Comparative analyses of the two groups found no statistically significant difference between the demographic data of the patients and the follicular-embryonal values. In the follicular fluid OSI ≤ 11.15 group (Group 1, n = 32), beta HCG positivity was seen in 65.6% of patients; live births were recorded in 50%. In the OSI > 11.15 group (Group 2, n = 48), beta HCG positivity was seen in 33.3% of patients; live births were recorded in 20.8%. As for follicular fluid OSI values, the difference in terms of both beta HCG positivity and live birth rates was seen to be statistically significant ($p = 0.003$,

$p = 0.007$).

In the follicular fluid IMA ≤ 0.509 group (Group 1, n = 24), beta HCG positivity was seen in 66.6% of patients, while live births were recorded in 50%. In the follicular fluid IMA > 0.509 group (Group 2, n = 56), beta HCG positivity was seen in 30.3% of patients; live births were recorded in 25%. In respect of the demographic and embryonal development parameters of the cases, the BMI value was significantly high in the IMA > 0.509 group ($p < 0.05$). No statistical difference was observed between the other parameters compared (Tables 1 and 2).

Discussion

To the best of our knowledge, this is the first study to have investigated the relationship between follicular-embryonal development, pregnancy success, and IMA levels in follicular fluid in patients undergoing IVF treatment for unexplained fertility. While the results of the study found no correlation between serum OSI and IMA values and the follicular and embryonal development parameters, a significant negative correlation was determined between the implantation and live birth rates and increased OSI and IMA values in the follicular fluid of the oocyte micro-environment. When patients were grouped according to the specified OSI and IMA cutoff points, a high BMI value was noticeable in the high IMA group.

In the current study, the implantation and live birth rates were found to be lower in the groups with high follicular fluid IMA and OSI values, likely due to increased oxidative stress. Reportedly, increased ROS decrease female fertility by modulating different reproductive functions includ-

Table 2. — Results of laboratory tests during IVF treatment and clinical outcomes of patients in groups formed according to the specified OSI and IMA cutoff values.

	Follicle Fluid OSI			Follicle Fluid IMA		
	OSI < 11.15 (n = 32)	OSI > 11.15 (n = 48)	<i>p</i>	IMA < 0.509 (n = 24)	IMA > 0.509 (n = 56)	<i>p</i>
M2 oocytes	8.15 ± 2.24	7.81 ± 2.48	0.53	7.45 ± 2.46	8.16 ± 2.34	0.23
M1 oocytes	1.53 ± 1.41	1.95 ± 1.59	0.22	1.54 ± 1.25	1.89 ± 1.63	0.35
GV-DG	1.12 ± 1.26	1.10 ± 1.17	0.94	1.37 ± 1.24	1.20 ± 1.17	0.20
No. of 2 N (two ro-nucleate) embryos (n)	5.71 ± 2.11	5.37 ± 2.20	0.49	5.16 ± 2.18	5.66 ± 2.15	0.35
Abnormal fertilisation rate (Anormal N/M2)	0.65 ± 0.74	0.81 ± 0.73	0.35	0.66 ± 0.70	0.78 ± 0.75	0.51
D3G1 Embryo (day 3 grade 1 embryo)	3.46 ± 1.50	3.25 ± 1.55	0.53	3.16 ± 1.78	3.41 ± 1.41	0.51
No. of blastocysts (n)	2.46 ± 1.21	2.33 ± 1.29	0.64	2.29 ± 1.30	2.42 ± 1.24	0.65
Transfer day	4.28 ± 0.75	4.06 ± 0.90	0.28	3.95 ± 0.95	4.23 ± 0.85	0.20
Implantation rate (β HCG > 20 U/L)	65.6%	33.3%	< 0.01	66.6%	30.3%	< 0.01
Live birth rate	50.0%	20.8%	< 0.01	50.0%	25.0%	< 0.01

OSI: Oxidative stress index, IMA: Ischemia-modified albumin, M2: Metaphase 2, M1: Metaphase 1, GV-DG: Germinal vesicle- degenerated oocytes, PN: Pronucleus.

ing ovarian steroidogenesis, oocyte maturation, ovulation, formation of blastocysts [16], sperm capacity and sperm-oocyte interaction [17], fertilization, implantation and early embryo development [18].

