Oxidative stress in the sera and seminal plasma of the infertile subjects in Babylon governorat*e*

Tariq H. Al-Khyatt
Kadhum J. Al-Hamdani
Pharmacy Awn Abdullah Mohammed
Dept. of Biochemistry, College of Medicine, University of Babylon

(K . J . Pharm . Sci)

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Abstract

This study was conducted to demonstrate the effect of serum and seminal oxidative stress (OS) on male infertility with different infertility potentials. To achieve this aim, the levels of malondialdehyde (MDA), glutathione (GSH) and catalase activity were determined by the spectrophotometric methods in the sera and the seminal plasma of 75 infertile males divided to: 25 azoospermics with age range (25-45 yr), 25 asthenozoospermics with age range (24-45 yr) and 25 oligozoospermics with age range (23-41 yr). Twenty five healthy fertile male with age range (22-45 yr) used as controls. The results indicated a significant increase in the seminal and serum levels of MDA in azoospermic group ($p< 0.05$, $p<0.001$ respectively), asthenozoospermic group ($p<0.001$ both) and oligozoospermic group ($p<0.001$ both). While the levels of seminal and serum GSH are significantly decreased in azoospermic group ($p<0.01$, $p<0.001$ respectively), asthenozoospermic group ($p<0.01$, $p<0.001$ respectively) and oligozoospermic group ($p<0.001$, $p<0.05$ respectively). Also the levels of seminal and serum catalase activity significantly decreased in azoospermic group ($p<0.05$, $p<0.001$ respectively), asthenozoospermic group ($p<0.01$, $p<0.05$ respectively) and oligozoospermic group ($p<0.01$ both). The obtained results showed a positive correlation between serum MDA with the seminal MDA but negative correlation with the seminal GSH and catalase activity, while the positive correlation between serum and seminal antioxidants has not been demonstrated in all study groups. The study reveals a negative correlation between OS and good seminal fluid analysis parameters. As a conclusion to this study, serum and seminal OS is involved in the pathogenesis of male infertility confirming that the use of antioxidant therapy can improve OS-induced male infertility.

الخلاصة

أجريت هذه الدراسة لتحديد تأثير مستوى الإجهاد التأكسدي في مصل الدم و بلازما السائل المنوي على حالات عقم الرجال المختلفة. و لتحقيق هذا الغرض، تم قياس كل من مستوى المالون ثنائي الألديهايد (MDA) ، الكلوتاثيون (GSH) و فعالية إنزيم الكاتاليز بواسطة الطرق المطيافية الضوئية في مصول الدم وبلازما السائل المنوي لخمس وسبعين مريضا مصابا بالعقم بواقع 25 منعدمي النطف تتراوح أعمارهم بين (25-45 سنة)، 25 مصابا بوهن النطف تتراوح أعمارهم بين (24-45 سنة) و 25 قليلي النطف تتراوح أعمارهم بين (23-41 سنة)، كما تم قياسها لخمس و عشرين رجلا مخصبا معافى تتراوح أعمارهم بين (22-45 سنة) استخدموا كمجموعة سيطرة. أظهرت الدراسة وجود ارتفاعا معنويا في مستوى المالون ثنائي الألديهايد (MDA) في بلازما السائل المنوي و مصول الدم لمنعدمي النطف ($p< 0.05$، 0.001 على التوالي)، واهني النطف ($p < 0.001$، كلاهما) و قليلي النطف ($p < 0.001$ كلاهما). بينما وجد انخفاضا معنويا في مستوى الكلوتاثيون (GSH) في بلازما السائل المنوي و مصول الدم لمنعدمي النطف ($p< 0.01$، $p< 0.001$ على التوالي)، واهني النطف ($p< 0.01$، $p < 0.001$ على التوالي) و قليلي النطف ($p< 0.001$، $p < 0.05$ على التوالي). كذلك وجد انخفاضا معنويا في مستوى فعالية إنزيم الكاتاليز في بلازما السائل المنوي و مصول الدم لمنعدمي النطف ($p< 0.05$، 0.001 على التوالي)، واهني النطف ($p< 0.01$، $p < 0.05$ على التوالي) و قليلي النطف ($p< 0.01$ كلاهما) عند مقارنتهم بمجموعة السيطرة. أظهرت النتائج المتوفرة ارتباطا معنويا موجبا بين مستوى المالون ثنائي الألديهايد (MDA) في الأمصال ومستواه في بلازما السائل المنوي لكنه سالبا مع مستوى الكلوتاثيون (GSH) و فعالية إنزيم الكاتاليز في بلازما السائل المنوي لكافة المجموعات تقريبا، بينما الارتباط الإيجابي بين مستويات مضادات الأكسدة في مصول الدم وبلازما السائل المنوي لم يتم الحصول عليه في كافة مجموعات الدراسة. أظهرت الدراسة ارتباطا معنويا سالبا بين الإجهاد التأكسدي ومتثابتات السائل المنوي الجيدة من عدد وحركية وشكل النطف. نستنتج من هذه الدراسة إن زيادة الإجهاد

