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Effects of reactive oxygen species, lipids peroxidation, and total antioxidant capacity on sperm morphological functional characteristics and quality embryological development ICSI outcomes

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ABSTRACT

The present study aims to evaluate the effects of seminal Reactive oxygen species (ROS), lipids peroxidation, and total antioxidant capacity (TAC) on the traditional and functional semen characteristic analysis and quality intracytoplasmic sperm injection (ICSI) outcomes. The study group demonstrated that the elevated levels of seminal ROS and lipids peroxidation greatly reduced sperm concentration, total sperm count, total motility, normal morphology, and seminal TAC and increased seminal leukocytes count compared to control group. In the study group, the elevated levels of seminal ROS and lipids peroxidation markedly attenuated fertilization, cleavage, and blastocysts formation rates and decreased D3 and D5 top quality embryos development grade compared to control group. Finally, the study results indicated that administration of antioxidant supplementation can improve sperm characteristic parameters and quality ICSI outcomes, decrease levels of seminal ROS and lipids peroxidation, and increase its TAC level.

Keywords: ART; ICSI Outcomes; sperm morphological functional characteristics; ROS; lipids peroxidation; oxidative stress biomarkers; TAC.

INTRODUCTION

Assisted reproduction techniques (ART) are procedures used to treat different infertility factors, which upregulate gametes interaction, fertilization, cleavage, embryos development, grading score, transfer, implantation, and pregnancy rates in subfertile patients. The successful rates of intracytoplasmic sperm injection (ICSI) method depends on maternal age, infertility reasons, gametes and embryos quality, and lifestyle factors (Nishihara *et al*., 2018). Human infertility represents 15% of the worldwide reproductive age population, which is defined as the inability to happen a pregnancy after one year or more from a regular unprotected sexual intercourse. Poor semen conventional and functional characteristics represent 25-30% from all infertility cases (Torres-Arce *et al*., 2021). In human body, oxidative stress response is considered as an imbalance between endogenous unstable free radicals generation and antioxidant capacity (Nishihara *et al*., 2018; Ritchie & Ko, 2021). Actually, 30–80% of infertile men have high concentrations of seminal reactive oxygen species (ROS) and low antioxidant capacity (Gualtieri *et al*., 2021; Scaruffi *et al*., 2021).

ROS are highly reactive free radicals produced from the mitochondrial enzymatic oxygen reduction process to generate energy (Tremellen, 2008; Majzoub & Agarwal, 2018; Ribas-Maynou & Yeste, 2020). The common forms of sperm ROS radicals include superoxide anion ($O_2^{\bullet-}$), hydroxyl ($^{\bullet}OH$), hydrogen peroxide (H_2O_2), singlet oxygen molecule ($^1O_2$), and nitric oxide (NO) (Agarwal *et*

al., 2014a; Ribas-Maynou & Yeste, 2020).Under physiological conditions, ROS critically regulate sperm maturation, capacitation, motility, hyperactivation, acrosome reaction, sperm-oocyte interaction, and fertilization rate (Agarwal & Bui, 2017; Majzoub & Agarwal, 2018; Alahmar, 2019). Under pathological conditions, ROS induce lipids peroxidation that alters sperm plasma membrane permeability and fluidity, elevates protein modifications, increases sperm DNA fragmentation, and develop male infertility characteristic features (Ribas-Maynou *et al*., 2020; Gualtieri *et al*., 2021). Under oxidative stress response, the high levels of ROS reduce antioxidant defense mechanisms of sperm cells and/or seminal plasma, sperm normal morphology, capacitation, sperm–oocyte interaction, oocytes fertilization, quality embryos development grade, and pregnancy rates (Ribas-Maynou *et al*., 2020; Scaruffi *et al*., 2021).