Using the thermo-chemiluminescence assay method, Wiener-Megnazi *et al.* found that the oxidative status in follicular fluid increased oxidative stress in follicular fluid following fertilization in processes such as cleavage and pregnancy. The authors indicate that a certain threshold of oxidative stress in the mature follicle may be necessary for good embryo quality, although high levels may be harmful [19].

Several studies show that oxidative stress could have harmful effects on embryo development through different mechanisms. The authors associated such mechanisms variously with mitochondrial alterations, embryo cell block caused by lipid peroxidation, adenosine triphosphate (ATP) depletion, and apoptosis [20-25].

In contrast, a recent study investigated the relationship between IVF success and culture media ROS levels, reporting no significant relationship between ROS levels in a culture medium and embryo quality, blastocyst formation or embryonal arrest [26].

Studies showing the relationship between IMA, a relatively new oxidative stress marker, and infertility and reproductive functions, have focused on PCOS, insulin resistance and hyperandrogenemia. In the first study on this subject, Guven *et al.* revealed a relationship between high serum IMA levels in patients with PCOS and testosterone levels, BMI, and Ferriman-Gallwey scores [13]. In support of that study, Çağlar *et al.* showed that in weak PCOS cases with high IMA levels, increased chronic hypoxia and associated secondary oxidative stress could be responsible for

metabolic problems in these patients [27].

In a similar study by Beyazit *et al.*, IMA levels were found to be high in PCOS cases; according to the authors, oxidative stress could play a role in the etiology of PCOS. They also determined a correlation between IMA and testosterone levels based on the negative effects of the follicular micro-environment on follicle and oocyte development [28]. In contrast, a study of PCOS patients by Çakir *et al.* found that TOS and TAS levels were similar to those of the control group, and while the IMA levels were high in PCOS patients, the elevation did not reach a level of statistical significance [29]. When our patients were examined and compared in two groups according to high or low IMA levels, increased BMI values were noticeable in the high IMA group. This is consistent with a study by Piva *et al.*, in which a relationship was found between high IMA and obesity [30].

The primary limitation of this study is that testosterone and insulin resistance levels were not examined. As most studies in literature have investigated high IMA and increased oxidative stress in PCOS patients, it has been suggested that the mechanism causing reduced fertility could be increased androgens and insulin resistance [13, 28]. Another limitation of the study was that there was no PCOS group. If a PCOS group had been included, a clearer conclusion could have been made as to whether oxidative stress was only a factor in unexplained fertility or also in PCOS.

In conclusion, in cases of unexplained infertility with high BMI, increased IMA but not OSI in follicular fluid during IVF treatment could be related to decreased implantation and live birth rates. Follicular fluid IMA levels below the cutoff value may predict pregnancy success.

Ethics Approval and Consent to Participate

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Kahramanmaraş Sutcu Imam University (session number: 2019/10, approval number: 15).

Acknowledgments

Thanks to all the peer reviewers and editors for their opinions and suggestions.

Conflict of Interest

The authors declare no conflict of interest.