التأكسدي في مصل الدم وبلازما السائل المنوي يشترك في النشوء المرضي لعُقمِ الذكور مؤكدا إن استعمال مضادات الأكسدة يمكن أن يقلل من حالات عقم الرجال بسبب الإجهاد التأكسدي.

Introduction

Infertility is defined as the failure of a couple to achieve pregnancy after at least one year of unprotected intercourse, if a pregnancy has not occurred after three years, infertility most likely will persist without medical treatment (1). Studies in the United States and Europe showed a one-year prevalence of infertility in 15% of couples, as shown in multicenter studies, 30-35% of subfertility can be attributed to predominantly female factors, 25-30 % to male factors, and 25-30% to problems in both partners; in the remaining cases, no cause can be defined (1).

Oxidative stress is a consequence of an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense mechanisms (2).Excessive production of free radicals or ROS can damage sperm. Superoxide anion, hydroxyl radicals and hydrogen peroxide are some of the major ROS present in seminal plasma. Cells living under aerobic conditions constantly face the oxygen paradox: oxygen is required to support life, but its metabolites such as ROS can modify cell functions, endanger cell survival, or both (3).

Recently OS has been considered as one of the major factors believed to be involved in idiopathic male infertility. Low levels of ROS are necessary for normal functions of spermatozoa like capacitation, hyperactivation, motility, acrosome reaction, oocyte fusion and fertilization (4). Subsequently, free radicals change lipid/protein ratio of membranes by affecting polyunsatured fatty acids and lipid peroxidation (LPO) causes functional irregularities of several cellular organelles (5).

Lipid peroxides are disintegrated quickly and form reactive carbon compounds. Among these, malondialdehyde (MDA) is an important reactive carbon compound which is used commonly as an indicator of LPO (6). Human spermatozoa are known to be susceptible to loss of motility in the presence of exogenous hydrogen peroxide, as a consequence of LPO (7). Studies concerning the chemistry of LPO in human spermatozoa imply that once this process has been initiated, its propagation is impeded, leading to the accumulation of lipid peroxides in the sperm plasma membrane (8).

There have been several studies on the existence of enzymatic defenses in spermatozoa and seminal plasma, such as superoxide dismutase, catalase (9), and glutathione peroxidase (10). Besides these enzymes small molecules present in semen could also act as ROS scavengers, such as vitamin C, urate, albumin, taurine, hypotaurine and pyruvate (11-13). Several independent studies identified hydrogen peroxide as the most toxic ROS to human spermatozoa (14). Protection against hydrogen peroxide is provided mainly by catalase and Glutathione peroxidase (15). Increased catalase-like activities were found in spermatozoa and seminal plasmas of ejaculates with increased ROS generation (9).

The other relevant system dealing with the detoxification of hydrogen peroxide is the glutathione (GSH) cycle. Glutathione peroxidase, a cytoplasmic selenoprotein, reduces hydrogen peroxide and lipidic or non-lipidic hydroperoxides while oxidizing 2 molecules of GSH (5γ-glutamyl-L-cysteinylglycine) (16). Therefore reduced GSH is necessary to detoxify peroxides.

In the present study attempt was made to assess the OS by measuring MDA levels of the serum and seminal plasma in human subjects with different infertility potential and at the same time to measure the levels of reduced glutathione and catalase activity in the sera and seminal plasma of the same subjects. Correlation between the MDA, GSH and catalase levels and fertility potential as well as MDA level and reduced glutathione and catalase were tested statistically in order to elucidate the antioxidant status and the OS in idiopathic male infertility.

