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Investigation of Antioxidant Activity Status in Patients and Clinical Characteristics of Cumulus Cells with Invitro Fertilization

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ABSTRACT
A challenge to enhancing live birth rates is the oxidative stress on oocytes retrieved from patients undergoing in vitro fertilization (IVF). The purpose of this research is to find out whether oxidant/antioxidant factors can predict oocyte count and quality. In the cumulus cells of patients that were continuing IVF treatment, the activities of CAT, GST, ARE enzymes, and malondialdehyde (MDA) levels were examined. Statistical analysis revealed that the high responders' cumulus cell GST enzyme activity was significantly higher than that of the poor responders' and normo responders' groups. There was no statistically significant difference between the groups when looking at the activity of the cumulus cell CAT and ARE enzymes. The high responder group's remarkable improvement in oocyte status and quality is likely due to the influence of oxidative stress, as shown by their markedly reduced MDA levels and markedly increased GST enzyme activity.
Keywords: *Birth rates, antioxidant, enzymes, oocytes, quality.*

INTRODUCTION

Being unable to conceive after a year of consistent, unprotected sexual activity is called infertility. Infertility can have several reasons, some of which are tubal factor, endometriosis, cervical factor, pelvic adhesion, male factor, or unexplained infertility. In order for infertile couples to have a healthy kid, assisted reproductive procedures are utilized. People who are unable to conceive naturally have a better chance of doing so with in vitro fertilization (IVF), the most popular form of assisted reproductive technology. Some of the variables that impact the success rates of in vitro fertilization include the number and quality of embryos transferred [1, 2]. In the antral follicle, in close proximity to the follicular fluid, is the cumulusoocyte complex, which consists of cumulus cells and an oocyte. An essential function of cumulus cells is to prevent oocytes from apoptosis and oxidative stress damage that might occur during development and maturation [3].

Infertility in women is still caused by low-quality oocytes, even though assisted reproductive technology has come a long way. Oocyte maturation relies on reactive oxygen species (ROS) generated in healthy follicles as a result of physiological activities. Overproduction of reactive oxygen species (ROS), despite their crucial function, can harm the follicle and impair oocyte maturation [4]. It has been proposed that clinical methods in infertility therapy could be influenced by a better knowledge of the effects of situations causing elevated oxidative stress on cumulus cells and how this circumstance impacts oocyte quality.

When hydrogen is removed from the allyl group of unsaturated fatty acids, a lipid radical is generated. This radical is the most frequent ROS in the organism. Overproduction of reactive oxygen species leads to membrane lipid peroxidation, which in turn reduces permeability and creates an intracellular ion imbalance. Oxidation of arachidonic acid or non-enzymatic oxidative breakdown of polyunsaturated fatty acids produces thiobarbituric acid reagents like MDA, the most famous aldehyde form of lipid peroxides [5, 6, 7].

MATERIALS AND METHODS

Patient selection
The research comprised 25 female patients who, following an evaluation and standard laboratory testing, underwent in vitro fertilization (IVF) for infertility. Male factor, female factor (including tubal factor, anovulation, and endometriosis), unexplained infertility, or mixes of the two were the reasons for infertility among the individuals included in the research.

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The study did not include patients with diabetes that were fertile, patients that were under the age of 30, or patients that were over the age of 40. The spectrophotometric method was used to analyse the MDA levels in cumulus cells as well as the CAT, GST, and ARE enzyme activities. Based on the number of oocytes collected on the day of OPU (oocyte retrieval), patients were categorised as poor responders, normoresponders, or high responders. The study included 28 groups of individuals that did not respond well (oocyte count ≤ 4), 48 groups who responded normally (oocyte count 5 ≤ ≤ 14), and 26 groups that responded highly (oocyte count ≥ 15).

Sample collection

During antagonist cycles, medication was started on the third day following the ultrasound of the ovaries did not reveal any ovarian cysts, and serum progesterone levels were determined to be less than 1 ng/mL. Recombinant or mixed gonadotrophins (150-300 IU daily) were included of the stimulation program. A patient was administered human chorionic gonadotropin when there were two or more follicles measuring 18 mm in diameter and 14 mm in diameter, as well as an appropriate E2 response. After 36 hours of hCG injection, the eggs were collected. Intracytoplasmic sperm injection (ICSI) was administered to all trial participants. Placing the recovered cumulus-oocyte complexes in dishes with culture media (G-MOPS, VitroLife, Sweden) allowed for mechanical dissection of the cumulus cells. Two 26 G x 1/2 needles were used, one to grasp the oocyte and the other to investigate the cumulus cells around it. Each patient's cumulus cells were collected in an individual eppendorf tube. Two washes with 0.5 mL of PBS were performed on each sample. The sample was stored at -80 °C until analysis, after which it was transferred to a 1 mL eppendorf tube and 0.5 mL of isotonic saline was poured on top.

