



Effect of Oral Zinc Supplementation on the Thiol Oxido-Reductive Index and Thiol-Related Enzymes in Seminal Plasma and Spermatozoa of Iraqi Asthenospermic Patients

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Abstract

A thiol group plays an essential role in sperm metabolism and the antioxidative defense state. Zinc is the second most abundant element in the human body, following iron. The present study was conducted to study the effect of zinc supplementation on the characteristics of semen along with thiol and thiol-related enzymes in semen of asthenospermic patients. Semen samples were obtained from 60 fertile and 60 asthenospermic men, from couples who had consulted the infertility clinic of Babil Hospital (Hillah city, Iraq). The subfertile group was treated with zinc; every participant took two 220 mg capsules of zinc sulfate per day for 3 months. Semen samples were obtained (before and after zinc supplementation). The levels of reduced thiol, oxidized thiol, thiol oxido-reductive index, and thiol-related enzymes activities were determined in spermatozoa and seminal plasma of patients and healthy groups. Oxidized thiol levels were significantly higher in the infertile patients compared to that in the fertile group. Conversely, reduced thiol level, sulfhydryl oxidase activity, and glutathione peroxidase activity significantly decreased in the infertile patients compared to that in the fertile group. Oxidized thiol levels, reduced thiol levels, and thiol-related enzymes activities of the infertile patients were restored to normal values after treatment with zinc. However, reduced and oxidized thiol levels in spermatozoa did not change significantly in the group treated with zinc. The quantitative values for RSH/RSSR and thiol-related enzymes may provide useful means to qualitatively express the oxidant/antioxidant balance in clinical and epidemiologic studies. ClinicalTrials.gov Identifier: NCT02985905

Keywords Oxidative stress · Zinc supplementation · Thiol · Asthenospermia · Glutathione peroxidase · Sulfhydryl oxidase activity

Abbreviations

GSSG	Oxidized glutathione
C.I.	Confidence interval for mean
GSH	Reduced glutathione
MTs	Metallothioneins
Se-GPx	Selenium-dependent glutathione peroxidase
SHO	sulfhydryl oxidase
RSSR	Oxidized thiol
RSH	Reduced thiol
RSH/RSSR	Thiol oxido-reductive index
GPx	Glutathione peroxidase

non-Se-GPx	non-selenium-dependent glutathione peroxidase
ROS	Reactive oxygen species
WHO	World Health Organization

Introduction

Male infertility is globally increasing and accounts for approximately 50% of infertile couples [1]. There are a number of factors responsible for male infertility, including excessive synthesis of reactive oxygen species (ROS). ROS symbolize a broad category of molecules that specify the collection of radicals and non-radical oxygen derivatives. ROS may participate in reactions that give rise to free radicals that injure cellular tissues [2]. Oxidative stress (OS) is a state that takes place when high levels of ROS exist comparative to antioxidant capacity [3]. Normal levels of ROS are necessary for complete functionality of the sperms, but excessive levels of

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ROS can harmfully affect sperm quality [4]. The OS-induced sperm damage has been elucidated to be a significant contributing aspect in 30–80% of all cases of male subfertility [5]. Oxidative stress has been shown to enhance protein oxidation and has been described in asthenospermia [6]. Protein carbonyl group can develop from the binding of aldehyde groups that produced from the lipid peroxidation, oxidative cleavage of the protein molecules, or by direct oxidation of amino acids such as arginine, lysine, proline, histidine, threonine, and glutamic acid [7].

It is therefore essential that the male reproductive system is well protected against oxidative damage. As a requirement of protection against oxidative stress, spermatozoa contain antioxidant enzymes, such as catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase [8] in addition to thiol-containing compounds [9]. Glutathione, ergothioneine, cysteine, and albumin [10] are the four essential thiol compounds in seminal fluids. Thiol groups (SH groups) play an essential role in sperm metabolism pathways and the antioxidative defense state. Glutathione is a major intracellular non-protein thiol compound. It has many biological functions such as maintenance of membrane protein thiol groups (SH groups) in the reduced form, as well as functions in catalysis, metabolism, transport, and in the protection of spermatozoa against foreign compounds such as ROS [9]. Buettner [11] summarized the reduction potentials of the selected group of the available antioxidants and free radicals; reduced glutathione (GSH) is one of these antioxidants that can act as a direct scavenger of almost all noxious free radicals, such as hydroxyl radicals (HO^\bullet), superoxide ($\text{O}_2^{\bullet-}$), and other carbon-, oxygen-, and nitrogen-centered free radicals, while it is converted into the relatively stable oxidized glutathione (GSSG). Glutathione functions as a substrate for several enzymes such as glutathione peroxidase, which catalyzes the detoxification of intracellular peroxides. Therefore, maintenance of glutathione levels is important in cellular defense against oxidative injury and for cellular integrity [12]. Thiols act as reducing agents that cause RSH to donate a hydrogen atom. They are highly reactive with free oxidizing radicals. The hydrogen atom abstraction is one of the most important properties of thiol reactivity, allowing it to function as an antioxidant [13].

