

Paraoxonase-1 Activity in Subfertile Men and Relationship to Sperm Parameters

FATMA FERDA VERIT,* AYHAN VERIT,† HALIL CIFTCI,‡ OZCAN EREL,§ AND HAKIM ÇELİK§

From the *Department of Obstetrics and Gynecology, the †Department of Urology, and the §Department of Clinical Biochemistry, Harran University Faculty of Medicine, Sanliurfa, Turkey; and the ‡Department of Urology, Sanliurfa State Hospital, Sanliurfa, Turkey.

ABSTRACT: Oxidative stress has been implicated in the pathogenesis of male infertility. Paraoxonase-1 (PON-1) is a high-density lipoprotein-associated antioxidant enzyme that prevents oxidative modification of low-density lipoprotein. Our aims in the study were to investigate 1) seminal PON-1 activity in subfertile men and 2) whether seminal PON-1 activity had any relationship to semen parameters. The study included 28 men with idiopathic subfertility, 32 subfertile men with abnormal semen parameters, and 30 fertile male volunteers. Seminal PON-1 activity was measured spectrophotometrically. Seminal total antioxidant status (TAS) and total oxidant status (TOS) were determined by using colorimetric methods. Oxidative stress index (OSI) was calculated as $[(TOS/TAS) \times 100]$. TOS and OSI were significantly higher and PON-1 activity and TAS were significantly lower in subfertile men with

abnormal semen parameters than in men with idiopathic subfertility and fertile donors. PON-1 activity was also strongly correlated with sperm concentration ($r = .68, P < .0001$), motility ($r = .58, P < .0001$), and morphology ($r = .62, P < .0001$) in the overall group. The receiver operating characteristic curve analysis revealed a high diagnostic value for PON-1 activity with respect to male-factor subfertility, with an area under curve of .95 (95% confidence interval = 0.89–1.01), sensitivity = 97%, and specificity = 88%. Men with abnormal semen parameters have decreased levels of PON-1 activity in their seminal plasma. This may play an important role in the pathogenesis of male-factor subfertility.

Key words: Oxidative stress, spermatozoa, male infertility.

J Androl 2009;30:183–189

Subfertility is a prevalent disorder occurring in approximately 10% of all couples during reproductive life. In about 30% of these couples, no etiology can be found (Snick et al, 1997).

All living aerobic cells are normally exposed to reactive oxygen species (ROS), and oxidative stress arises as a consequence of excessive production of ROS and impaired antioxidant defense mechanisms (Sikka, 2001). It is proposed that oxidative stress precipitates the range of pathologies that currently are thought to afflict the reproductive function (Sikka, 2001). Increased levels of seminal oxidative stress have been correlated with sperm dysfunction through different mechanisms that include lipid peroxidation of sperm plasma membrane and impairment of sperm metabolism, motility, and fertilizing capacity (Saleh et al, 2003). It has been reported that asthenozoospermic, asthenoteratozoospermic, and oligoasthenoteratozoospermic sub-

fertile males had significantly lower values of catalase activity and total antioxidant capacity when compared with a normozoospermic group (Khosrowbeygi and Zarghami, 2007). Studies have demonstrated that 25%–88% of nonselected subfertile patients have high levels of seminal ROS (Lewis et al, 1995).

Paraoxonase-1 (PON-1) is a high-density lipoprotein (HDL)-associated antioxidant enzyme with paraoxonase, arylesterase, and dyazoxonase activities (Aslan et al, 2007). PON-1 activity has been suggested to be inversely associated with oxidative stress (Rozenberg et al, 2003). It is responsible for the antioxidant effect of HDL (Durrington et al, 2001). PON-1 has been shown to prevent low-density lipoprotein (LDL) and HDL oxidation and has also been proposed to stimulate cholesterol efflux, the first step in reverse cholesterol transport (Mackness et al, 1993). Reduced PON-1 activities have been reported in several groups of patients with diabetes, hypercholesterolemia, and cardiovascular disease who are under increased oxidative stress (Mackness et al, 1993; Ayub et al, 1999). Concerning the role of oxidative stress in male subfertility, the aims of the study were to investigate 1) seminal PON-1 activity as an antioxidant enzyme in subfertile men with normal and abnormal semen parameters with other markers of oxidative stress and

Correspondence to: Dr Fatma Ferda Verit, Department of Obstetrics and Gynecology, Harran University, Faculty of Medicine, Tr-63100 Sanliurfa, Turkey (e-mail: fverit@harran.edu.tr; fverit@gmail.com).