Living organisms have antioxidant defense system to scavenge, neutralize, and reduce ROS damage. Cellular antioxidant defense modulators include enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and glutathione peroxidase (GPx) as well as small non-enzymatic molecules such as ascorbic acid (VitC), α-tocopherol (VitE), β-carotene, and reduced glutathione (GSH) (Koracevic *et al*., 2001; Ritchie & Ko, 2021). Under normal conditions, semen enzymatic and non-enzymatic antioxidant defense modulators positively interact with each other to downregulate ROS response (Alahmar, 2019). In sperm cells, SOD, CAT, GPx enzymes, and reduced GSH are considered as a main endogenous total antioxidant capacity (TAC), which improve sperm concentration, morphology, viability, and motility (Alahmar, 2019; Ribas-Maynou & Yeste, 2020). The co-operation among different endogenous antioxidant modulators greatly provides a protection against excessive ROS generation compared to the efficacy of each individual antioxidant compound (Torres-Arce *et al*., 2021). In plasma and body fluids, TAC markedly introduces great biological protective information compared to individual antioxidant components (Koracevic *et al*., 2001).

In this study the effects of seminal ROS, lipids peroxidation, and TAC on the traditional and functional semen characteristic analysis and quality ICSI outcomes were studied.

PATIENTS AND METHODS

1- Study Group Population

Ethical approval was obtained from the National Medical Research Ethics Committee of the University of Sadat City, Menoufia, Egypt. In the present study, 160 infertile couples were recruited to perform an ICSI application. The duration of infertility was 3-11 years. This study was conducted between October 2018 and August 2021.The age of male was 25-50 year, and the age of their female partners was 20-39 year.From 160 recruited infertile couples, 110 samples were excluded as the following reasons: poor response to oocytes hyperstimulation (12 couples), <4-5 oocytes retrieved (38 couples), and severe abnormal semen analysis (60 couples). The current study was performed on the remained 50 infertile couples (study group/positive oxidative stress biomarkers) and 15 normal healthy unexplained infertile couples (control group/negative oxidative stress biomarkers) in a clinical assisted reproductive centre that called Ingab Fertility Centre (IFC), Nasr City, Cairo, Egypt.

1.1-Laboratory Semen Collection, Preparation, and Evaluation

After recruitment, written informed consent was obtained from all participants. Semen samples were collected by masturbation at clinical siteinto sterile containers. The men asked to abstain from ejaculation for at least 48 hours before

semen collection. After liquefaction, all basic semen analyses were manually performed within one hour after semen samples collection including semen ejaculate volume (mL), sperm concentration (*10^6/mL), total sperm count (*10^6/ ejaculate), total sperm motility (%), normal sperm morphology (%), and seminal leukocytes count (*10^6/mL) as described by Shekarriz *et al*. (1995); Guzick *et al*. (2001) and Agarwal *et al*. (2014b). Sperm viability was evaluated using eosin-nigrosin staining (Yánez-Ortiz *et al.*, 2021). A normal seminal fluid analysis was carried out according to the World Health Organization (WHO) criteria (WHO, 5th edition, 2010) (Cooper *et al*., 2010). Processed sperm samples were prepared by a discontinuous two-layer density gradient centrifugation method (Pure Sperm® Wash (PSW-100), Pure Sperm® 40/80 (PSK-020), Nidacon, Sweden) according to the manufacturer's instructions before the ICSI cycles (Zorn *et al*., 2003; Bedaiwy *et al*., 2010).

1.2-Oocytes Retrieval, Culture Conditions, Fertilization, and Embryo Assessment

Controlled ovarian hyperstimulation, oocytes recovery (Rago *et al*., 2017), ICSI technique, embryos culture media, and top quality morphological assessment of embryos were performed as described by Bedaiwy *et al*. (2010) and Massarotti *et al*. (2021). The retrieved oocytes were processed and incubated in GMHTF media (Gynemed GmbH& Co. KG, Lensahn, Germany) to develop mature metaphase II (MII) and fertilized oocytes using a standard ICSI technique. In ICSI cycles, the fertilization rate was confirmed by formation of two pronuclei (2PN) zygote and extrusion of a second polar body. The fertilized oocytes were cultured in groups of 4-5 oocytes in 1 mL of global® total™ (LifeGlobal, Ontario, Canada) until D5 (blastocysts formation stage) (Bedaiwy *et al*., 2010). The D3 and D5 quality ICSI embryos development grade were evaluated and classified using modified criteria of the Veeck's morphological grading system (Nagy *et al*., 2008). A good quality embryos graded score was described as a four cell stage on D2 and seven cell stage on D3, which had no (grade I) and/or <20% cytoplasmic fragments (grade II). In D5 embryos, ICSI blastocysts formation was also evaluated (Lan *et al*., 2019).