The human glutathione peroxidase group is classified into eight classes (specifically GPx1 to GPx8) depending on their substrate specificity, primary sequence, and subcellular localization [14]. GPx1–4 contain selenocysteine groups. GPx1 and GPx4 (also, named phospholipid hydroperoxide GPx) are both cytosolic enzymes abundant in most mammalian tissues. GPx4 has been established as having dual functions in spermatozoal metabolism by being enzymatically active in spermatids but insoluble and functioning as a structural binding protein in mature spermatozoa [15]. GPx occupies an important position in the cascade of ROS reusing reactions, permitting for small physiological alterations in the levels of hydrogen peroxide or other substrates, such as organic

peroxides. In human male reproductive tissues, GPx plays a major role. A number of GPx proteins are activated either on spermatozoa or in close proximity during its movement through the epididymal duct [16]. Alvarez and Storey [17] were among the first researcher to indicate the role of GPx in protecting human spermatozoa from loss of motility initiated by spontaneous lipid peroxidation. Subsequent scientific development was completed by Foresta et al. [18], who reported that failure of the expression of GPx in the spermatozoa was associated with subfertility in humans. Crucial scientific development in this area includes development of mouse GPx knockout models that are accompanied with subfertility or infertility or both [15]. It can be concluded from the results of previous studies that GPx plays central roles in sperm physiology.

The sulfhydryl oxidase (SHO) enzyme is sometimes also referred to thiol oxidase, an enzyme that also oxidizes thiol groups using oxygen as an electron acceptor, reducing it to water [19]. SHO catalyzes the production of de novo disulfide bonds between SH groups with the subsequent reduction of oxygen to hydrogen peroxide. Moderate thiol levels are also known to have a defensive function upon sperm motility and metabolism under in vitro conditions [11].

Zinc participates as a cofactor for more than 80 metalloenzymes involved in protein synthesis and DNA transcription. These two processes are major mechanisms of germ cell development; for this reason, zinc is predicted to be essential for reproduction. Furthermore, zinc has anti-apoptotic activity [20] and antioxidant properties [21]. The linkage of zinc with different proteins, such as α -2 macroglobulin [α -2M] and metallothioneins [MTs], is essential for immune efficiency during aging [22]. MT is involved in zinc metabolism and defense against certain metal toxicities [23].

Although several researches have studied the relationship between subfertility and thiol compound levels in semen, no studies have been published on the effects of asthenospermia treatments, such as oral zinc supplementation, on the activity of thiol-related enzymes, which are important in fertility of the individual. The present study was organized to analyze the effect of zinc supplementation on the qualitative and quantitative characteristics of semen, along with reduced thiol, oxidized thiol, and thiol-related enzymes in the seminal plasma of asthenospermic patients.

Methods

Study Subjects

This study included 60 subfertile male partners from couples who had consulted the infertility clinic of Babylon Hospital of Maternity (Babylon governorate, Hillah city, Iraq) between July 2011 and July 2012. A complete medical history was recorded and physical examination was completed for each

Table 1 The parameters of ejaculated seminal fluids of infertile patients and healthy donors (Mean \pm SD)

	Volume (mL)	Sperm count ($\times 10^6$)	Progressive sperm motility (%)	Normal sperm form (%)
Healthy donors (G1)	2.8 \pm 0.53	77 \pm 9	69 \pm 8	38 \pm 9
Patients before treatment (G2)	1.83 \pm 0.66* (<i>P</i> value = 0.042)	47 \pm 21* (<i>P</i> value = 0.023)	21 \pm 9* (<i>P</i> value = 0.00)	21 \pm 11
Patients after treatment (G3)	2.39 \pm 0.9** (<i>P</i> value = 0.037)	70 \pm 15 (<i>P</i> value = 0.03)	39 \pm 14** (<i>P</i> value = 0.05)	33 \pm 7** (<i>P</i> value = 0.041)

*Significance versus group I (healthy donors)

**Significance versus group II (patients before treatment)

participant. Subjects taking antioxidant supplementation or any medication at the time were excluded from the study. The written consent of every participant included in the study and approval of the institutional research ethics committee were obtained. The inclusion criteria were asthenozoospermia, absence of endocrinopathy, varicocele, and female factor infertility. Smokers were excluded from the study because of their distinguished high seminal ROS concentrations and reduced antioxidant concentrations. The selection criteria of the fertile group (60 male) were having a child born within a birth in the last year and the absence of asthenozoospermia, varicocele, and endocrinopathy. Routine semen analyses were performed according to the 2010 WHO recommendation. These analyses include pH, sperm motility, semen volume, concentration, normal sperm, and round cell morphology [24].

