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Association Between Systemic and Local Oxidative Stress of Infertile Women Undergoing Ivf/Icsi

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Abstract

Oxidative stress (OS) may affect *in vitro* fertilization (IVF) outcomes in infertile women undergoing *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI). The aim of this study is to explore the possible relationships between OS parameters in serum and follicular fluid (FF) from infertile women with male cause infertility (n=40), women with polycystic ovary syndrome (PCOS) (n=40), and women with unexplained infertility (UI)(n=45) undergoing IVF/ICSI. The collection of blood and FF samples was done at the day of oocyte aspiration. Total peroxide (TPX) level, total antioxidant capacity (TAC), and malondialdehyde (MDA) level were measured in serum and FF; whereas, glutathione-s-transferase (GST) activity and superoxide dismutase (SOD) activity were measured in FF. Also, oxidative stress index (OSI) that is the percentage ratio of TPX to TAC, was calculated. In the control group, correlation analysis reveals the presence of a significant positive association between FF OSI with serum OSI, FF TPX with FF OSI, and serum TPX with FF GST activity. In the PCOS group, there was a significant negative association between: FF TPX and serum TAC level. However, non-significant relationship was found between serum and FF OS status parameters in the UI group. It's concluded from the present study that systemic OS may give valuable information about local OS occurrence (blood OS reflects FF OS) only in control group and PCOS group. Such information could be useful for a better understanding of the pathological OS mechanisms involved in IVF failure for patients with different causes of infertility.

Keywords: Oxidative stress, infertility, follicular fluid, antioxidants, *in vitro* fertilization, assisted reproductive techniques.

العلاقة بين الشد التأكسدي العام والموضعي للنساء العقيمات الخاضعات للتلقيح الخارجي/ الحقن المجهري

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الخلاصة

قد يؤثر الشد التأكسدي على نتائج التلقيح الخارجي في النساء العقيمات الخاضعات للتلقيح الخارجي/ الحقن المجهري. الغرض من الدراسة هو لأيجاد العلاقات المحتملة لمتغيرات الشد التأكسدي بين مصل الدم والسائل الجريبي للنساء غير المنجبات بسبب ازواجهن (n=40) والنساء العقيمات بسبب متلازمة تكيس المبايض PCOS (n=40). والنساء العقيمات لسبب مجهول (UI) (n=45) و الخاضعات للتلقيح الخارجي/

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الحقن المجهرى. تم جمع عينات الدم والسائل الجريبي في يوم سحب البويضات. تم قياس تركيز البيروكسيد الكلي TPX، وقدرة مضادات الأكسدة الكلية TAC، وتركيز المالونداالدهايد MDA في مصل الدم والسائل الجريبي، إضافة الى قياس الفعالية النوعية لأنزيم جلوتاثاين ترانسفيراز GST، ولأنزيم سوپر أوكسيد ديسميوتاز SOD في السائل الجريبي. وقد تم أيضا حساب مؤشر الأجهاد التأكسدي (OSI) وهو النسبة المئوية بين تركيز البيروكسيد الكلي الى مضادات الأكسدة الكلية. أظهر تحليل العلاقات في مجموعة السيطرة، علاقة معنوية موجبة بين OSI في السائل الجريبي مع مثيله في مصل الدم و مع TPX في السائل الجريبي وكذلك بين فعالية انزيم GST في السائل الجريبي و TPX في مصل الدم. ظهرت في مجموعة PCOS علاقة معنوية سالبة بين TPX في السائل الجريبي مع TAC في مصل الدم، بينما في مجموعة UI لم تظهر اية علاقة معنوية بين متغيرات حالة الشد التأكسدي بين مصل الدم والسائل الجريبي. أظهرت الدراسة الحالية أن الشد التأكسدي العام قد يعطي معلومات ذات قيمة حول تواجد الشد التأكسدي الموضعي في مجموعتي السيطرة ومريضات متلازمة تكيس المبيض PCOS فقط. وقد تكون هذه المعلومات مفيدة لمعرفة ميكانيكية الشد التأكسدي المرضية والتي لها علاقة بفشل التلقيح الخارجي للعقيمت بأختلاف مسببات العقم.

Introduction

Oxidative stress (OS) is a state caused by an imbalance between Reactive Oxygen Species (ROS) or Reactive Nitrogen Species production and the antioxidant defense systems. This process is responsible for adaptive response consisting in the induction of antioxidant response and following antioxidant depletion occurs in dysfunction and cellular injury [1, 2].

Indeed, overproduction of ROS and resulting OS may affect female reproduction and gamete health; therefore, the loss in the balance between ROS and antioxidants in serum and follicular fluid (FF) have been implicated in poor reproductive outcomes in infertile patients treated by *in vitro* fertilization (IVF) [3].

In vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) are widely used as assisted reproductive techniques (ART) to solve human infertility. These techniques provide great benefits for couples who have struggled with infertility disorders [4]. In previous reports, researchers concentrated on the microenvironment surrounding the oocyte. They found that OS affects the reproductive potential [5-7]. Furthermore, OS is an important cause for IVF failure [8]. All these studies highlighted the complex connection amongst ROS and antioxidants in the ovaries.