Submitted: December 12, 2019

Accepted: April 29, 2020

Published: December 15, 2020

References

- [1] The Practice Committee of the American Society for Reproductive Medicine.: "Definitions of infertility and recurrent pregnancy loss". *Fertil. Steril.*, 2008, 90, 60.
- [2] Pellicer A., Albert C., Mercader A., Bonilla-Musoles F., Remohi J., Simon C.: "The follicular and endocrine environment in women with endometriosis: local and systemic cytokine production". *Fertil. Steril.*, 1998, 70, 425-431.
- [3] Agarwal A., Gupta S., Sekhon L., Shah R.: "Redox considerations in female reproductive function and assisted reproduction: from molecular mechanisms to health implications". *Antioxid. Redox. Signal.*, 2008, 10, 1375-1403.
- [4] Rizzo A., Roscino M.T., Binetti F., Sciorsci R.L.: "Roles of reactive oxygen species in female reproduction". *Reprod. Domest. Anim.*, 2012, 47, 344-352.
- [5] Agarwal A., Gupta S., Sikka S.: "The role of free radicals and antioxidants in reproduction". *Curr. Opin. Obstet. Gynecol.*, 2006, 18, 325-332.
- [6] Guerin P., El Moutassim S., Menezo Y.: "Oxidative stress and protection against reactive oxygen species in the preimplantation embryo and its surroundings". *Hum. Reprod. Update.*, 2001, 7, 175-189.
- [7] Jurisicova A., Varmuza S., Casper R.F.: "Programmed cell death and human embryo fragmentation". *Mol. Hum. Reprod.*, 1996, 2, 93-98.
- [8] Lippi G., Montagnana M., Guidi G.C.: "Albumin cobalt binding and ischemia modified albumin generation: an endogenous response to ischemia?". *Int. J. Cardiol.*, 2006, 108, 410-411.
- [9] Turedi S., Gunduz A., Mentese A., Topbas M., Karahan S.C., Yenioçak S., et al.: "The value of ischemia-modified albumin compared with d-dimer in the diagnosis of pulmonary embolism". *Respir. Res.*, 2008, 9, 49.
- [10] Borderie D., Allanore Y., Meune C., Devaux J.Y., Ekindjian O.G., Kahan A.: "High ischemia-modified albumin concentration reflects oxidative stress but not myocardial involvement in systemic sclerosis". *Clin. Chem.*, 2004, 50, 2190-2193.
- [11] Piwowar A., Knapik-Kordecka M., Warwas M.: "Ischemia-modified albumin level in type 2 diabetes mellitus - preliminary report". *Dis. Markers.*, 2008, 24, 311-317.
- [12] Ozturk M., Keskin U., Ozturk O., Ulubay M., Alanbay İ., Aydin A., et al.: "Are serum gamma-glutamyl transferase, high-sensitivity C-reactive protein, and ischaemia-modified albumin useful in diagnosing PCOS?". *J. Obstet. Gynaecol.* 2016, 36, 929-934.
- [13] Guven S., Karahan S.C., Bayram C., Ucar U., Ozeren M.: "Elevated concentrations of serum ischaemia-modified albumin in PCOS, a novel ischaemia marker of coronary artery disease". *Reprod. Biomed. Online*, 2009, 19, 493-500.
- [14] Bar-Or D., Curtis G., Rao N., Bampos N., Lau E.: "Characterization of the Co(2+) and Ni(2+) binding amino-acid residues of the N-terminus of human albumin. An insight into the mechanism of a new assay for myocardial ischemia". *Eur. J. Biochem.*, 2001, 268, 42-47.
- [15] Kosecik M., Erel O., Sevinc E., Selek S.: "Increased oxidative stress in children exposed to passive smoking". *Int. J. Cardiol.*, 2005, 100, 61-64.
- [16] Suzuki T., Sugino N., Fukaya T., Sugiyama S., Uda T., Takaya R., et al.: "Superoxide dismutase in normal cycling human ovaries: immunohistochemical localization and characterization". *Fertil. Steril.*, 1999, 72, 720-726.