Preparation of Spermatozoa and Seminal Plasma for Biochemical Analysis

One hour after semen collection of each sample, spermatozoa were separated from the seminal plasma, by centrifugation of 2 ml of seminal fluid at 1600g for 15 min at 2 °C, and maintained in -30 °C until investigation. The pellet was re-suspended in ten volumes of a suitable medium (NTPC medium) and centrifuged at 1600g for 10 min at 2 °C. This washing process was repeated three times. 0.1% Triton

Table 2 Reduced thiol levels (μ M/L) in seminal plasma of infertile patients and healthy donor groups

	Mean	SD	95% C.I.		Compared groups	<i>P</i> value
			Lower bound	Upper bound		
G1	163.9	42.0	103.9	223.8	1, 2, 3	0.05*, 0.001*
G2	127.2	33.51	82.6	171.8	2, 3	0.05*, 0.000*
G3	320.5	100.15	226.09	414.9	3, 1, 2	0.001*, 0.001*, 0.000*

*The mean difference is statistically significant at $P \leq 0.05$

X-100 was added to the pellets obtained. After that, the pellet samples were mixed vigorously and centrifuged again at 8000g for 30 min in a refrigerated centrifuge [8]. The supernatant was used for biochemical measurements in spermatozoa. The samples were frozen (-20 °C) until analyzed. The medium was composed of 20 mM Tris (0.242 g/100 ml); 1.5 mM D-glucose (0.0027 g/100 ml); 0.4 mM EDTA (0.0148 g/100 ml); 113 mM NaCl (0.66 g/100 ml); 2.5 mM Na₂HPO₄ (0.0355 g/100 ml); 2.5 mM NaH₂PO₄ (0.3 g/100 ml); and 1.7 mM CaCl₂ (0.0188 g/100 ml). The medium was adjusted to pH 7.4 with HCl.

Reagents and Solutions

All reagents and solutions were obtained from Sigma Chemical Co. and used without further purification.

Biochemical Methods

Determination of Total Thiol (RSH) Concentration

RSH was measured according to the procedure followed by Riddles et al. [25]. Total thiol group was assessed using 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB). This reagent reacts with the thiol groups to produce a yellow-colored complex which has a peak absorbance at 412 nm. RSH was estimated in the samples by comparison with a standard curve consists of known concentrations of a cysteine.

Determination of Total Disulfide Linkage (RSSR) Concentration

Total disulfide linkage (RSSR) concentration was measured according to the protocol specified by Tannhauser et al. [26]. The disulfide bonds of proteins can be cleaved by reaction with excess sodium sulfite. Total RSSR was measured using 2-nitro-5-thiosulfobenzoate (NTSB). This reagent reacts with the cleavage disulfide bonds to produce a yellow-colored complex which has a peak absorbance at 412 nm. RSSR was estimated in a sample by using a molar extinction coefficient of resulted product (11,400 M⁻¹ cm⁻¹).

Table 3 Reduced thiol levels ($\mu\text{M}/10^8$ spermatozoa) in spermatozoa of infertile patients and healthy donor groups

	Mean	SD	95% C.I.		Compared groups	P value
			Lower bound	Upper bound		
G1	204.7	43.15	141.31	268.25	1 2	0.05*
					3	0.016*
G2	131	29.8	108.9	153.09	2 1	0.05*
					3	0.001*
G3	109.4	33.1	91.1	127.7	3 1	0.016*
					2	0.001*

*The mean difference is statistically significant at $P \leq 0.05$

Determination of the Thiol Oxide-Sensitive Index (RSH/RSSR)

The thiol oxide-sensitive index (RSH/RSSR) of seminal plasma was calculated by dividing RSH concentration by the RSSR concentration. This value indicates the measurement of the balance between pro-oxidant and antioxidant-reduced thiol levels.