Additionally, ROS production in the ovaries perform plasma membrane damage by lipid peroxidation of polyunsaturated fatty acids and give rise to cell injury [3, 9]; thus, Malondialdehyde (MDA) is a byproduct of the lipid peroxidation process is used as a marker for this oxidation [10]. In a previous report, it was stated that elevation in MDA levels and depletion in antioxidant levels in serum and FF may affect the fertilization rate, the quality of oocytes and embryo in patients undergoing ART [11].

Antioxidant enzymes inside granulosa cells, cumulus cells, and FF each play a basic part in the protection of the oocyte. Also, glutathione system plays an important role in cell defense against ROS [3]. Glutathione peroxidase, glutathione reductase and glutathione-s-transferase (GST) are antioxidant enzymes expressed in mammalian oviducts and play an important role in the balance between intra and extracellular redox system [12]. Superoxide dismutase (SOD) enzyme catalyzes the dismutation of superoxide anion radicals to form hydrogen peroxide and molecular oxygen. Thus, SOD plays an important role in the first line of antioxidant defense [13].

Several authors have demonstrated signs of OS in serum and in FF of infertile patients [14, 3]. Moreover, other reports studied OS parameters, both at systemic and local levels. An earlier publication had studied total peroxide (TPX) concentrations, total antioxidant capacity (TAC), and oxidative stress index (OSI) in plasma and FF of patients undergoing IVF program [15]. Also, researchers studied the relationship between ROS, TAC, and redox index in each of plasma FF, and granulosa cell of infertile women undergoing ART procedure [16]. But, these data lack the simultaneous presence of OS in serum and FF; as well as, the relationship between OS parameters in both of systemic and local levels in regard to different etiological infertile patients. Thus, the aim of this study is to assess serum and FF OS status parameters, and to explore possible relationships between the assessed parameters in serum and FF of infertile women with male cause infertility, poly

cystic ovary syndrome (PCOS), and unexplained infertility (UI) undergoing *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI).

Subjects, Materials, and Methods

Subjects

A prospective case control study conducted at Kamal Al-Samarai Hospital in Baghdad-Iraq, from December 2017 to June 2018. The medical ethics committee in University of Baghdad approved this study protocol. All patients signed a written informed consent.

The study included 125 infertile women undergoing IVF/ICSI, They were divided according to the etiology of infertility into: 40 infertile women (PCOS group) (mean age 31.02 ± 0.93 years), 40 women with male cause of infertility (control group) (mean age 30.87 ± 1.98 years), and 45 women with UI group (mean age 30.68 ± 1.23 years).

The diagnosis of PCOS was done according to the revised Rotterdam European Society of Human Reproduction and Embryology ESHRE/American Society for Reproductive Medicine ASRM Criteria [17]. Patients diagnosed with UI based on standard infertility tests, according to the guidelines of the Practice Committee of the ASRM [18]. These tests included assessments of spermogram, ovulation, hysterosalpingogram, and, if indicated, ovarian reserve tests and laparoscopy. If the results of all these tests were normal, patients were accepted as UI.

Exclusion criteria: presence of tumors, women older than 38 years of age, women who laparoscopically diagnosed with endometriosis, poor ovarian reserve, endocrine disorders (such as hyperprolactinemia and thyroid dysfunction), pathologies of ovarian or fallopian tubes (e.g. Adenomyosis, Hydrosalpinx), severe pelvic adhesions, diabetes mellitus, and cardiovascular diseases. All subjects were hyperstimulated by gonadotropin releasing hormone (GnRH) antagonist protocol. The administration of 150-225 IU of recombinant FSH (Gonal-F®) injection was done from day two of menstrual cycle. The GnRH antagonist (Cetrorelix) was given (0.25 mg) daily when the follicle reached 12-14mm, as detected by ultrasound. Cetrorelix and Gonal-F® were continued together until either two or three follicles reach 17-18 mm in the ovary. Then, ovulation induction using recombinant human chorionic gonadotropin administration (rhCG 6500 IU, Ovitrelle®; Merck Serono, Italy) was done.

Oocytes were picked up after 34 - 36 hours from hCG injection using needle aspiration with the guidance of transvaginal ultrasound transducer. Venous blood (5ml) was withdrawn at the day of oocyte aspiration from all subjects and allowed to clot in a gel tube and then centrifuged. The separated serum was stored in sterile eppendorf tubes at -20°C until use. Uncontaminated FF samples were centrifuged at $3000 \times g$ for 10 min at room temperature. The clear supernatants of FF were transferred to sterile eppendorf tubes and stored at -20°C until assayed.

Analysis

Measurement of TPX levels

Concentrations of TPX in serum and FF samples were measured using the modified xylenol orange assay (FOX2) [19]. The assay is based on oxidation of ferrous to ferric ion by several kinds of peroxides within the samples to give ferric-xylenol orange complex. The absorbance of the solution was measured at 560 nm using spectrophotometer. The TPX contents of samples were measured by a standard solution of hydrogen peroxide.