Received for publication January 8, 2008; accepted for publication July 1, 2008.

DOI: 10.2164/jandrol.108.004929

2) whether seminal PON-1 activity has any relationship to semen parameters.

Materials and Methods

In this prospective study, semen samples were collected from 28 men with idiopathic subfertility, 32 subfertile men with abnormal semen parameters, and 30 fertile volunteers who attended a male infertility clinic. The study was approved by Harran University's Institutional Review Board and informed consent was obtained from each participating man. A detailed medical history was obtained from all subjects, including reproductive history and infertility evaluation of the female partner. An experienced urologist performed the genital examinations. All the patients had normal female partners aged between 18 and 35 years who had normal reproductive history, normal ovulation (by follicular ultrasound scan, luteal phase progesterone levels, and endometrial biopsy), and tubal patency (by hysterosalpingogram). All couples presenting for infertility evaluation had engaged in unprotected intercourse for at least 1 year. Male-factor subfertility was defined by the presence of at least one of the sperm anomalies oligozoospermia, asthenozoospermia, and/or teratozoospermia. The idiopathic subfertility group had normal standard semen parameters on repeated analyses and normal genital exams. The exclusion criteria were age older than 35, smoking, alcohol drinking, coronary artery disease, unstable angina, myocardial infarction, any operation or cardiovascular intervention within the previous 3 months, hypertension, hyperlipidemia, rheumatologic or endocrine conditions such as diabetes, acute-chronic liver diseases, renal dysfunction, anemia, parasitic diseases, systemic or local infection, leukocytospermia, any history of cancer in the past 5 years, or therapeutic interventions known to influence antioxidants such as supplemental vitamins. A group of healthy subjects of proven fertility (initiated a successful pregnancy within the last 12 months before participation in the study) who volunteered without payment served as the control group.

Hyperlipidemia was defined as follows: serum LDL cholesterol ≥ 160 mg/dL, total cholesterol ≥ 240 mg/dL, triglyceride ≥ 200 mg/dL, HDL cholesterol < 40 mg/dL (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2001). A patient was considered as diabetic with a fasting plasma glucose level ≥ 126 mg/dL.

Semen Analysis

Semen samples were collected by masturbation in a clean specimen container after sexual abstinence for 3–5 days, were allowed to liquefy at 37°C, and were evaluated immediately according to World Health Organization recommendations (ejaculate volume, pH, time to liquefaction, sperm concentration, motility, and morphology). Morphology smears were scored using the Kruger strict criteria (Kruger et al, 1986). Sperm concentration was expressed as sperm per milliliter of semen, and motility and morphology were expressed as percentages. Sperm parameters were considered normal when

sperm concentration was $\geq 20 \times 10^6$ /mL semen, motility was $\geq 50\%$, and normal sperm forms were $> 14\%$ by the Kruger strict criteria (Kruger et al, 1986). Seminal leukocytes were quantified by a myeloperoxidase staining test, and values were considered to be normal at concentrations of $\leq 1 \times 10^6$ peroxidase-positive leukocytes per milliliter of semen. The remaining semen samples were centrifuged at $1500 \times g$ for 10 minutes to obtain the seminal plasma. The separated seminal plasma was then stored at -80°C until further analysis of TOS, total antioxidant status (TAS), and PON-1 measurement.

Measurement of TOS in Seminal Plasma

The TOS of semen samples was determined by using a new automated colorimetric measurement method (Erel, 2005). The assay is based on the oxidation of ferrous ion to ferric ion in the presence of various oxidant species in acidic medium and the measurement of the ferric ion by xylenol orange. Within- and between-batch precision values were lower than 3%. The results were expressed as μmol hydrogen peroxide (H_2O_2) equivalent per liter (Verit et al, 2006).