1.3-Estimation of Seminal Oxidative Stress Biomarkers and Total Antioxidant Capacity

Seminal plasma was obtained by centrifugation of fresh liquefied semen at 3000 rpm for 10 min at 4°C, recovered, and stored at -20°C until the biochemical analysis. To evaluate the seminal levels of intracellular ROS production (measured as $O_2^{\bullet-}$ radical levels) in the control and study semen samples, yellow nitroblue tetrazolium (NBT) molecules directly interacted with seminal $O_2^{\bullet-}$ radical molecules (basal ROS levels) that produced by sperm and leukocytes to form insoluble formazan crystals (blue pigment) (OxiSperm® Halotech, HT-OS20, Madrid). The concentration of formazan crystals is directly proportional with the concentration of seminal ROS as explained by Kefer *et al.* (2009) and Castleton *et al*. (2022). In all seminal plasma specimens, malondialdehyde (MDA) was measured as a thiobarbituric acid reactive substance (TBARS) using a Ohkawa *et al*. method (Ohkawa *et al*., 1979) with specific modifications as described previously (Kasperczyk *et al*., 2015; Hamilton *et al*., 2016). Furthermore, seminal total antioxidant capacity (TAC) was determined using an antioxidant assay kit (Biodiagnostic, Egypt) according to the manufacturer's instructions.

1.4-Statistical Analysis

Data values were statistically expressed as means ± standard errors of

the means (s.e.m) for control (15 couples) and study groups (50 couples) as male and female partners. Statistical significance differences between the experimental groups ($p < 0.05$; $p < 0.01$) were evaluated using analysis of compare means by Independent-Samples T test of SPSS Windows Version 16.0 (SPSS, Inc., Chicago, IL, USA) (Zhu *et al*., 2018; Degola *et al*., 2019). The relationships between seminal functional characteristic parameters and quality ICSI outcomes as variables within the experimental groups were evaluated using a bivariate pearson correlation standardized coefficients, which determined bivariate standardized coefficient values (**r**) with a 2-tailed significant ($p< 0.01$).

RESULTS

In the study group, the levels of seminal leukocytes, ROS, and lipids peroxidation (TBARS/MDA) were significantly ($p< 0.01$) increased compared to their levels of the control group (Table 1). Also, it was obvious that the study group demonstrated elevated levels of seminal leukocytes, ROS, and TBARS/MDA significantly ($p< 0.01$) reduced the level of seminal TAC and significantly ($p< 0.05$) induced poor quality sperm characteristic parameters including sperm concentration, total count, total motility, and normal morphology compared to their findings of the control group. This potentially affected on functional parameters of the normal healthy sperm cells and significantly ($p< 0.05$) decreased their quality ICSI outcomes, which significantly ($p< 0.05$) attenuated numbers of D1 fertilized oocytes, D3 cleavage-stage embryos, and D5 developed blastocysts and rates of fertilization, cleavage, D3 top quality embryos development, blastocysts development, and D5 top quality embryos development compared to their results of the control group (Table 2).

Table 1. Effects of seminal ROS, lipids peroxidation, and TAC on semen characteristic parameters.

Routine and Semen Parameters	Control Group (n=15)	Study Group (n=50)
Age of Males (Years)	38.41 ± 8.52	35.76 ± 7.89
Age of Females (Years)	30.13 ± 6.64	28.72 ± 5.87
Duration of Infertility (Years)	6.81 ± 2.78	6.81 ± 2.59
Ejaculate Volume (mL)	3.93 ± 0.52	3.43 ± 0.72
Sperm Concentration (*10^6/mL)	50.11 ± 5.11	27.34 ± 5.11[a]
Total Sperm Count (*10^6/ejaculate)	196.51 ± 31.42	91.37 ± 14.48[a]
Total Sperm Motility (%)	68.21 ± 3.23	49.96 ± 4.15[a]
Normal Sperm Morphology (%)	25.13 ± 2.45	10.42 ± 1.74[a]
Leukocytes (WBCs*10^6/mL)	0.129 ± 0.008	0.804 ± 0.016[b]
Seminal ROS (Abs/10^6 sperm/mL)	0.167 ± 0.009	0.702 ± 0.023[b]
Seminal TBARS/MDA (nM/mL)	7.37 ± 0.13	40.36 ± 2.97[b]
Seminal TAC (μM/L)	1540.53 ± 40.13	354.02 ± 14.11[b]