Measurement of TAC

Estimation of TAC depends on reaction of $[\text{Fe}^{2+}\text{-O-dianisidine}]$ complex with hydrogen peroxide to produce hydroxyl radicals. Hydroxyl radical oxidize colorless O-dianisidine to dianisidyl radicals that is yellow-brown color, hence can be measured by spectrophotometer at 444 nm. Antioxidants in the sample suppress color degree depending on their concentrations. Ascorbic acid (2.0 mM) was used as standard [20a].

Calculation of OSI index

The percentage ratio of TPX to TAC was used to measure OSI index [21].

Measurement of MDA levels

The reaction of MDA with thiobarbituric acid (TBA) gives a red compound, which can be measured by spectrophotometer at 535 nm [9].

Determination of GST activity

Activity of GST was measured, as previously described [22], according to the conjugation reaction of 1-chloro-2,4-dinitro-benzene (CDNB) with reduced glutathione (GSH). The reaction mixture

contains phosphate buffer saline (1 M, pH 6.5), GSH (100 mM), CDNB (100 mM), and FF sample (100 μ l). The increase in absorbance at 340 nm at 25°C was recorded. One unit of enzyme activity was defined as one μ mol of GSH conjugated/min at 25°C.

GST activity (U/ml) = $[(\Delta A_{340}/\text{time min})/0.0096] \times (V_t/V_s)$

$\Delta A_{340}/\text{min} = (A_{340}/\text{min}) \text{ sample} - (A_{340}/\text{min}) \text{ blank}$; Molar extinction coefficient of CDNB at (340nm) = 0.0096 $\mu\text{M}^{-1}/\text{cm}$; V_t = volume of test; V_s = volume of sample

GST specific activity (U/mg) = $\frac{\text{GST activity U/ml}}{\text{protein Conc. mg/ml}}$

Determination of SOD activity

The activity of SOD was estimated using indirect method (riboflavin/ nitro blue tetrazolium NBT method), as previously described [23]. The working mixture (15.125 ml) consisted of phosphate buffer (131.19mM, pH 7.8), L-Methionine (300 mg/10ml), NBT-2HCl (14.1 mg/10ml), Triton X-100 (100mg/10ml). To this mixture, (100 μ l) of FF sample and 10 μ l of Riboflavin solution (4.4 mg/100ml) were added followed by illumination at 25 °C in aluminum foil lined box containing two fluorescent lamps (20-Watts) for 7 minutes. The absorbance was measured immediately at wavelength 560 nm.

One unit of SOD was defined as that amount of sample that causes a 50% decrease of the SOD inhibition NBT reduction in this assay. Therefore, the SOD activity in the sample can be expressed in riboflavin/NBT assay unit (U) using the following equation:

SOD activity (U/ml) = $\text{Sample inhibition \%} \times 2 \times 1000 / \text{Max.inhibition \%} \times V_s (\mu\text{l})$

V_s = volume of the sample.

Maximum inhibition was calculated from inhibition curve of each group.

Sample inhibition % = $(AB_2-AB_1) - (AS_2-AS_1) / (AB_2-AB_1) \times 100$.

Where:

AS1: absorbance of the sample before illumination; AS2: absorbance of sample after illumination; AB1: absorbance of blank before illumination; AB2: absorbance of blank after illumination.

SOD specific activity (U/mg) = $(\text{SOD activity (U/ml)}) / (\text{protein Conc. (mg/ml)})$

Protein assay

The protein concentration was determined by the modified biuret method, using bovine serum albumin (BSA 10 mg/ml) as the standard [24].

Statistical analysis

Data analysis was done by utilizing SPSS for Windows, version 22 (SPSS Inc. Chicago, Illinois, United States). Data appeared as mean \pm standard deviation. Statistical analysis performed by one-way ANOVA followed by Tukey's Post Hoc test, and Person correlation. A p value less than 0.05 was considered statistically significant [25].

Results

1. The OS status parameters in serum and FF:

As shown in Table-1A, mean TPX concentration and OSI in PCOS groups were significantly ($P < 0.05$) higher compared to control group and UI group; while, non-significant ($P > 0.05$) differences were seen in MDA and TAC concentrations among the three groups.

Table 1 A-Oxidative stress statue parameters in serum of the three studied groups.

Parameter	Control group (n=40)	PCOS group (n=40)	UI group (n=45)	P value
TPX (μM)	4.33 \pm 1.55	7.96 \pm 2.30 ^{a, b}	5.72 \pm 2.30	0.000
TAC (mM)	0.81 \pm 0.11	0.77 \pm 0.09	0.81 \pm 0.14	0.271
OSI %	0.53 \pm 0.21	1.09 \pm 0.31 ^{a, b}	0.73 \pm 0.30	0.000
MDA (μM)	0.53 \pm 0.28	0.55 \pm 0.38	0.34 \pm 0.14	0.450

^a $P < 0.05$ compared with control group; ^b $P < 0.05$ compared with UI group.