Measurement of TAS in Seminal Plasma

TAS of semen samples was determined by using a novel automated measurement method developed by Erel (2004). In this method, the hydroxyl radical, the most potent radical, is produced via Fenton reaction and consequently the colored dianisidiny radical cations, which are also potent radicals, are produced in the reaction medium of the assay. Antioxidant capacity of the added sample against these colored potent free radical reactions measured the total antioxidant capacity. The assay has excellent precision values; within- and between-laboratory precision values are lower than 3%. The results were expressed as millimoles of Trolox equivalent per liter (Verit et al, 2006).

Measurement of PON-1 Activity in Seminal Plasma

PON-1 activity was determined by using paraoxon as a substrate and measured by increases in the absorbance at 412 nm because of the formation of 4-nitrophenol as already described (Verit et al, 2008). Briefly, the activity was measured at 25°C by adding 50 μL of seminal plasma to 1 mL Tris-HCl buffer (100 mM at pH 8.0) containing 2 mM CaCl_2 and 5.5 mM of paraoxon. The rate of generation of 4-nitrophenol was determined at 412 nm. Enzymatic activity was calculated by using the molar extinction coefficient $17\,100\ \text{M}^{-1}\ \text{cm}^{-1}$.

Oxidative Stress Index

The percentage ratio of TOS to TAS gave the oxidative stress index (OSI), an indicator of the degree of oxidative stress ($[\text{TOS}/\text{TAS}] \times 100$; Verit et al, 2006).

Statistical Analysis

Results were expressed as $\bar{x} \pm \text{SD}$ for all continuous variables. Differences between control and male infertility groups were assessed by using ANOVA followed by a Tukey test. Associations between TOS, TAS, OSI, and PON-1 activity

Table 1. Demographic characteristics, semen characteristics, and seminal TOS, TAS, OSI, and PON-1 activity in men with male-factor subfertility, men with idiopathic subfertility, and fertile donors

	Male-Factor Subfertility, $\bar{x} \pm SD$ (n = 32)	Idiopathic Subfertility, $\bar{x} \pm SD$ (n = 28)	Fertile Donors, $\bar{x} \pm SD$ (n = 30)
Age, y	31.0 \pm 3.0	31.4 \pm 2.6	30.7 \pm 3.1
Duration of infertility, y	3.1 \pm 1.2	3.3 \pm 1.3	—
Semen parameters			
Concentration, $\times 10^6$ /mL	13.6 \pm 8.7 ^{a,b}	58.3 \pm 9.0	58.5 \pm 9.4
Motility, % motile sperm	27.5 \pm 14.3 ^{a,b}	59.2 \pm 10.6	63.0 \pm 12.9
Morphology (Kruger criteria), % normal sperm	10.0 \pm 5.3 ^{a,b}	25.8 \pm 4.8	27.0 \pm 5.1
TOS, μ mol H ₂ O ₂ Eq/L	18.5 \pm 1.8 ^{a,b}	14.8 \pm 2.0	13.9 \pm 2.0
TAS, mmol Trolox Eq/L	11.9 \pm 1.3 ^{a,b}	15.3 \pm 1.2	15.9 \pm 1.2
OSI, AU	157.2 \pm 20.0 ^{a,b}	97.9 \pm 18.2	87.7 \pm 17.0
PON-1, U/L	1.0 \pm 0.8 ^{a,b}	2.8 \pm 0.9	3.0 \pm 0.9

Abbreviations: OSI, oxidative stress index; PON-1, paraoxonase-1; TAS, total antioxidant status; TOS, total oxidant status.

^a $P < .0001$ vs idiopathic subfertile group.

^b $P < .0001$ vs fertile donors.

and semen parameters were evaluated by Pearson's correlation test. The area under the receiver operating characteristic curve (ROC) was used to assess the discriminative ability of PON-1 activity in male-factor subfertility and in idiopathic infertility.

Results

Demographic characteristics, semen characteristics, and seminal TOS, TAS, and PON-1 activity in men with male-factor subfertility, men with idiopathic subfertility, and fertile donors are summarized in Table 1.