Data values are expressed as means ± s.e.m. Statistical significance differences ($p < 0.05$; $p < 0.01$) were evaluated using analysis of compare means by Independent-Samples T test of SPSS. [a]$p< 0.05$ and [b]$p< 0.01$ vs. control group. ROS (reactive oxygen species); MDA, malondialdehyde; TAC, total antioxidant capacity. Control group, negative oxidative stress group; study group, positive oxidative stress group.

Table 2. Effects of seminal ROS, lipids peroxidation, and TAC on ICSI outcomes.

ICSI Outcomes	Control Group (n=15)	Study Group (n=50)
Retrieved Oocytes	15.00 ± 1.46	13.80 ± 1.74
Mature MII Oocytes	13.07 ± 1.62	11.78 ± 1.81
D1 Fertilized Oocytes (2PN Zygotes)	11.40 ± 1.45	7.78 ± 1.81[a]
Fertilization Rate (%)	87.31 ± 4.21	65.21 ± 5.64[a]
D3 Cleavage-Stage Embryos	8.13 ± 1.25	4.40 ± 1.13[a]
Cleavage Rate (%)	71.18 ± 3.36	56.51 ± 3.94[a]
D3 Top Quality Embryos Development(%)	87.73 ± 1.79	68.72 ± 5.48[a]
D5 Developed Blastocysts	5.80 ± 0.86	2.48 ± 0.65[a]
Blastocysts Development Rate (%)	71.38 ± 3.14	56.94 ± 7.43[a]
D5 Top Quality Embryos Development (%)	80.87 ± 4.55	55.44 ± 8.02[a]

Data values are expressed as means ± s.e.m. Statistical significance difference ($p < 0.05$) was evaluated using analysis of compare means by Independent-Samples T test of SPSS. [a]$p< 0.05$ vs. control group. Control group, negative oxidative stress group; study group, positive oxidative stress group.

) demonstrates strength, **r** values (positive or negative), and types (directly or inversely proportional) of the bivariate pearsoncorrelation standardized coefficient relationships between each two different variable parameters (sperm concentration, total count, total motility, normal morphology, leukocytes, seminal ROS, TBARS/MDA, and TAC) within two experimental groups. It is clear that the normal sperm morphology was negatively ($p< 0.01$) related to seminal ROS (-0.950r) and TBARS/MDA (-0.939r) and positively related to seminal TAC (0.955r). Seminal ROS was negatively ($p< 0.01$) related to sperm concentration (-0.869r), total count (-0.924r), total motility (-0.871r), and normal morphology (-0.950r) and positively related to seminal leukocytes (0.994r). Seminal TAC was positively ($p< 0.01$r) related to sperm concentration (0.887r), total count (0.918r), total motility (0.890r), and normal morphology (0.955r) and negatively related to seminal leukocytes (-0.998r).

Table 3. Bivariate Pearson correlation standardized coefficient (**r**) values between each two variables (semen functional characteristic parameters) within two experimental groups.

Pearson's Correlation (r values)	ROS	TBARS/MDA	TAC
Sperm Concentration	-0.869	-0.866	0.887
Total Sperm Count	-0.924	-0.886	0.918
Total Sperm Motility	-0.871	-0.897	0.890
Normal Sperm Morphology	-0.950	-0.939	0.955
Leukocytes	0.994	0.985	-0.998

N=65. Correlation is significant at the 0.01 level (2-tailed).