The OS status parameters in FF were shown in (Table-1B), mean TPX concentration and OSI in PCOS group showed higher levels compared to control group, but the differences were statistically significant ($P < 0.05$) only in OSI. Also, mean TPX concentration and OSI in UI group showed significant ($P < 0.05$) higher levels compared to control groups and PCOS group. Non-significant ($P > 0.05$) differences were seen in MDA and TAC concentrations among the three groups.

Table 1 B-Oxidative stress statue parameters in FF of the three studied groups

Parameter	Control group (n=40)	PCOS group (n=40)	UI group (n=45)	P value
TPX (μM)	5.42±2.12	6.47±2.19 ^b	8.31±1.32 ^a	0.000
TAC (mM)	0.93±0.08	0.89±0.12	0.89±0.11	0.276
OSI %	0.58±0.24	0.72±0.27 ^{a, b}	0.94±0.16 ^a	0.000
MDA (μM)	0.31±0.25	0.41±0.27	0.24±0.13	0.115

^aP< 0.05 compared with control group; ^bP< 0.05 compared with UI group.

2. Antioxidant enzymes activity in FF:

As shown in (Table-2), both of GST and SOD activity and specific activity were lower in PCOS group and UI group compared to control group, and the only statistically significantly (P < 0.05) lower value was shown in SOD specific activity of PCOS group compared to control group.

Table 2-Antioxidant enzymes activity in FF of the three studied groups

Enzyme activity	Control group (n=40)	PCOS group (n=40)	UI group (n=45)	P value
GST activity (U/ml)	66.50±28.50	57.66±24.06	54.21±26.46	0.185
GST specific activity (U/mg)	1.19±0.58	0.98±0.52	0.98±0.48	0.177
SOD activity (U/ml)	13.03±2.62	12.11±4.28	11.94±2.29	0.376
SOD specific activity (U/mg)	0.23±0.09	0.18±0.06 ^a	0.18±0.04	0.004

^aP< 0.05 compared with control group.

3. Association between OS status parameters in both serum and FF of control group:

In the present results, there was a significant (P< 0.05) positive association between: FF OSI and serum OSI (Figure-1A), FF TPX and FF OSI (Figure-1B), and serum TPX and FF GST (Figure-1C).

4. Association between OS status parameters in serum and FF of PCOS group:

In the present results, there was a significant (P< 0.05) negative association between: FF TPX and serum TAC (Figure-2).

5. Association between OS status parameters in UI group:

In the present study, non-significant relationship was found between serum and FF OS status parameters in UI group.

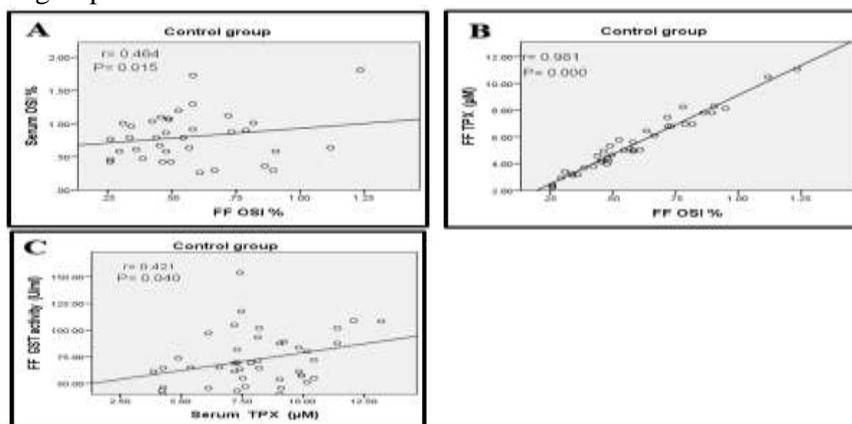


Figure 1- The relationship between: A. FF OSI and serum OSI; B. FF TPX and FF OSI; C. serum TPX and FF GST of control group.

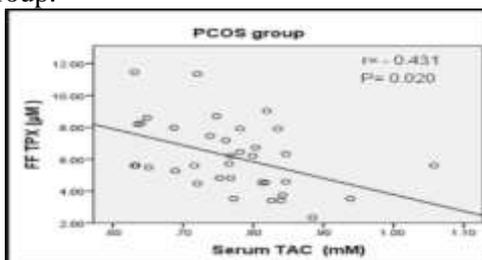


Figure 2- The relationship between FF TPX and serum TAC of PCOS group.

Discussion

As in many other systems, a physiological amount of ROS may be indicative of healthy developing oocytes, whereas excessively high levels may be indicative of OS [9].

In the present study, intrafollicular and systemic balances of the pro oxidant-antioxidant system were studied in patients undergoing IVF program. To achieve this object, markers reflecting OS status were measured (TPX, TAC, and MDA). Also, OSI was calculated. However, OSI is an indicator of OS reflecting the redox balance between oxidant and antioxidant, thus, shows higher values if there was elevation in OS level [20b, 26].