Material and Methods

The study was conducted in Babylon governorate, from October 2009 to May 2010.Seventy five infertile men with different infertility potential who were referred to Babylon maternity and pediatric teaching hospital were subjected to this study. Those selected infertile men were divided into three groups according to infertility type:-

- ***The azoospermic group*** includes 25 infertile men with an age range (25-45) years with primary infertility and sperm count equal to zero.
- ***The oligozoospermic group*** includes also 25 patients with age range (23-41) years with primary infertility and sperm count less than 20 million/ milliliter with or without asthenozoospermia.
- ***The asthenozoospermic group*** includes also 25 patients with age range (24-45) years with primary infertility and the sperm motility less than 25% (grad A according to World Health Organization (WHO) 1999) with or without oligozoospermia.
- ***The control group*** includes twenty five apparently healthy fertile volunteers with normal seminal parameters according to WHO (1999).

Exclusion Criteria

Those patients were admitted to hospital for further investigations, monitoring, and treatment. Worthy to mention that those patients were not smokers, not alcoholics and not suffering from any other serious systemic illnesses like diabetes mellitus, cardiac diseases, renal diseases and hepatic diseases, not taking any drug in the last year and not have any hormonal disturbances, so as not to interfere with the result of measured parameters and the outcome of the study.

Collection of Blood and Serum Preparation

Five milliliters of blood were obtained from infertile men and healthy controls, then collected in tubes without anticoagulants and were left for 15 minutes at room temperature to clot. After that, the blood samples were centrifuged at 1500xg for approximately 10 minutes. The sera were aspirated and stored at (–20 ◌̊ C) until time of use.

Collection of Semen and Seminal Plasma Preparation

Semen samples were collected from patient and control groups. All specimens were collected into sterile plastic containers by masturbation after an abstinence period of 72-96 hrs and analyzed within 1hr of collection. After allowing at least 30 min for liquefaction to occur, semen analysis was performed to measure sperm concentration, percentage progressively motile sperms and normal sperm morphology in accordance with the recommendations of the WHO (1999). Sperm concentration was determined by diluting a semen sample in a semen diluting fluid [5 g NaCl and 100 ml formaldehyde (40%) made up to 100 ml with distilled water]. Thereafter, some quantity of diluted sample transferred to neubauer chamber and sperm count was determined under the microscope. Sperm motility was expressed as the percentage of spermatozoa that showed forward progression (sum of grade a and b sperms). Liquefied semen samples were centrifuged at 15000xg for 15 minutes. The supernatant seminal plasma was then carefully removed and transferred to Eppendrof tubes. The seminal plasma was frozen at (–20 ◌̊ C) until examination.

Determination of Serum Malondialdehyde Concentration

Malondialdehyde was colorimetrically determined of MDA after its reaction with thiobarbituric acid (TBA) at 90-100° C and pH 2-3 for 15 minutes to form pink colored product. The intensity of the color formed was measured at a wavelength of 532 nm (17). To 0.15 ml serum add 1 ml TCA (17.5 %) and 1 ml TBA (0.67 %) then mix by vortex and put in boiling water bath for 15 minutes, then allowed to cool at room temperature. One milliliter of TCA 70% was added, and let to stand at room temperature for 20 minutes. Later centrifuge at 3000 xg for 15 minutes and the supernatant was taken out to measure the absorbance of a sample at 532 nm and the concentration was calculated using the extinction coefficient (1.56×10^5 mol/L/cm).

Determination of Seminal Plasma Malondialdehyde Concentration

MDA levels were measured as per Thiobarbituric Acid (TBA) method described by Yao-Yuan Hsieh *et al*. (18). Semen sample was centrifuged at 3000xg for 10 minutes after liquefaction to get the seminal plasma. Then 0.1 ml of seminal plasma was added to 0.9 ml of distilled water in a glass tube, to it 0.5 ml of TBA reagent (0.67 gm of 2–thiobarbituric acid dissolved in 100 ml of distilled water with 0.5 gm of NaOH and 100 ml of glacial acetic acid) was added and then heated for 1 hr in a boiling water bath. After cooling the tube was centrifuged for 10 minutes at 4000 xg and the supernatant absorbance was read on a spectrophotometer at 534 nm.

Determination of Serum Glutathione Concentration

Determination of serum GSH depends on the action of sulfhydryl group (19). Serum GSH is determined by using a modified procedure utilizing Elman's reagent (DTNB), to 0.2 ml serum add 0.8 ml distilled water and 0.1 ml TCA (50%), then mix in vortex for 10-15 minutes, and centrifuge for 15 minutes at 3000 xg. Then to 0.4 ml supernatant add 0.8 ml Tris–EDTA buffer and 0.02 ml DTNB reagent, then mix in vortex, and the absorbance was read by spectrophotometer at wavelength of 412nm within 5 minute of the addition of DTNB. Then the concentration is calculated from the standard curve in µmol/l.