Table 4) demonstrates strength, **r** values (positive or negative), and types (directly or inversely proportional) of the bivariate pearson correlation standardized coefficient relationships between each two different variable parameters (sperm

concentration, total count, total motility, normal morphology, leukocytes, ROS, TBARS/MDA, TAC, D1 fertilized oocytes, fertilization rate, cleavage oocytes, cleavage rate, D3 top quality embryos, developed blastocysts, blastocysts formation rate, D5 top quality embryos) within two experimental groups. It was obvious that the number of D5 developed blastocysts was positively ($p<$ 0.01) correlated with sperm concentration (0.813r), total count (0.783r), total motility (0.826r), normal morphology (0.874r), and seminal TAC (0.897r) and negatively correlated with seminal leukocytes (-0.894r), ROS (-0.889r), and TBARS/MDA (-0.878r). Fertilization rate (%) was positively ($p<0.01$) correlated with sperm concentration (0.798r), total count (0.787r), total motility (0.776r), normal morphology (0.823r), and seminal TAC (0.867r) and negatively correlated with seminal leukocytes (-0.870r), ROS (-0.864r), and TBARS/ MDA (-0.858r).

Table 4. Bivariate Pearson correlation standardized coefficient (**r**) values between each two variables (semen functional characteristic parameters and quality ICSI outcomes) within two experimental groups.

Pearson's Correlation (r values)	Sperm Con.	Total Sperm Count	Total Sperm Motility	Normal Sperm Morphology	Leukocytes	ROS	TBARS/ MDA	TAC
D1 Fertilized Oocytes (2PN Zygotes)	0.622	0.574	0.629	0.648	-0.667	-0.659	-0.656	0.665
Fertilization Rate (%)	0.798	0.787	0.776	0.823	-0.870	-0.864	-0.858	0.867
D3 Cleavage-Stage Embryos	0.739	0.709	0.762	0.790	-0.809	-0.805	-0.790	0.809
Cleavage Rate (%)	0.752	0.777	0.787	0.817	-0.853	-0.855	-0.819	0.855
D3 Top Quality Embryos Development (%)	0.788	0.786	0.758	0.830	-0.857	-0.855	-0.836	0.854
D5 Developed Blastocysts	0.813	0.783	0.826	0.874	-0.894	-0.889	-0.878	0.897
Blastocysts Development Rate (%)	0.603	0.597	0.600	0.657	-0.673	-0.669	-0.663	0.681
D5 Top Quality Embryos Development (%)	0.704	0.771	0.749	0.786	-0.825	-0.825	-0.821	0.829

N=65. Correlation is significant at the 0.01 level (2-tailed).

DISCUSSION

Excessive leukocytes, debris, non-germ cells, immature, dead and/or damaged spermatozoa highly elevate production of seminal reactive oxygen species (ROS) that destroy normal functional spermatozoa, disturb normal sperm morphological characteristics, reduce fertilization potentials, and induce male infertility (Agarwal *et al*., 2014a; Alahmar, 2019). Seminal leukocytes produce up to 1000 times more ROS than immature spermatozoa, which develop oxidative stress response (Roychoudhury *et al*., 2016). Both male and female reproductive systems have antioxidant defense mechanisms to regulate ROS production and restore equilibrium between pro- and antioxidants (Agarwal *et al*., 2014a). Assisted reproduction techniques (ART) decrease levels of seminal ROS, select normal morphological and functional sperm cells, improve quality intracytoplasmic sperm injection (ICSI) outcomes, and increase implantation, pregnancy, and delivery

rates (Gupta *et al*., 2010; Gualtieri *et al*., 2021). Double-density gradient centrifugation (DDGC), a sperm preparation technique, is used to separate fractions of mature and highly motile spermatozoa free from debris, leukocytes, non-germ, and degenerated germ cells depending on their motility, size, density, vitality, and normal morphological characteristic features (Gupta *et al.*, 2010; Gualtieri *et al*., 2021; Ritchie & Ko, 2021).