The OS parameters were studied by Becattiet al. which included forty five infertile women undergoing IVF program vs. forty five control group women (with tubal factor or male factor infertility). The study displayed significantly lower oxygen radical absorbance capacity (account for TAC) and significantly higher thiobarbituric acid-reactive substances level (as index of lipid peroxidation) in plasma samples, taken at the day of egg retrieval of infertile patients. They concluded that the presence of OS in infertile patients was also confirmed by the significantly higher ROS production in leukocytes (lymphocyte, monocyte, and granulocyte) and granulosa cells compared to control [16].

Additionally, in a recent study, total oxidant, TAC, and OSI levels were studied in serum samples (at early follicular phase) of PCOS patients and control group. They showed significant higher level of oxidant and OSI; as well as, significant lower TAC levels in PCOS group compared to control. They suggested that higher levels of OS parameters might be related to PCOS [27].

Appasamyet al. studied TAC of infertile women with different etiologies (male cause infertility, UI, tubal factor, PCOS, and endometriosis) in plasma samples obtained at the time of oocyte recovery. In contrast to the present study findings, the study was found plasma TAC was higher in UI, PCOS, and endometriosis groups than control group (male cause infertility) [5]. This discrepancy in results may be due to their lower number of patients (PCOS= 15, UI= 36) and different stimulation protocol (GnRH agonist); as well as, different TAC assay methods.

In a previous Turkish study by Ozturket al., that studied serum (total oxidant status, TAC, and OSI) in high responder patients, normoresponder patients, and poor responder patients. They revealed there were non-significant differences between groups in terms of studied parameters. They concluded that; even if, these parameters did not change significantly between the groups, but serum TAC could affect OS in FF by its relationship with FF TAC [28].

The higher TPX and OSI but lower TAC levels in FF of PCOS group than control group that displayed by the present study is in agreement with previous reports [29, 30].

Artimani et al. studied prooxidant-antioxidant balance and inflammatory cytokines in twenty one infertile women with PCOS and twenty one control group whom underwent IVF program due to tubal obstruction or male factor infertility. They found significant higher total oxidant levels and lower TAC level in PCOS group than control group. They proved that increased OS is associated with inflammation in PCOS patients [29].

Göktolga et al. failed to detect a significant difference in total oxidants, TAC, and OSI levels in FF between patients with PCOS and patients with male factor infertility whom underwent ART using ICSI. It is seemly that total oxidants and OSI were higher; whereas, TAC was lower in PCOS group than control group, but the data did not reach the statistical significance difference. A definitive conclusion in their study has not been drawn regarding the role of OS with infertility in those women [30].

In the present study, concerning the OS in the two body fluids (serum & FF) of UI group, it seems that the overall OSI in FF of UI was higher than that in serum of the same group. These results haven't shown before in literatures.

This may be interpreted by Gonadotropin stimulation which may have a direct impact on OS markers. However, IVF cycles have been associated with the production of ROS and perturbation in the oxidant-antioxidant balance [31,32]. This mean that the higher level of local OS of UI group may come from systemic OS through the blood-follicle barrier [33] plus the OS due to stimulation protocol in IVF cycles [31, 32]. Indeed, this elevation in FF may reflect the active metabolism in the follicle and subsequently the whole ovary as a result of multiple follicle stimulation [15]. Oxidative metabolism is implicated in every stage of ovarian follicular growth and oocyte maturation [34]. Also, high ovarian ROS concentrations are a byproduct of steroidogenesis [35].

The present study showed that MDA levels were higher in serum and FF of PCOS group as compared with control group. In agreement with this result; the study of Artimani *et al.* who showed that PCOS women had an elevated concentration of MDA compared to normal ovulatory controls treated with IVF/ICSI, as well as they suggested in their study that increased OS in PCOS is associated with inflammation that support insulin resistance which is closely linked to this syndrome [29]. The increased MDA levels in patients with PCOS treated with IVF/ICSI also documented by other studies [36, 37].

Our study showed, despite the OSI index in FF of UI group was higher than control group and PCOS group, but, MDA concentration in serum and FF were lower than the other two groups. The scientific interpretation of this conflict observation is that the antioxidant concentration in serum and FF may be able to work as buffer to oppose the high toxic level of MDA [38] and reduce its level in serum and FF of UI group.

Unfortunately, the lack of a reference value in ordinary healthy women (unstimulated ovaries) makes it hard to decide whether the TPX levels observed in FF are in the pathological or physiological range. Despite there is a control group (male cause infertility group), but it may be possible that some normal women have an alternate response to ovarian hyperstimulation which leads to altered ROS levels [31, 32].

Fujimoto *et al.* studied GST activity in FF samples (that obtained on the day of ovum pick up) and its effect on embryo morphology of infertile patients who underwent gonadotropin stimulation. They failed to find significant association between GST activity and both of embryo cell number and embryo fragmentation score. They suggested that GST activity within FF is not associated with the quality of embryos [39].