Determination of Selenium, Non-selenium-Dependent, and GPx Activities

Selenium, non-selenium-dependent, and total GPx activities were measured according to the procedure followed by Rotruck et al. [27]. Briefly, reaction solution consists of 0.2 ml of serum, 0.1 ml of 10 mM sodium azide, 0.2 ml of 0.4 M Tris buffer pH 7.0, 0.2 ml glutathione, and 0.1 ml of 2.5 mM Tert-butyl hydroperoxide for the assessment of selenium-dependent GPx or 0.2 mM cumene hydroperoxide for the assessment of the total GPx. The reaction mixture was incubated at 37 °C for 10 min. The enzymatic reaction was stopped by 400 μl of 10% trichloroacetic acid and centrifuged. Supernatant was assayed for un-reacted glutathione content by using Ellman's reagent (20 mg of 5,5'-dithiobisnitro benzoic

Table 4 Oxidized thiol levels ($\mu\text{M}/\text{L}$) in seminal plasma of infertile patients and healthy donor groups

	Mean	SD	95% C.I.		Compared groups	P value
			Lower bound	Upper bound		
G1	160.7	35.86	130.9	190.5	1 2	0.05*
					3	0.017*
G2	185.9	28.04	164.4	207.5	2 1	0.05*
					3	0.358
G3	205.0	52.43	176.0	234.1	3 1	0.017*
					2	0.358

*The mean difference is statistically significant at $P \leq 0.05$

Table 5 Oxidized thiol levels ($\mu\text{M}/10^8$) in spermatozoa in infertile patients and healthy donor groups

	Mean	SD	95% C.I.		Compared groups	P value
			Lower bound	Upper bound		
G1	135.3	50.7	48.3	222.3	1 2	0.89
					3	0.93
G2	142.8	23.5	123.1	162.5	2 1	0.89
					3	0.95
G3	139.5	37.1	63.5	215.4	3 1	0.93
					2	0.95

*The mean difference is statistically significant at $P \leq 0.05$

acid (DTNB) and was dissolved in 100 ml of 0.1% sodium nitrate).

Non-Se-Dependent Glutathione Peroxidase

Non-Se-dependent glutathione peroxidase activity = [Total glutathione peroxidase activity] – [Se-dependent glutathione peroxidase activity] by Rotruck et al. [27].

Determination of the SHO Activity

SHO activity was measured according to the method specified by Hadwan et al. [28] which depends upon the reaction between resulted hydrogen peroxide with DCFH diacetate to generate fluorescent dichlorofluorescein (at Ex/Em = 495/520 nm). The reaction mixture was composed of 30 μl of sample and 1 ml of 50 mM sodium phosphate buffer (pH 7.4) containing 0.3 mM glutathione and 50 mM 3-aminotriazole. After incubation time for 5 min at 37 °C, SHO activity was assessed by mixing peroxidase mimics and 500 μl of 40 $\mu\text{M}/\text{ml}$ DCFH diacetate to produce fluorescent dichlorofluorescein (at Ex/Em = 495/520 nm). SHO activity was calculated from the standard curve that plotted

Table 6 Sulfhydryl oxidase (SHO) activity (nmol/mg protein. min) in seminal plasma of infertile patients and healthy donor groups

	Mean	SD	95% C.I.		Compared groups	P value
			Lower bound	Upper bound		
G1	68.27	31.29	50.9	85.6	1 2	0.05*
					3	0.007*
G2	56.15	24.32	42.1	70.2	2 1	0.05*
					3	0.000*
G3	88.37	4.75	86.2	90.4	3 1	0.007*
					2	0.000*

*The mean difference is statistically significant at $P \leq 0.05$

Fig. 1 Thiol oxido-reductive index value in seminal plasma of infertile and healthy donor groups. *Significance versus group I (healthy donors). **Significance versus group II (patients before treatment)