There was no significant difference in terms of age among the groups. Seminal TOS was significantly higher and seminal TAS and PON-1 activity were significantly lower in the male-factor subfertility group as compared with idiopathic subfertile men and fertile donors (Table 1).

The relationship between seminal TOS, TAS, OSI, and PON-1 activity and semen parameters in the overall group (n = 90) is shown in Table 2. There were negative correlations between TOS and OSI and sperm parameters such as concentration, motility, and morphology ($P <$

.0001 for all; Table 2). In addition, TAS and PON-1 activity had significant positive correlations with all sperm parameters in the study ($P < .0001$ for all; Table 2).

PON-1 had a positive correlation with seminal TAS ($r = .76$, $P < .0001$) and was negatively correlated with seminal TOS ($r = -.61$, $P < .0001$) and OSI ($r = -.73$, $P < .0001$) in the study.

ROC analysis revealed a high diagnostic value for PON-1 activity with respect to male-factor subfertility, with an area under curve (AUC) of 0.95 (95% confidence interval = 0.89–1.01), sensitivity = 97%, and specificity = 88% with a cutoff value of 1.75 U/L (lower than that value was related to male-factor subfertility), shown in the Figure.

However, PON-1 activity was not effective statistically in the diagnosis of idiopathic infertility (AUC < 0.5).

Discussion

In this study, we found that TOS and OSI were increased and TAS and PON-1 activity were decreased

Table 2. Correlations (r) between sperm parameters and seminal TAS, TOS, OSI, and PON-1 activities in the overall group (n = 90)

Sperm Parameters	TOS, μ mol H ₂ O ₂ Eq/L	TAS, mmol Trolox Eq/L	OSI, AU	PON-1, U/L
Concentration, sperm $\times 10^6$ mL)	-.67 ^a	.73 ^a	-.79 ^a	.68 ^a
Motility, % motile sperm	-.58 ^a	.58 ^a	-.65 ^a	.58 ^a
Morphology (Kruger criteria), % normal sperm	-.57 ^a	.64 ^a	-.68 ^a	.62 ^a

Abbreviations: OSI, oxidative stress index; PON-1, paraoxonase-1; TAS, total antioxidant status; TOS, total oxidant status.

^a $P < .0001$.

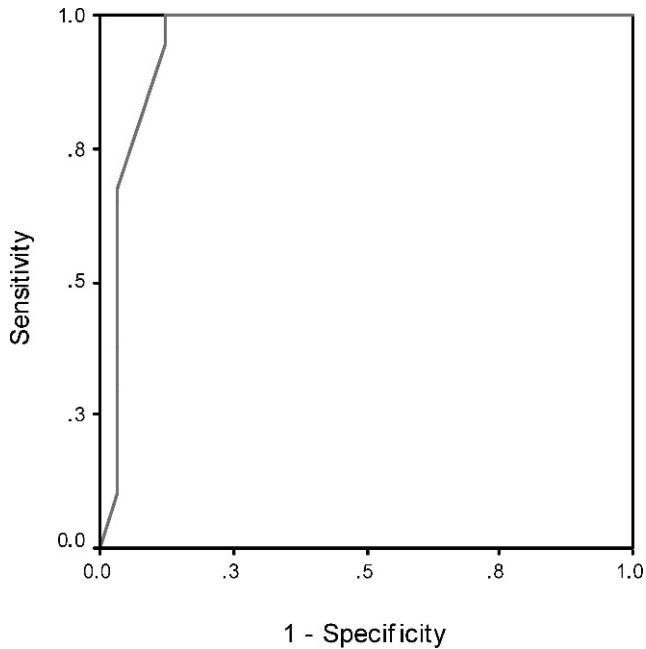


Figure. Receiver operating characteristic curve (ROC; gray line) of paraoxonase-1 (PON-1) activity in male-factor subfertility.

in male subfertile men with abnormal semen parameters as compared with an idiopathic subfertile group and fertile donors. There was a close relationship between PON-1 activity and abnormal semen parameters in the study. Moreover, ROC curve analysis revealed a good predictive power to discriminate subfertile patients from other populations.