Ito *et al.* studied GST theta 1 expression in the mural and cumulus granulosa cells obtained from age related infertile patients. They reported that GST theta 1 (nonfunctional) in mural and cumulus granulosa cells is a possible indicator of aging of the reproductive cells. They strongly speculate that aging could cause decline of oocyte quality and could be associated with OS that induced apoptosis defined by glutathione-s-transferase theta 1 up regulation [40].

Carbone *et al.* studied the antioxidant enzyme (GST and SOD) activities in human FF during reproductive ageing; as well as, the expression of these enzymes [41].

The study found a reduced level of GST activity and a higher level of SOD activity in FF of older women group than younger women group. Moreover, the study revealed that ageing was associated with decreased protein expression of GST Pi isoform and did not affect SOD expression. The study was proved that reproductive ageing is accompanied by the change in the expression and activity of GST, which may lead to OS in the follicular milieu [41].

In regard to the result of mean SOD activity and specific activity in PCOS group and control group, the current study is in consistence with others [42, 43].

Sabatini *et al.* indicated that SOD activity in FF of PCOS patients is lower than that in the control group. They suggested that SOD may have a role in the pathophysiology of PCOS [42].

In a case control study by Saleem *et al.*, who studied FF SOD activity as well as Cu/Zn-SOD mRNA in PCOS patients, they found that both of SOD activity in FF, and Cu, Zn SOD mRNAs in cells isolated from FF, of PCOS cases lower than that of control group. Even if, lowered SOD activity in FF, they did not find relationships with fertilization rate and embryo quality after ICSI in PCOS patients [43].

Similarly, other researchers [44] proved that the concentration of SOD in FF of PCOS patients is lower than that in control group. They study the effect of using myoinositol as a therapy in PCOS women undergoing IVF cycles. They demonstrated that SOD concentration reaches levels that were observed among control group when PCOS patients were treated with myoinositol, suggesting the importance of myoinositol therapy before and during IVF program [44].

By contrast to the findings of the present study, a previous study by Pেকেlet *et al.* displayed higher FF SOD activity in PCOS group and UI group than that in control group. They explained that this increase may be to maintain oxidant-antioxidant balance in their patients' groups [45].

The results of the current study showed that TPX and TAC concentrations were independent as regards FF and serum, while OSI correlated well between the two body fluids of control group.

In a previous study that failed to find a significant association between serum total oxidant level and FF total oxidant level; whereas, they found a significant association between serum TAC level and FF TAC level. They suggested that serum TAC level could affect OS in FF [28].

The scientific interpretation of the direct relationship between FF GST activity and serum TPX level is that the intrafollicle antioxidant enzymes may work to reduce the high toxic level of systemic oxidants in control women group. Since, physiological oxidants level may be crucial for healthy growing oocyte [9].

Based on that, there is cooperation between local and systemic OS status parameters (necessarily through the blood follicle barrier) [33], to reach prooxidant-antioxidant balance and reduce OS. Interestingly, this observation may be supported by the present study where higher FF GST activity and lower serum TPX level found in control group.

In the present investigations, since serum TAC concentration was lower in PCOS group compared to control group and has negative effect on FF TPX concentration in PCOS women. Thus, as a cause-effect relationship, we can hypothesize that lower serum TAC level may increase intrafollicle oxidants level, therefore, may increase local OS.

Conclusion

Global OS may give valuable information about local OS occurrence (FF OS reflects systemic OS) only in control group and PCOS group. Such information would be useful for a better understanding of the pathological OS mechanisms involved in IVF failure for patients with different causes of infertility.

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References

1. Fink, G. **2007**. *Encyclopedia of stress*. Second Edition. Academic Press.
2. Marrocco, I., Altieri, F. and Peluso, I. **2017**. Measurement and clinical significance of biomarkers of oxidative stress in humans, *Oxid Med Cell Longev*, 1-32.
3. Agarwal, A., Aponte-Mellado, A., Premkumar, B.J., Shaman, A. and Gupta, S. **2012**. The effects of oxidative stress on female reproduction: a review, *Reprod Biol Endocrinol*, **10**(1): 1-49.
4. Agarwal, A., Gupta, S. and Sharma, R.K. **2005**. Role of oxidative stress in female reproduction, *Reprod Biol Endocrinol*, **3**(1): 1-28.
5. Lu, Y.H., Wang, N., and Jin, F. **2013**. Long-term follow-up of children conceived through assisted reproductive technology, *J Zhejiang Univ Sci B*, **14**(5): 359-371.
6. Appasamy, M., Jauniaux, E., Serhal, P., Al-Qahtani, A., Groome, N.P. and Muttukrishna, S. **2008**. Evaluation of the relationship between follicular fluid oxidative stress, ovarian hormones, and response to gonadotropin stimulation, *Fertil Steril*, **89**(4): 912-921.
7. Fujimoto, V.Y., Bloom, M.S., Huddleston, H.G., Shelley, W.B., Ocque, A. J. and Browne, R.W. **2011**. Correlations of follicular fluid oxidative stress biomarkers and enzyme activities with embryo morphology parameters during in vitro fertilization, *Fertil Steril*, **96**(6): 1357-1361.
8. Pereira, A.C. and Martel, F. **2014**. Oxidative stress in pregnancy and fertility pathologies, *Cell Biol Toxicol*, **30**(5): 301-312.
9. Borowiecka, M., Wojsiat, J., Polac, I., Radwan, M., Radwan, P. and Zbikowska, H.M. **2012**. Oxidative stress markers in follicular fluid of women undergoing in vitro fertilization and embryo transfer, *Syst Biol Reprod Med*, **58**(6): 301-305.
10. Das, S., Chattopadhyay, R., Ghosh, S., Ghosh, S., Goswami, S.K., Chakravarty, B.N. and Chaudhury, K. **2006**. Reactive oxygen species level in follicular fluid—embryo quality marker in IVF?, *Hum Reprod*, **21**(9): 2403-2407.
11. Aitken, J. and Fisher, H. **1994**. Reactive oxygen species generation and human spermatozoa: the balance of benefit and risk, *Bioessays*, **16**(4): 259-267.
12. Berker, B., Kaya, C., Aytac, R., and Satiroglu, H. **2009**. Homocysteine concentrations in follicular fluid are associated with poor oocyte and embryo qualities in polycystic ovary syndrome patients undergoing assisted reproduction, *Hum Reprod*, **24**(9): 2293-2302.
13. El Mouatassim, S., Guérin, P. and Ménéz, Y. **2000**. Mammalian oviduct and protection against free oxygen radicals: expression of genes encoding antioxidant enzymes in human and mouse, *Eur J Obstet Gynecol Reprod Biol*, **89**(1): 1-6.