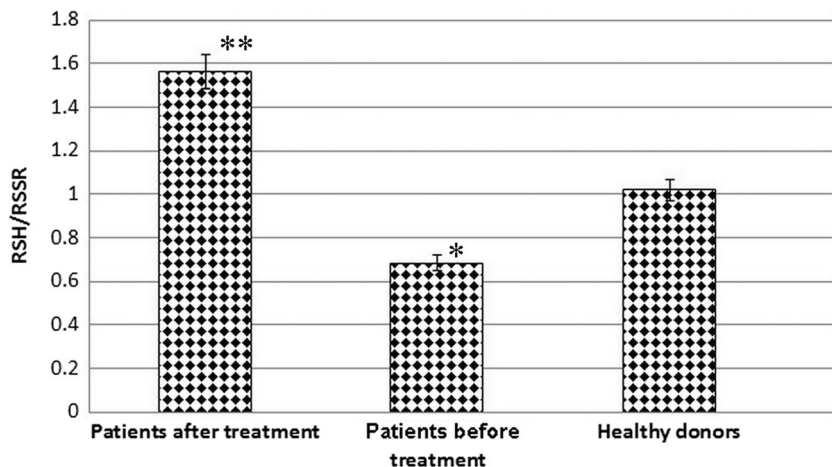
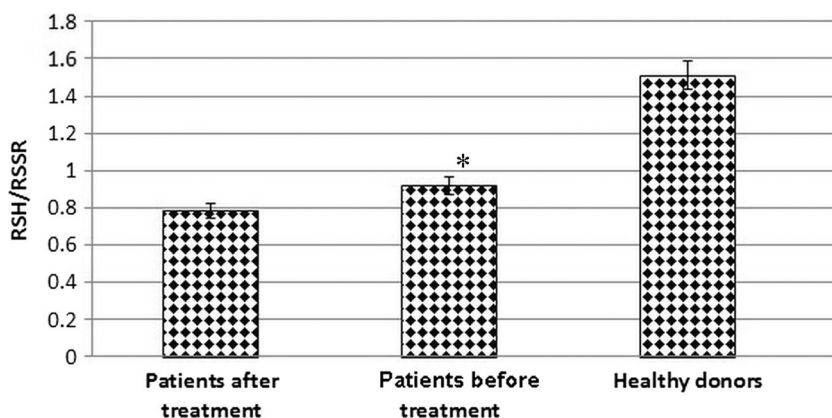


Fig. 2 Thiol oxido-reductive index value in spermatozoa of infertile and healthy donor groups. *Significance versus group I (healthy donors)



between fluorescence intensity and suitable concentrations of hydrogen peroxide.

Statistical Analysis

The result analysis was completed using SPSS 21 for Windows (SPSS Inc., Chicago, IL, USA). Between-

group comparisons of physiological and biochemical parameters were assessed by ANOVA to assess the differences in intervention group (before and after zinc supplementation) and adjust the baseline values. The Kolmogorov–Smirnov test was used to confirm if the data followed a normal distribution. A *P* value of <0.05 was considered as a significant value.

Fig. 3 Glutathione peroxidase activity (μ katal/L) in seminal plasma of infertile and healthy donor groups. *Significance versus group I (healthy donors). **Significance versus group II (patients before treatment)

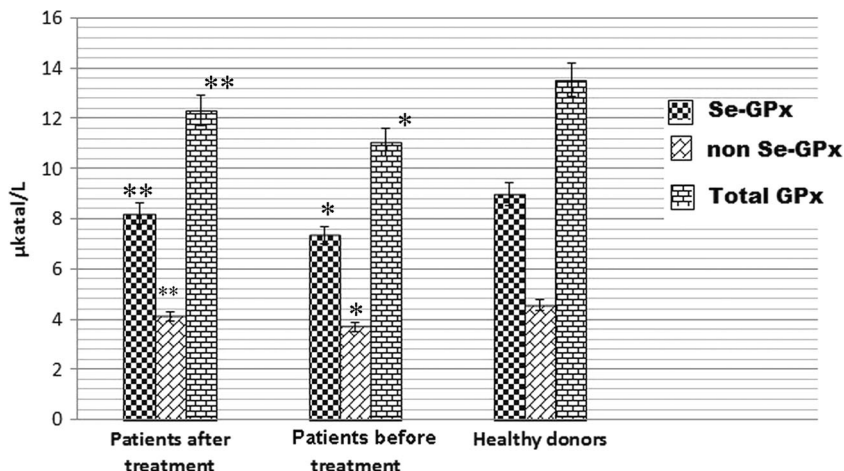
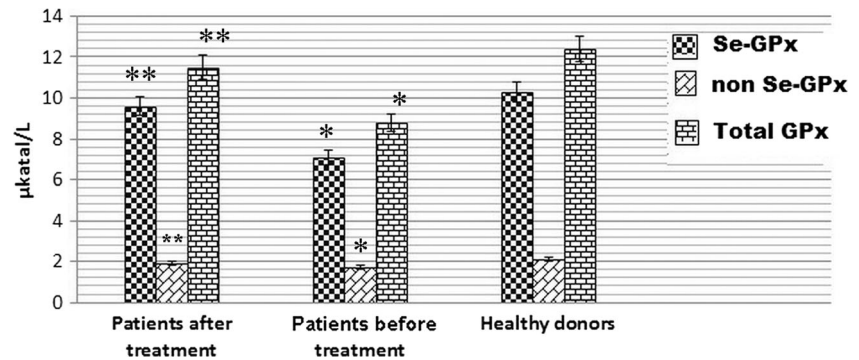