- [17] de Lamirande E., Leclerc P., Gagnon C.: "Capacitation as a regulatory event that primes spermatozoa for the acrosome reaction and fertilization". *Mol. Hum. Reprod.*, 1997, 3, 175-194.
- [18] Sakkas D., Urner F., Bizzaro D., Manicardi G., Bianchi P.G., Shoukir Y., et al.: "Sperm nuclear DNA damage and altered chromatin structure: effect on fertilization and embryo development". *Hum. Reprod.*, 1998, 13 Suppl 4, 11-19.
- [19] Wiener-Megnazi Z., Vardi L., Lissak A., Shnizer S., Reznick A.Z., Ishai D., et al.: "Oxidative stress indices in follicular fluid as measured by the thermochemiluminescence assay correlate with outcome parameters in in vitro fertilization". *Fertil. Steril.*, 2004, 82, 1171-1176.
- [20] Kowaltowski A.J., Vercesi A.E.: "Mitochondrial damage induced by conditions of oxidative stress". *Free. Radic. Biol. Med.*, 1999, 26, 463-471.
- [21] Nasr-Esfahani M.H., Aitken J.R., Johnson M.H.: "Hydrogen peroxide levels in Mouse oocytes and early cleavage stage embryos developed in vitro or in vivo". *Development*, 1990, 109, 501-507.
- [22] Noda Y., Matsumoto H., Umaoka Y., Tatsumi K., Kishi J., Mori T.: "Involvement of superoxide radicals in the mouse two-cell block". *Mol. Reprod. Dev.*, 1991, 28, 356-360.
- [23] Hyslop P.A., Hinshaw D.B., Halsey W.A. Jr., Schraufstatter I.U., Sauerheber R.D., Spragg R.G., et al.: "Mechanisms of oxidant-mediated cell injury. The glycolytic and mitochondrial pathways of ADP phosphorylation are major intracellular targets inactivated by hydrogen peroxide". *J. Biol. Chem.*, 1988, 263, 1665-1675.
- [24] Pierce G.B., Parchment R.E., Lewellyn A.L.: "Hydrogen peroxide as a mediator of programmed cell death in the blastocyst". *Differentiation*, 1991, 46, 181-186.
- [25] Yang H.W., Hwang K.J., Kwon H.C., Kim H.S., Choi K.W., Oh K.S.: "Detection of reactive oxygen species (ROS) and apoptosis in human fragmented embryos". *Hum. Reprod.*, 1998, 13, 998-1002.
- [26] Lan K.C., Lin Y.C., Chang Y.C., Lin H.J., Tsai Y.R., Kang H.Y.: "Limited relationships between reactive oxygen species levels in culture media and zygote and embryo development". *J. Assist. Reprod. Genet.*, 2019, 36, 325-334.
- [27] Caglar G.S., Oztas E., Karadag D., Pabuccu R., Demirtas S.: "Ischemia-modified albumin and cardiovascular risk markers in polycystic ovary syndrome with or without insulin resistance". *Fertil. Steril.*, 2011, 95, 310-313.
- [28] Beyazit F., Yilmaz N., Balci O., Adam M., Yaman S.T.: "Evaluation of oxidative stress in women with polycystic ovarian syndrome as represented by serum ischemia modified albumin and its correlation with testosterone and insulin resistance". *Intern. Med.*, 2016, 55, 2359-2364.
- [29] Kahir E., Ozbek M., Ozkaya E., Colak N., Cakal E., Sayki M., et al.: "Oxidative stress markers are not valuable markers in lean and early age of polycystic ovary syndrome patients". *J. Endocrinol. Invest.*, 2011, 34, e178-e182.
- [30] Piva S.J., Duarte M.M., Da Cruz I.B., Coelho A.C., Moreira A.P., Tonello R., et al.: "Ischemia-modified albumin as an oxidative stress biomarker in obesity". *Clin. Biochem.*, 2011, 44, 345-347.

Corresponding Author:

MURAT BAKACAK, M.D.

Department of Obstetrics and Gynecology and IVF Center,
Kahramanmaraş Sutcu Imam University,

School of Medicine, Kahramanmaraş (Turkey)

e-mail: muratbakacak46@gmail.com