14. Ighodaro, O.M. and Akinloye, O.A. **2018**. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid, *Alexandria J Med*, **54**(4): 287-293.
15. Oyawoye, O., Abdel Gadir, A., Garner, A., Constantinovici, N., Perrett, C. and Hardiman, P. **2003**. Antioxidants and reactive oxygen species in follicular fluid of women undergoing IVF: relationship to outcome, *Hum Reprod*, **18**(11): 2270-2274.
16. Velthut, A., Zilmer, M., Zilmer, K., Kaart, T., Karro, H. and Salumets, A. **2013**. Elevated blood plasma antioxidant status is favourable for achieving IVF/ICSI pregnancy, *Reprod Biomed Online*, **26**(4): 345-352
17. Becatti, M., Fucci, R., Mannucci, A., Barygina, V., Mugnaini, M., Criscuoli, L. and Evangelisti, P. **2018**. A biochemical approach to detect oxidative stress in infertile women undergoing assisted reproductive technology procedures, *Int J Mol Sci*, **19**(2): 585-592.
18. Rotterdam. **2004**. ESHRE/ASRM- Sponsored PCOS Consensus Workshop Group Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome, *Fertil Steril*, **81**(1): 19.
19. ASRM. **2006**. Effectiveness and treatment for unexplained infertility Practice Committee of the American Society for Reproductive Medicine, *Fertil Steril*, **86**(5 Suppl 1): S111.
20. Harma, M., Harma, M. and Erel, O. **2003**. Increased oxidative stress in patients with hydatidiform mole, *Swiss Med Wkly*, **133**(41-42): 563-566.
21. **20(a)**. Erel, O. **2004**. A novel automated method to measure total antioxidant response against potent free radical reactions, *Clin Biochem*, **37**(2): 112-119.
22. **20(b)**. Erel, O. **2005**. A new automated colorimetric method for measuring total oxidant status, *Clin Biochem*, **38**(12): 1103-1111.
23. Horoz, M., Bolukbas, C., Bolukbas, F.F., Aslan, M., Koylu, A.O., Selek, S. and Erel, O. **2006**. Oxidative stress in hepatitis C infected end-stage renal disease subjects, *BMC Infect Dis*, **6**(1): 114-118.
24. Mannervik, B. **1985**. The isoenzymes of glutathione transferase, *Adv Enzymol Relat Areas Mol Biol*, **57**: 357-417.
25. Kakkar, P., Das, B. and Viswanathan, P. **1984**. A modified spectrophotometric assay of superoxide dismutase, *Indian J Biochem Biophys*, **21**: 130-132.
26. Janairo, G., Linley, M.S., Yap, L., Llanos-Lazaro, N. and Robles, J. **2015**. Determination of the sensitivity range of biuret test for undergraduate biochemistry experiments, *Eur J Lipid Sci Technol*, **6**(5): 77-83.
27. Glover, T., and Mitchell, K. **2008**. *An Introduction to Biostatistics*. Waveland Press.
28. Rabus, M., Demirbağ, R., Sezen, Y., Konukoğlu, O., Yildiz, A., Erel, O. and Yakut, C. **2008**. Plasma and tissue oxidative stress index in patients with rheumatic and degenerative heart valve disease, *Türk Kardiyol Dern Ars*, **36**(8): 536-540.
29. Sak, S., Uyanikoglu, H., Incebiyik, A., Incebiyik, H., Hilali, N. G., Sabuncu, T. and Sak, E. **2018**. Associations of serum fetuin-A and oxidative stress parameters with polycystic ovary syndrome, *Clin Exp Reprod Med*, **45**(3): 116-121.
30. Ozturk, E., Dilbaz, S., Ozdegirmenci, O., Dede, H., Isikoglu, S. and Goktolga, U. **2013**. Measurement of the serum and follicular fluid total oxidant status, total antioxidant status, oxidative stress index in patient with high, normal or poor response, *Fertil Steril*, **100**(3): S427.
31. Artimani, T., Karimi, J., Mehdizadeh, M., Yavangi, M., Khanlarzadeh, E., Ghorbani, M. and Kheiripour, N. **2018**. Evaluation of pro-oxidant-antioxidant balance (PAB) and its association with inflammatory cytokines in polycystic ovary syndrome (PCOS), *Gynecol Endocrinol*, **34**(2): 148-152.
32. Göktolga, Ü., Topçu, H. O., Çinar, Ö., Işıkoğlu, S. and Demir, P. **2017**. Comparison of the Paraoxonase and Arylesterase Activities in Follicle Fluid of Females with PCOS in ICSI Cycle, *Türk Üreme Tıbbi ve Cerrahisi Dergisi*, **1**(1): 1-6.
33. Aurrekoetxea, I., Ruiz-Sanz, J.I., del Agua, A.R., Navarro, R., Hernández, M.L., Matorras, R. and Ruiz-Larrea, M.B. **2010**. Serum oxidizability and antioxidant status in patients undergoing in vitro fertilization, *Fertil Steril*, **94**(4): 1279-1286.