Fig. 4 Glutathione peroxidase activity ($\mu\text{katal}/10^8$ spermatozoa) of infertile and healthy donor groups. *Significance versus group I (healthy donors). **Significance versus group II (patients before treatment)



Results

Table 1 shows the baseline values of the semen parameters represented in the subfertile and fertile groups (G1—healthy donors; G2—patients before treatment; G3—patients after treatment). These factors significantly decreased in the subfertile group compared to that in the healthy donor group ($P < 0.05$). Progressive sperm motility, percentage, total normal sperm count, and volume of semen were enhanced after zinc supplementation.

The levels of reduced thiol, oxidized thiol, and SHO activity of seminal plasma and spermatozoa of patients and healthy groups are presented in Tables 2, 3, 4, 5, and 6, respectively. The results show a decrease in reduced thiol level, an increase in oxidized thiol level, and a decrement in sulfhydryl oxidase (SHO) activity in seminal plasma and spermatozoa of patients (G2) compared with the healthy subjects (G1). However, Zn supplementation restored to normal levels the reduced thiol level and sulfhydryl oxidase (SHO) activity in seminal plasma of the treated patients (G3). On the other hand, reduced thiol level and oxidized thiol level in spermatozoa did not change significantly in the group treated with zinc (G3).

The results of the present study (Figs. 1, 2, 3, and 4) show a significantly decrement ($P < 0.05$) in thiol oxido-reductive index value and glutathione peroxidase activities of seminal plasma and spermatozoa of patients (G2) compared with the

healthy group (G1). However, Zn supplementation restored to normal levels the thiol oxido-reductive index value and glutathione peroxidase activities in seminal plasma and spermatozoa of the treated patients (G3). All differences were statistically significant ($P < 0.05$).

Discussion

The key findings of the current study include three outcomes. First, the measurement of total RSH levels is more reliable than glutathione because it shows considerable levels in seminal plasma. Second, the RSH/RSSR ratio is a more accurate measurement of oxidative damage than solely measuring the concentrations of reduced or oxidized thiol. Third, zinc supplementation improves the synthesis of thiol-related enzymes in seminal plasma. Several studies examined glutathione levels in the seminal plasma of subfertile patients because glutathione acts as the mother of all antioxidants [29–32]. The results obtained from the previous studies are inconsistent in terms of the exact quantity of glutathione in seminal plasma. These differences may be owing to its very low levels in the semen, with the mean average glutathione seminal plasma level being $0.5 \mu\text{M}$ (range $0.01\text{--}0.55 \mu\text{M}$; mean $0.19 \pm 0.11 \mu\text{M}$) [33, 34]. Surprisingly, Yeung et al. [35] documented that glutathione was non-detectable in seminal plasma

Table 7 Total carbonyl group levels ($\mu\text{M}/L$) in seminal plasma of infertile patients and healthy donor groups

	Mean	SD	95% C.I.		Compared groups	<i>P</i> value	
			Lower bound	Upper bound			
G1	22.2	7.0	10.9	33.4	1	2	0.05*
						3	0.05*
G2	33.8	10.6	23.9	43.7	2	1	0.05*
						3	0.899
G3	32.2	9.6	16.7	49.4	3	1	0.05*
						2	0.899

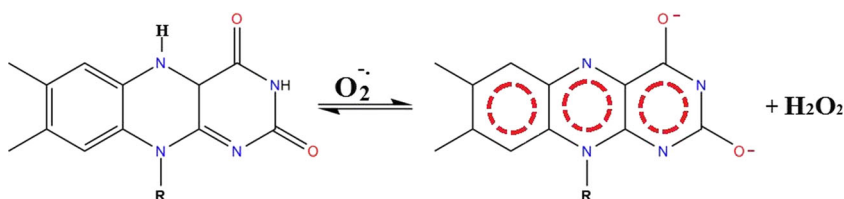
*The mean difference is statistically significant at $P \leq 0.05$

Table 8 Total carbonyl group levels ($\mu\text{M}/10^8$ spermatozoa) in spermatozoa of infertile patients and healthy donor groups

	Mean	SD	95% C.I.		Compared groups	<i>P</i> value	
			Lower bound	Upper bound			
G1	35.4	4.3	31.4	39.4	1	2	0.751
						3	0.302
G2	41.0	8.1	32.4	49.5	2	1	0.751
						3	0.538
G3	40.2	14.5	28.6	51.8	3	1	0.302
						2	0.538

The mean difference is statistically significant at $P \leq 0.05$

Fig. 5 The conversion of flavin from active form (reduced) to non-active form (oxidized)



because it had levels less than 2.5 μM . For these reasons, the measurement of total RSH levels is more reliable because it shows considerable levels in seminal plasma. In addition, thiol levels in the semen are much higher than the detection limit of Ellman's reagent. Thiol compounds in seminal fluids consist of glutathione [36], ergothioneine [37], cysteine, and albumin [38].