In the plasma membranes and cytoplasm, spermatozoa rich with polyunsaturated fatty acids (PUFAs) that are highly susceptible to the harmful effects of ROS(Ribas-Maynou & Yeste, 2020; Gualtieri *et al.*, 2021). ROS radicals promote sperm lipid peroxidation to produce reactive lipid aldehydes, accelerate intracellular oxidative injury and membranes integrity dysfunction, increase membrane permeability and fluidity as well as reduce normal quality sperm morphological characteristics, sperm-oocytes fusion, and fertilization rates (Agarwal *et al*., 2014a; Alahmar, 2019; Gualtieri *et al*., 2021). Furthermore, the elevated levels of seminal ROS develop male infertility features, poor conventional and functional semen parameters (Shemshaki *et al.*, 2021), sperm DNA fragmentation, apoptosis, and antioxidant defenses dysfunction (Agarwal *et al*., 2006; Tremellen, 2008; Agarwal *et al*., 2014b). Malondialdehyde (MDA) is considered as a final endproduct of the decomposition of sperm lipid peroxides, which represents ROS response (Ribas-Maynou & Yeste, 2020). Kefer *et al.* (2009) reported that concentration of seminal MDA was negatively correlated with normal sperm parameters and sperm-oocyte fusion capacity. Ritchie & Ko (2021) in their review of literature demonstrated that several studies greatly introduced a negative correlation between MDA concentrationsand sperm count, motility, and morphology, which induced male infertility features. In normal healthy men, the high levels of seminal MDA were positively associated with low sperm progressive motility and sperm abnormalities (Kurkowska *et al*., 2020).

The current study aimed to evaluate the effects of seminal oxidative stress biomarkers (ROS and lipids peroxidation) and the total antioxidant capacity (TAC) on top quality sperm characteristic parameters, fertilization efficiency, and embryological ICSI outcomes. In the study group, the elevated levels of seminal leukocytes greatly increased levels of seminal ROS and lipids peroxidation, developed poor quality sperm functional characteristic features (sperm concentration; total count; total motility; normal morphology), and retarded top quality ICSI outcomes (D1 fertilized oocytes; fertilization rate; D3 cleavage oocytes; cleavage rate; D3 top quality embryos; D5 developed blastocysts; blastocysts formation rate, D5 top quality embryos) compared to the control group.

In infertile men, the increased levels of seminal lipid peroxides/MDA negatively correlated with normal spermatozoa morphology, vitality, seminal antioxidants, and fertilization rate (Agarwal *et al*., 2014a). In native semen samples and culture media of oocytes and embryos, the increased values of oxidative-reduction potentials (ORP) were negatively correlated with fertilizing capacity, excellent embryos transfer, and pregnancy rate (Sallam *et al*., 2021). In infertile men, the elevated levels of seminal ROS production, lipids peroxidation, and DNA fragmentation index markedly attenuated conventional and functional semen parameters including sperm concentration, total count, progressive motility, normal morphology, and antioxidant capacity compared to fertile men group (Mayorga-Torres *et al*., 2017). In D1 culture media, the elevated levels of ROS were greatly associated with low fertilization, cleavage, and blastocysts

formation rates, high embryonic fragmentation yield and low pregnancy rates in the IVF/ICSI cycles (Bedaiwy *et al*., 2004). In D3 embryos culture media, the reduced levels of ROS were positively correlated with top quality embryos development, blastocysts formation rate, and good pregnancy yield in the conventional IVF/ICSI cycles (Bedaiwy *et al*., 2010). In infertile men, the elevated levels of seminal ROS were negatively correlated with testicular volume, quality semen functional characteristics, fertilization rate, blastocysts formation yield, embryos development grade, and quality conventional IVF/ICSI outcomes as well as implantation and pregnancy rates (Zorn *et al*., 2003). All these previous studies confirmed our results.

Seminal fluid highly includes enzymatic and non-enzymatic antioxidants that are produced by sperm cells and seminal plasma against oxidative stress response (Majzoub & Agarwal, 2018). Seminal TAC value represents cumulative effect of all seminal antioxidants that greatly scavenge oxidative free radicals (Roychoudhury *et al*., 2016). Seminal antioxidants improvespermatozoa quality to upregulate successful IVF/ICSI outcomes (Agarwal *et al*., 2014a). In the present study, study infertile men (positive oxidative stress group) were markedly reduced in the levels of seminal TAC that positively correlated with the elevated levels of seminal leukocytes, ROS, and lipids peroxidation and negatively correlated with the conventional and functional semen parameters and top quality ICSI outcomes compared to control infertile men (negative oxidative stress group). In study group, excessive oxidative stress response (seminal leukocytes, ROS, lipids peroxidation) markedly depleted levels of seminal TAC, which greatly induced poor quality semen parameters and ICSI outcomes compared to the control group.