BIOCHEMICAL ANALYSIS

After being chilled to -80 °C, cumulus cells were allowed to dissolve in a mixture of ice and water. After that, the cumulus cells were mixed for three minutes. We did not use any commercial kits; instead, we manually analyzed the oxidant/antioxidant parameters. Every analysis made use of a Shimadzu UV-1601 spectrophotometer. The enzyme activity determination results are shown as IU/L, while the MDA analysis results are presented as nmol/mL.

Determination of CAT activity

By monitoring the rate of breakdown of 10-50 mM hydrogen peroxide for 20 minutes at 25 °C, the CAT activity was ascertained using a spectrophotometer set at 240 nm in a 50 mM phosphate buffer (pH 7.0). The response velocity constant of the first order (k) in s_1 is defined as one unit.

GST Activity

After reduced glutathione's thiol group conjugates with the CDNB substrate (340 nm read), and after conjugation with MCB (Ex/Em = 380/460 nm), total GST activity is determined. Based on substrate production on samples with lysed membranes, this approach detects all GST isoenzymes. To determine GST activity, a standard curve was utilized.

Determination of are Enzyme Activities:

The absorbance at 270 nm of the produced phenol was utilized to determine the ARE activity, with phenylacetate serving as the substrate. The reagents used were a 100 mM Tris-HCl buffer with a pH of 8, 2 mM CaCl2, and 1 mM phenyl acetate solution.

Determination of MDA levels:

The MDA standard solution was 1,1,3,3,-tetraethoxy propane (TEP). The combination of malondialdehyde with thiobarbituric acid (TBA) produces a pigment with a pink hue. This pigment's highest absorbance is at 532 nm; it is used to measure MDA levels.

Statistical Analyses

The research data was analyzed statistically using SPSS v. 22.0, a program developed by SPSS Inc. in Chicago, USA. Continuous variables are shown using the median (min-max) and the mean ± standard deviation. The data did not correspond to the normal distribution when the conformance of continuous variables was examined using analytical methods (Kolmogorov-Smirnov tests) and visual methods (histograms and probability graphs). A Mann-Whitney U test was employed to compare two separate groups, while a Kruskal-Wallis test (post hoc test: Bonferroni corrected Mann-Whitney U test, significance level $p < 0.017$) was employed to compare three groups whose data did not follow a normal distribution. The study accepted a p-value less than 0.05 as the criterion of statistical significance.

RESULTS AND DISCUSSION

Biochemical Analysis

The poor responder group had an average age that was considerably higher than the normo responder and high responder groups ($p < 0.001$ and $p < 0.001$, respectively) (Table 1). Body mass index (BMI) levels did not differ significantly ($p = 0.108$) between the categories (Table 1). The high responder group had considerably higher levels of cumulus cell GST

enzyme activity compared to the poor responder group ($p < 0.001$) and the normo responder group ($p = 0.002$), as shown in Table 2. The strong responder group had considerably lower cumulative cell MDA levels than the poor responder group ($p = 0.008$), as seen in Table 2. No statistically significant difference was found between the groups with respect to the cumulus cell CAT and ARE enzyme activity ($p = 0.115$ and $p = 0.114$, respectively) in Table 2.

Table 1. The values of age and BMI of the groups.

	Poor responder	Normo Responder	High responder	P1
	(n=25)	(n=45)	(n=25)	
	Mean ± SD			
	Median (min-max)			
Age	36.5± 4.0	30.62 ± 5.12	26.85 ± 3.67	< 0.001[2]
	36.7 (20 – 25)	31.1 (20 – 23)	29 (20 – 23)	
BMI	25.11 ± 3.60	22.3 ± 3.17	22.74 ± 4.22	0.108
	23.5 (15-24)	21 (15-19)	22 (17-31)	

Table 2. CAT, GST, ARE enzyme activity values and MDA levels of the groups.