Determination of Seminal Plasma Glutathione Concentration

The GSH concentration in seminal plasma was measured according to the method of Beutler *et al*. (20). 0.2 ml of seminal plasma and 1.8 ml of distilled water were mixed with 3 ml of precipitating solution (metaphosphoric acid 1.67gm, disodium EDTA 0.2 gm, NaCl 30 gm in 100 ml distilled water). After allowing to stand for 5 minutes, the solution was filtered. 1 ml of clear filtrate, 4 ml of freshly made disodium hydrogen phosphate (4.6 gm/L) solution and 0.5 ml of DTNB reagent (5,5'dithiobis-2-nitro benzoic acid: 20 mg in 100 ml of citrate buffer) were added. Absorbance of the yellow colour developed was read in spectrophotometer at 412 nm.

Measurement of Serum and Seminal plasma Catalase Activity

Catalase activity was estimated by the method of Aebi (21). Catalase degrades hydrogen peroxide, which decreases the absorption with time and can be measured directly at 240 nm. The difference in the extinction per unit time was a measure of catalase activity, which then calculated from the change in the absorbance. The enzyme activity is expressed as the rate constant of first order reaction (K).

Statistical Analysis

Student's *t*-test has been used to determine the significant difference between two groups. P values less than 0.05 is considered significant. While the correlation between two variables have been determined using Pearson's correlation coefficients.

Results

There were a significant increase in the seminal and serum levels of MDA of azoospermics ($p<0.05$, $p<0.001$ respectively), asthenozoospermics ($p<0.001$, $p<0.001$ respectively) and oligozoospermics ($p<0.001$, $p<0.001$ respectively). There were a significant decrease in the seminal and serum levels of GSH of azoospermics ($p<0.01$, $p<0.001$ respectively), asthenozoospermics ($p<0.01$, $p<0.001$ respectively) and oligozoospermics ($p<0.001$, $p<0.05$ respectively. There were also a significant decrease in the seminal and serum levels of catalase of azoospermics ($p<0.05$, $p<0.001$ respectively), asthenozoospermics ($p<0.01$, $p<0.05$ respectively) and oligozoospermics ($p<0.01$, $p<0.01$ respectively). Those results are summarized in Table 1.

The serum MDA has a significant positive correlation with seminal MDA of all study groups, and a significant negative correlation with seminal GSH of all study groups except that of azoospermics and a significant negative correlation with seminal catalase of all study groups except that of oligozoospermics. The serum GSH has a significant negative correlation with only seminal MDA of control group, a significant positive correlation with seminal GSH and catalase of control and oligozoospermic. The serum catalase has a significant negative correlation with only seminal MDA of oligozoospermic group, no significant positive correlation with seminal GSH of any group and a significant positive correlation with seminal catalase of azoospermic and asthenozoospermic groups. Those results are summarized in table 2.

Count, motility (linear activity) and the percent of normal sperm have been correlated to the measured OS indicators in the study groups. There was a significant negative correlation between seminal MDA and measured spermiograms of the study groups except with the count of the asthenozoospermic group. The seminal GSH has a significant positive correlation with the spermiograms of the study groups except with the percent of normal sperms of the asthenozoospermic group. The seminal catalase has a significant positive correlation with the spermiograms of the study groups except with the count of the oligozoospermic, the linear activity of the asthenozoospermic and the percent of normal sperms of both control and oligozoospermic groups. Serum MDA has a significant negative correlation with all spermiograms of the study groups. Despite it is positive there is no significant correlation between serum GSH and spermiograms of the study groups except with the count of control and the percent of normal sperms of both asthenozoospermic and oligozoospermic groups. Serum catalase was correlated positively only with the active sperms of oligozoospermic and with the count of control group. Table 3 summarize the results of correlations between count, motility (linear activity) and the percent of normal sperms with the measured OS indicators in the study groups.