In infertile men, the elevated levels of seminal MDA greatly decreased sperm motility, normal morphology, and seminal total antioxidant status (TAS) compared with fertile men, which confirmed our results (Hosen *et al*., 2015). The reduced levels of seminal TAC were highly related to develop oxidative stress and male infertility characteristic features, which confirmed our results (Pahune *et al*., 2013; Roychoudhury *et al*., 2016). In D1 culture media, the elevated levels of TAC were greatly associated with high fertilization, cleavage, and blastocysts formation rates, low embryonic fragmentation yield, and high clinical pregnancy rates in the assisted IVF/ICSI cycles (Bedaiwy *et al*., 2006). Under oxidative stress response, the elevated levels of oxidative free radicals greatly impaired antioxidant defense mechanisms, reduced follicular levels of TAC, and induced poor oocytes fertilization rates (Luddi *et al*., 2016). Jana *et al*. (2016) demonstrated that the decreased levels of follicular TAC were positively correlated with poor quality oocytes and embryos development and low fertilization rates. Oyawoye *et al.* (2003) reported that the elevated levels of TAC highly increased number of fertilized oocytes and embryos viability. TAC levels were positively correlated with number of mature follicles and high quality oocytesand embryos grade in the IVF model (Pasqualotto *et al*., 2004). In infertile patients, follicular antioxidant capacity was impaired compared to controls under the IVF method (Pekel *et al*., 2015). In infertile men, antioxidant α-lipoic acid supplementation significantly increased the levels of seminal TAC and improved sperm functional parameters. In idiopathic oligoasthenospermic patients, clinical tamoxifen treatment markedly increased the levels of serum and seminal TAC and decreased spermatozoa intracellular ROS, which indicated potent antioxidant activity of tamoxifen supplementation (Roychoudhury *et al*., 2016).

In conclusion, in study group, the elevated levels of seminal leukocytes,

ROS, and lipids peroxidation markedly reduced sperm concentration, total count, total motility, normal morphology, seminal TAC level, and top quality ICSI outcomes compared to control group. For study group, it is recommend that antioxidant supplementation can improve quality semen functional characteristic parameters, reduce levels of seminal leukocytes, ROS, and lipids peroxidation as well as upregulate yield of ICSI outcomes compared to control group.

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Effects of reactive oxygen species, lipids peroxidation, and total antioxidant capacity on sperm morphological functional characteristics and quality embryological development ICSI outcomes

تأثيرات أنواع الأكسجين التفاعلية ، وبيروكسيد الدهون ، والقدرة الإجمالية لمضادات الأكسدة على الخصائص الوظيفية المورفولوجية للحيوانات المنوية ونتائج التطور الجنيني النوعي للحقن المجهري

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المستخلص

أستهدفت هذه الدراسة تقييم آثار المؤشرات الحيوية للإجهاد التأكسدي على معايير الخصائص الوظيفية للحيوانات المنوية ونتائج الحقن المجهري. في مجموعة الدراسة ، أدت المستويات المرتفعة من كريات الدم البيضاء، وROS ، و بيروكسيد الدهون بالسائل المنوي إلى إنخفاض ملحوظ في تركيز الحيوانات المنوية ، والعدد الكلي ، والحركة الكلية ، والتشكل الطبيعي لها، ومستوى TAC بالسائل المنوي، و تقليل جودة نتائج الحقن المجهري مقارنة بمجموعة التحكم السالبة. بالنسبة للمجموعة موضع الدراسة . ويتضح من النتائج أن تناول المكملات الغذائية الغنية بمضادة للأكسدة يمكن أن تحسن المعايير الحيوية والخصائص الوظيفية للسائل المنوي ، وتقلل مستويات كريات الدم البيض البيضاء ، وROS ، وبيروكسيد الدهون بالسائل المنوي، وكذلك تنظيم إنتاجية مخرجات الحقن المجهري مقارنة بمجموعة التحكم السالبة.