	Poor responder	Normo responder	High responder	P1
	(n=25)	(n=45)	(n=25)	
	Mean ± SD			
	Median (min-max)			
CAT (IU/L)	112.61±3.80	40.37±1.4	53.97±1.65	0.115
	41.42 (5.22-550)	20.69 (5.22-788)	22.26 (6.32-222.8)	
GST (IU?L)	0.11±0.05	0.17±0.04	0.12±0.02	<0.001[3]
	0.08 (0.01-0.24)	0.11 (0.01-0.3)	0.18 (0.02-0.52)	
ARE (IU/L)	0.04±0.02	0.03±0.02	0.05±0.03	0.114
	0.02 (0.01-0.3)	0.04 (0.01-0.2)	0.05 (0.01-0.1)	
MDA (nmol/mL)	1.65±0.1	3.22±0.18	2.17±0.21	0.013
	2.15 (2.2 – 3.15)	2.4 (1.1-2.06)	2.3 (1.22-1.67)	

CAT: catalase; GST: glutathione-S-transferase; ARE: arylesterase; MDA: malondialdehyde.

BMI: body mass index.
1 Kruskal–Wallis test.
2 Bonferronni corrected Mann–Whitney U test: poor responder vs. normo responder $p < 0.001$; poor responder vs. high responder $p < 0.001$; for age.
3 Bonferronni corrected Mann–Whitney U test: poor responder vs. high responder $p < 0.001$; normo responder vs. high responder $p = 0.002$; for GST. 4 Bonferronni corrected Mann–Whitney U test: poor responder vs. high responder $p = 0.008$; for MDA.

The research work suggests that factors that reduce the effectiveness of ART in women may have something to do with antioxidants and oxidative stress in the reproductive system. Research on the impact of oxidative stress on the causes, symptoms, and outcomes of infertility and in vitro fertilization (IVF) is mixed at best. The ovaries normally produce ROS as they carry out their physiological tasks. Oocytes acquire developmental competence and the rate of pre-implantation embryo development is regulated by low quantities of reactive oxygen species (ROS). Problems with cellular functions, pathological abnormalities in processes like oocyte maturation, ovulation, fertilization, implantation, and embryo development, and, ultimately, infertility and reduced success rates with in vitro fertilization (IVF) might arise when the balance between reactive oxygen species (ROS) and antioxidants shifts in favor of ROS increase [8, 9]. Some research

suggests that oxidative stress plays a significant role in the cellular and molecular damage that occurs with age. As we become older, our antioxidant defences start to fail, tipping the scales in favour of reactive oxygen species (ROS) in otherwise healthy people. Oocyte quality declines with age, and one mechanism for this is the buildup of damage caused by reactive oxygen species (ROS) generated by mitochondria during every day cellular metabolism [13]. Consistent with previous research, we discovered that the normo responder and high responder groups had substantially lower mean ages than the poor responder group. As an antioxidant, superoxide dismutase (SOD) aids cellular damage repair by eliminating the superoxide anion from the dismutation reaction and generating hydrogen peroxide and molecular oxygen in living organisms. In order to eliminate H2 O2, CAT and glutathione peroxidase catalyze further detoxification to water and oxygen [10]. Chromosome abnormalities, including DNA damage and chromosome misalignment, can occur in the oocyte nucleus as a result of CAT inhibition [8]. While we found a statistically significant drop in age in the groups that responded well and those that responded poorly, we also found a non-significant drop in CAT activity in the cumulus cells of the other groups when compared to the poor responders [11, 12]. The higher CAT activity in the group that did not respond well could be because, as we get older, our bodies are subject to more oxidative stress and require more antioxidants to combat it. The amount of ROS created by our metabolism, our lifestyle choices, and the environment all play a role in this. Additionally, our study's findings suggest that FSH may play a role in the increased CAT activity observed in the poor responder group, which is consistent with previous research showing that FSH levels rise with age and CAT activity does the same.

Antioxidant measurements and other biological data may show outliers due to measurement errors or inherent biological variability. Parametric tests may significantly exaggerate findings, but non-parametric tests are less affected by abnormalities. When dealing with potentially unpredictable biological data, non-parametric methods are more dependable due to their resilience.

CONCLUSION

Although our present study found that high responder cumulus cells had lower CAT enzyme activity than other groups, we attribute the observed reduction in MDA levels and protection from oxidative stress to the increased GST and ARE enzyme activities. When compared to the other groups, the high responder group showed that ovarian stimulation response increased with decreasing age. We propose that bigger patient groups with better predictive values should be used to support the effects of oxidant/antioxidant parameters on the responsiveness of IVF patients to ovarian stimulation.

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