RSH levels were found to significantly decrease in the semen of asthenospermic patients compared with healthy controls. Furthermore, while RSSR shows significant higher values, the RSH/RSSR ratio was remarkably lower relative to the control. The decrease in RSH and the increase in RSSR could be correlated with the increase in the oxidation of protein, as shown by the high levels of total carbonyl groups in the semen of asthenospermic patients, as indicated in Tables 7 and 8. Total carbonyl levels were elevated significantly ($P=0.05$) in seminal plasma of asthenospermic patients, but the elevation was non-significant ($P=0.751$) in spermatozoa. That means the oxidative stress was high in seminal plasma and moderate in spermatozoa. On the other hand, zinc supplementation was not affected with the total carbonyl levels in seminal plasma or in spermatozoa.

High levels of copper in the semen of asthenospermic patients [39] act to accelerate the rate of protein oxidation. The elevation of peroxynitrite in the semen of asthenospermic patients may be the central basis for the decrease in RSH concentrations. Peroxynitrite radical preferably attacks the RSH

groups such as MTs to create thiol radicals and sulfenic acid end products [40]. These products are active and react rapidly with RSH to form RSSR.

The increase in the production rate of ROS in the seminal plasma of asthenospermic patients will cause the consumption of RSH and convert it to RSSR.

From the above results, the RSH/RSSR ratio is a more accurate measurement of oxidative damage than solely measuring the concentrations of reduced or oxidized thiol. The depletion of RSH levels in the present study, when compared with a healthy control group, supports the suggestion that RSH acts as a protective factor against the development of oxidative damage.

Zinc supplementation restores RSH to normal concentrations and thus increases the RSH/RSSR ratio by two mechanisms. Firstly, zinc supplementation improves the synthesis of MTs (the most vital reduced thiol in the seminal fluid), which have antioxidant properties [8]. Secondly, zinc works to inhibit the oxidation of protein.

As shown in Figs. 3 and 4, GPx activities were shown to be decreasing in the seminal plasma of patients with asthenospermia. The reduction of GPx activity may be owing to its broader protective spectrum than catalase in catalyzing the reduction of hydroperoxides [41]. However, the elevated concentration of $\text{O}_2^{\cdot-}$ in the seminal plasma of asthenospermic patients causes a depression in GPx activity. $\text{O}_2^{\cdot-}$ has been shown to inactivate GPx and activate CAT [42]. The increase