Table 1. Levels of serum and seminal MDA, GSH and catalase in infertile and control groups

Group	MDA (µmol/L)		GSH (µmol/L)		Catalase (K/ml)	
	Serum	Seminal	Serum	Seminal	Serum	Seminal
Control	4.28 ± 0.96	1.38±0.54	19.67 ± 2.66	4.19 ± 1.55	0.368 ± 0.043	0.329 ± 0.089
Azoospermia	5.74 ± 0.94 ***	1.77 ± 0.52 *	16.62 ± 2.59 ***	2.89 ± 1.20 **	0.295 ± 0.040 ***	0.278 ± 0.041 *
Asthenozoospermia	6.01 ± 0.84 ***	2.38 ± 0.63 ***	16.49 ± 2.96 ***	2.92 ± 1.21 **	0.342 ± 0.037 *	0.267 ± 0.052 **
Oligozoospermia	5.42 ± 0.90 ***	2.14 ± 0.67 ***	17.81 ± 2.56 *	2.49 ± 1.25 ***	0.326 ± 0.046 **	0.262 ± 0.053 **

*** values significantly different from control.(p< 0 .001)
** values significantly different from control.(p< 0 .01)
* values significantly different from control.(p< 0 .05)

Table 2. Correlation among serum and seminal MDA, GSH and catalase in infertile and control groups

Group	Serum / Seminal	MDA		GSH		Catalase	
		r	P value	r	P value	r	P value
Control	MDA	0.80	0.001	-0.63	0.001	-0.28	N.S.
Azoospermic		0.55	0.01	-0.35	N.S.	-0.32	N.S.
Asthenozoospermic		0.43	0.05	-0.23	N.S.	0.15	N.S.
Oligozoospermic		0.63	0.001	-0.22	N.S.	-0.47	0.05
Control	GSH	-0.54	0.01	0.48	0.05	0.24	N.S.
Azoospermic		-0.39	N.S.	0.26	N.S.	0.33	N.S.
Asthenozoospermic		-0.43	0.05	0.30	N.S.	0.19	N.S.
Oligozoospermic		-0.48	0.05	0.58	0.01	0.33	N.S.
Control	Catalase	-0.51	0.01	0.58	0.01	0.25	N.S.
Azoospermic		-0.53	0.01	0.08	N.S.	0.43	0.05
Asthenozoospermic		-0.43	0.05	0.33	N.S.	0.40	0.05
Oligozoospermic		-0.29	N.S.	0.46	0.05	0.32	N.S.

Table 3. Correlations between count, percent of linear activity and percent of normal sperms with the serum and seminal MDA, GSH and catalase in infertile and control groups

		Count		% Active		% Normal	
Parameter	**Group**	**r**	**P value**	**r**	**P value**	**r**	**P value**
Seminal MDA (µmol/L)	**Control**	**-0.64**	**0.001**	**-0.62**	**0.001**	**-0.66**	**0.001**
	Asthenozoospermic	**-0.13**	**N.S.**	**-0.61**	**0.001**	**-0.59**	**0.01**
	Oligozoospermic	**-0.66**	**0.001**	**-0.51**	**0.01**	**-0.55**	**0.01**
Seminal GSH (µmol/L)	**Control**	**0.44**	**0.05**	**0.47**	**0.05**	**0.60**	**0.001**
	Asthenozoospermic	**0.46**	**0.05**	**0.65**	**0.001**	**0.23**	**N.S.**
	Oligozoospermic	**0.44**	**0.05**	**0.68**	**0.001**	**0.53**	**0.01**
Seminal Catalase (K/ml)	**Control**	**0.43**	**0.05**	**0.41**	**0.05**	**0.40**	**N.S.**
	Asthenozoospermic	**0.45**	**0.05**	**0.28**	**N.S.**	**0.54**	**0.01**
	Oligozoospermic	**0.39**	**N.S.**	**0.65**	**0.001**	**0.29**	**N.S.**
Serum MDA (µmol/L)	**Control**	**-0.44**	**0.05**	**-0.56**	**0.01**	**-0.76**	**0.001**
	Asthenozoospermic	**-0.47**	**0.05**	**-0.59**	**0.01**	**-0.43**	**0.05**
	Oligozoospermic	**-0.49**	**0.05**	**-0.44**	**0.05**	**-0.55**	**0.01**
Serum GSH (µmol/L)	**Control**	**0.45**	**0.05**	**0.36**	**N.S.**	**0.28**	**N.S.**
	Asthenozoospermic	**0.29**	**N.S.**	**0.22**	**N.S.**	**0.42**	**0.05**
	Oligozoospermic	**0.17**	**N.S.**	**0.34**	**N.S.**	**0.51**	**0.01**
Serum Catalase (K/ml)	**Control**	**0.51**	**0.01**	**0.27**	**N.S.**	**0.10**	**N.S.**
	Asthenozoospermic	**0.21**	**N.S.**	**0.13**	**N.S.**	**0.12**	**N.S.**
	Oligozoospermic	**0.33**	**N.S.**	**0.55**	**0.01**	**0.07**	**N.S.**