34. Palini, S., Benedetti, S., Tagliamonte, M. C., De Stefani, S., Primiterra, M., Polli, V. and Canestrari, F. **2014**. Influence of ovarian stimulation for IVF/ICSI on the antioxidant defence system and relationship to outcome, *Reprod Biomed Online*, **29**(1): 65-71.
35. Siu, M.K. and Cheng, C.Y. **2012**. *The Blood-Follicle Barrier (BFB) in Disease and in Ovarian Function: Advances in Experimental Medicine and Biology*. Springer.
36. Krisher, R. **2004**. The effect of oocyte quality on development, *J Anim Sci*, **82**(suppl_13): E14-E23.
37. Fujii, J., Iuchi, Y. and Okada, F. **2005**. Fundamental roles of reactive oxygen species and protective mechanisms in the female reproductive system, *Reprod Biol Endocrinol*, **3**(1): 1-10.
38. Nasiri, N., Moini, A., Eftekhari-Yazdi, P., Karimian, L., Salman-Yazdi, R., Zolfaghari, Z. and Arabipour, A. **2015**. Abdominal obesity can induce both systemic and follicular fluid oxidative stress independent from polycystic ovary syndrome, *Eur J Obstet Gynecol Reprod Biol*, **184**: 112-116.
39. Rahsepar, M., Mahjoub, S., Esmaelzadeh, S., Kanafchian, M. and Ghasemi, M. **2017**. Evaluation of vitamin D status and its correlation with oxidative stress markers in women with polycystic ovary syndrome, *Int J Reprod Biomed*, **15**(6): 345.
40. Pasqualotto, E.B., Agarwal, A., Sharma, R.K., Izzo, V.M., Pinotti, J.A., Joshi, N.J. and Rose, B. I. **2004**. Effect of oxidative stress in follicular fluid on the outcome of assisted reproductive procedures, *Fertil Steril*, **81**(4): 973-976.
41. Fujimoto, V.Y., Bloom, M.S., Huddleston, H.G., Shelley, W.B., Ocque, A.J. and Browne, R.W. **2011**. Correlations of follicular fluid oxidative stress biomarkers and enzyme activities with embryo morphology parameters during in vitro fertilization, *Fertil Steril*, **96**(6): 1357-1361.
42. Ito, M., Muraki, M., Takahashi, Y., Imai, M., Tsukui, T., Yamakawa, N. and Iwasaki, W. **2008**. Glutathione S-transferase theta 1 expressed in granulosa cells as a biomarker for oocyte quality in age-related infertility, *Fertil Steril*, **90**(4): 1026-1035.
43. Carbone, M., Tatone, C., Monache, S. D., Marci, R., Caserta, D., Colonna, R. and Amicarelli, F. **2003**. Antioxidant enzymatic defences in human follicular fluid: characterization and age-dependent changes, *MHR: Basic Science of Reproductive Medicine*, **9**(11): 639-643.
44. Sabatini, L., Al Shawaf, T., Wilson, C., Lower, A. and Grudzinskas, J. **2000**. Follicular Fluid Superoxide Dismutase (SOD) Activity in Women with Polycystic Ovarian Syndrome (PCOS), *Fertil Steril*, **74**(3): 253-254.
45. Seleem, A.K., El Refaeey, A.A., Shaalan, D., Sherbiny, Y. and Badawy, A. **2014**. Superoxide dismutase in polycystic ovary syndrome patients undergoing intracytoplasmic sperm injection, *J Assist Reprod Genet*, **31**(4): 499-504.