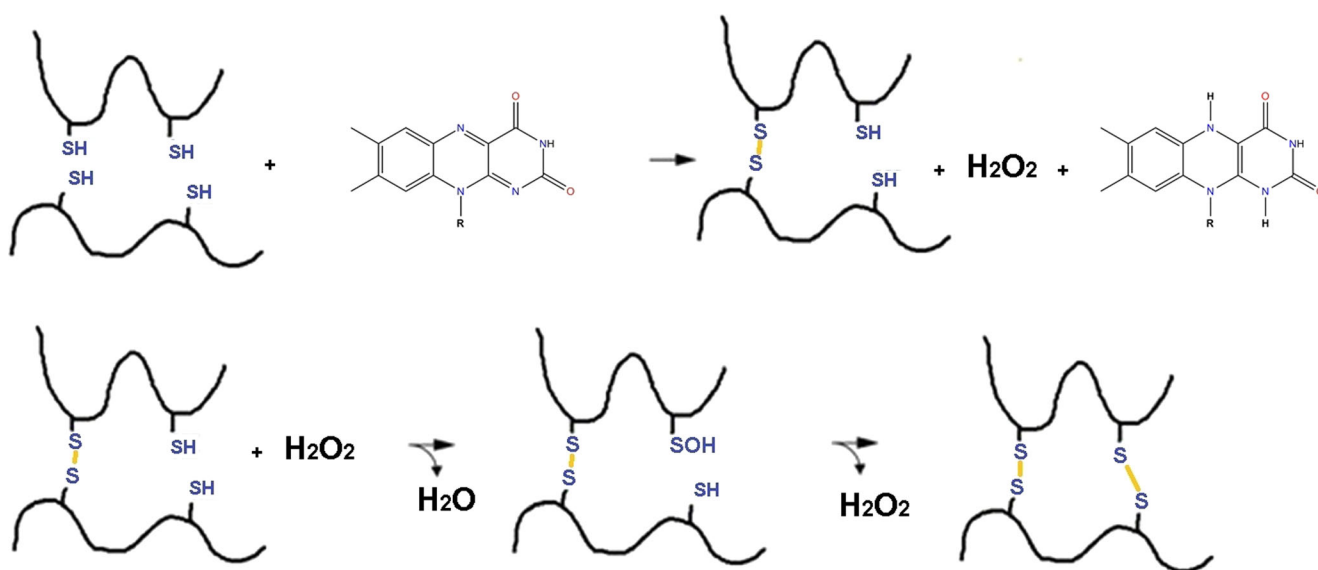


Fig. 6 Sulfhydryl oxidase (SHO) acts to stabilize cell membrane via formation of a disulfide linkage

in homocysteine levels in the seminal plasma of asthenospermic patients [43] could directly explain the decrement of GPx activity. Upchurch et al. [44] showed that elevated homocysteine concentrations suppressed GPx expression in endothelial cells in vitro and proposed that this effect may account, in part, for the vascular oxidant stress of hyperhomocysteinemic states. The low GPx levels could be demonstrated clearly by the low selenium concentration discovered in the seminal plasma of asthenospermic subjects [45], since selenium is a cofactor of GPx. Consequently, low selenium levels necessitate low GPx levels, which may cause raised oxidative stress.

Zinc supplementation restores GPx activity to normal levels. This could be due to the role of zinc in the inhibition of XO activity, thus inhibiting superoxide radical production, which works to inhibit glutathione peroxidase. You et al. [46] have documented that zinc compounds act as xanthine oxidase inhibitors. Another mechanism could be related to the improvement of the synthesis of MTs via zinc supplementation [23]. As a result, zinc supplementation resulted in decreased oxidative stress and enhanced GPx levels.

The dose chosen in the current research was well matched with those selected in former studies. Zinc sulfate has been used as an antioxidant in three clinical studies [47–49]. The amount of zinc sulfate used in former trials ranged from 66 to 500 mg, while the treatment period ranged from 13 to 26 weeks. The results of previous trials have presented positive benefits from the use of zinc sulfate. Additionally, negative results due to the use of zinc sulfate were not registered; hence, investigators inferred that the used dosage was safe.

The absence of a control group or placebo and not determining zinc status of subjects were represented as limitations to the present study. Also, another potential for bias was included in the assessments of enzymes without blinding the investigator to the experimental group. However, potential bias was decreased by following the standardized protocol by the researcher and random assignment of participants.

As shown in Table 6, SHO activities were found to be decreasing in the seminal plasma of asthenospermic subjects. The decrease in SHO activity may be related to the increase in ROS in the seminal plasma of patients with asthenospermia. Free radicals such as superoxide $O_2^{\cdot-}$ have the ability to convert the cofactor of SHO (flavin) from the active form (reduced) to non-active form (oxidized), as shown in Fig. 5.

Zinc supplementation restores SHO activities in the seminal plasma of asthenospermic subjects to normal levels, which could be owing to increasing total antioxidant levels. Secondly, it may also play a role in the synthesis of MTs, which act as antioxidants in the seminal plasma [39]. SHO acts to stabilize the cell membrane via formation of a disulfide linkage, as shown in Fig. 6.

The oxidation of sperm SH group occurs in spermatozoa during epididymal maturation as a noticeable transformation.

Caput epididymal spermatozoa and immature testicular are rich in SH groups (reduced thiol) and deficient in disulfide bonds (oxidized thiol) [50]. During epididymal migration, the majority of these sperm sulfhydryl groups are oxidized to disulfides. Consequently, the disulfide bonds are increased in mature spermatozoa from the cauda epididymidis [51]. The present data clearly show that SHO acts as an effective protection factor in cell membranes.

Conclusion

In conclusion, these results suggest that quantitative values for RSH/RSSR and thiol-related enzymes may provide a useful means to qualitatively express the oxidant/antioxidant balance in clinical and epidemiologic studies. Zinc supplementation restores the oxido-reductive index and thiol-related enzyme activities to normal ranges in seminal plasma and in spermatozoa of asthenozoospermic subjects.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval

Ethical Committee
Iraq: Ethics Committee (University of Babylon/College of Science), Reference number of approval: 545; Date: 22/6/2011.

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