Discussion

Plasma membrane of the mammalian sperm, which is rich in unsaturated fatty acids, is vulnerable to ROS-related LPO. The cellular structure, motility, survival, and metabolic functions of the sperm can be impaired as a result of LPO caused by ROS (22). In the present study, MDA, which is an index of LPO, were increased in semen and sera of infertile men when compared to fertile men. This is in agreement with the findings of Landat *et al.* (23) and Tavilani *et al.* (24). Moreover, OS has been considered as a potential mechanism of sperm DNA damage in infertile men. It was found that sperm DNA integrity was correlated with seminal total antioxidant capacity. The combined index from ROS generation and total antioxidant status score is reported to be a better marker of OS. Other studies stated that the total antioxidant levels in patients with idiopathic infertility were significantly less compared to fertile group, they concluded that total antioxidant capacity might contribute to the pathophysiology of male infertility irrespectively of clinical diagnosis (25). The system dealing with the detoxification of hydrogen peroxide is the GSH cycle. Glutathione peroxidase, a cytoplasmic selenoprotein, reduces hydrogen peroxide and lipidic or non-lipidic hydroperoxides while oxidizing 2 molecules of GSH. Therefore GSH is necessary to detoxify peroxides. Hydrogen peroxide produced via dismutation reaction of O_2 is mainly removed by catalase and glutathione peroxidase (26).

Although human spermatozoa are known to possess major antioxidants defense systems including catalase and the other enzymatic and non enzymatic antioxidants, which neutralize the effects of free radicals, but their effectiveness is impaired by their limited concentration.

Moreover, the decrease in cytoplasmic volume during spermatogenesis further decreases the antioxidants present in the sperm (22).

In contrast to Siciliano *et al*., we observed a significant decrease in catalase activity in men with asthenozoospermia compared to normozoospermic men (27).

Those results explain that there are a correlation between the serum OS and that occur in the seminal plasma meaning that the factors causing a systemic OS can cause local seminal OS, consequently systemic antioxidant therapies can reduce oxidative damage to sperms and improve seminal quality of infertile patients. In the other hand, the correlations among serum and seminal antioxidants are still controversial since it is not improved in all study groups giving that they may be regulated in the seminal plasma in a manner which differ from that of the blood.

A negative correlation observed between sperm concentration, motility and normal morphology and seminal MDA levels was in agreement with the findings of Yao-Yuan Hsieh *et al.* (18). In our present study, high LPO was found to be associated with poor semen quality. We observed a positive correlation between sperm concentration, motility and normal sperm morphology and the levels of seminal GSH. These findings were compatible with that observed by Bhardwaj *et al.* (28). Therefore, GSH might have some fertility enhancing role by reducing LPO. It could therefore be proposed that the concentration of GSH, a non-enzymatic antioxidant of seminal plasma, could be used as a chemical parameter to assess male fertility, whereas high levels of MDA can stand for poor semen quality. The beneficial role of GSH to minimize oxidative damage to the sperms can make it a suitable candidate for therapeutic usage in the treatment of male infertility. A series of clinical trial is needed to explore the possibility. Detoxification of hydrogen peroxide is carried out by catalase activity. Therefore, catalase activity in semen is important for maintaining sperm motility. In asthenozoospermic patients catalase adsorption to the cell membrane is less than that of normozoospermic men causing a higher degree of LPO of sperm membrane (25).

The main conclusions of the present study include OS is implicated in the pathogenesis of male infertility, there are a negative correlations between OS marker (MDA) with the antioxidants (GSH and catalase) levels and with the good seminal parameters in the seminal plasma and the systemic (serum) OS can cause local (seminal) OS, giving that the antioxidant therapy can improve male infertility. Thus based on the results, we recommend use of OS analysis in the seminal plasma as a routine measurement to evaluate male infertility especially in idiopathic cases and the importance of the antioxidants in the treatment of male infertility that can be a dose-related. However, further studies are required to demonstrate the other enzymatic and non-enzymatic antioxidants levels in the serum and seminal plasma of infertile males in relevance to seminal fluid analysis and to study the effect of OS on the molecular level at the stages of spermatogenesis.

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