ROS have been found to have a dual effect on human spermatozoa. Sperm plasma membrane has a high concentration of polyunsaturated fatty acids, which can undergo lipid peroxidation initiated by ROS (Saleh et al, 2003). Such peroxidative damage to the sperm plasma membrane leads to a loss of membrane fluidity and integrity, as a result of which the spermatozoa lose their competence to participate in the membrane fusion events associated with fertilization (Aitken and Fischer, 1994; Alvarez and Storey, 1995; Storey, 1997). In addition, ROS are also known to attack DNA, inducing strand breaks and oxidative base damage in human spermatozoa (Hughes et al, 1996; Kodoma et al, 1997).

It has been indicated that levels of ROS are negatively correlated with the quality of sperm in the original semen (Gomez et al, 1998). High levels of ROS production in human ejaculates may originate from morphologically abnormal spermatozoa and/or seminal leukocytes (Aitken and West, 1990). Many studies have reported that spermatozoa from oligozoospermic or asthenozoospermic men showed a greater production of oxidative stress (Aitken et al, 1992; Sharma and Agarwal, 1996; Griveau and de Lannou, 1997; Pasqua-

lotto et al, 2000). We also found that serum TOS was significantly higher in subfertile men with abnormal semen parameters in this study. Moreover, TOS was negatively correlated with semen parameters such as concentration, motility, and morphology in the study.

Previous reports have described that patients with idiopathic male infertility have elevated levels of ROS (Pasqualotto et al, 2000; Saleh et al, 2003). It has been also suggested that lipid peroxidation of sperm membrane may be one of the key mechanisms involved in the pathophysiology of idiopathic male infertility (Alkan et al, 1997). However, the correlation between oxidative stress and male idiopathic infertility is not clear, and there were some limitations in these studies. Saleh et al (2003) found significant correlations between abnormal sperm parameters including leukocytospermia and oxidative stress, but did not state the level of leukocytospermia in each group in the study population. Moreover, subfertile men with normal semen parameters had increased oxidative stress compared with normozoospermic fertile donors in this study, and it is a matter of debate how oxidative stress was increased in that group with normal semen parameters. In another study that was reported by Pasqualotto et al (2000), the study size was small, and the study was poorly designed because the normospermic group was not homogenous but also included varicocele patients. Many studies have demonstrated that oxidative stress is increased in varicocele even in the presence of normal semen parameters (Agarwal et al, 2006; Smith et al, 2006; Pasqualotto et al, 2008). In this study, we found that there was no difference in seminal oxidative stress between men with idiopathic subfertility and fertile donors, which supports our previous work (Verit et al, 2006). Moreover, Ochsendorf et al (1998) found that spermatozoa of oligozoospermic patients contained much lower concentrations of the endogenous antioxidant thiol glutathione than did those of normozoospermic men. Another study also demonstrated that oxidative stress was increased in asthenozoospermic patients compared with normozoospermic men (Tavilani et al, 2005). We suggest that oxidative stress is dependent on sperm parameters but is not directly related to the diagnosis of male-factor infertility.

Antioxidants are important in maintaining the oxidant-antioxidant balance in tissues. Among the well-known biological antioxidants, superoxide dismutase, catalase, and the glutathione peroxidase/reductase system have a significant role in protecting the sperm against peroxidative damage (De Lamirande and Gagnon, 1993; Sharma and Agarwal, 1996). Depressed seminal antioxidant capacity has been implicated in male subfertility. TAS levels have been shown to be lower in the semen of subfertile men as compared with

fertile men (Lewis et al, 1995, 1997; Smith et al, 1996). More specifically, Raijmakers et al (2003) reported significantly higher seminal plasma thiol glutathione concentrations in fertile men compared with subfertile men. In accordance with this finding, it has been reported that ascorbate levels were significantly reduced in seminal plasma of asthenozoospermic subfertile men (Lewis et al, 1997). Furthermore, studies have suggested that subfertile men empirically treated with antioxidants have demonstrated improved semen characteristics, fertilization in vitro, and higher pregnancy rates in the treatment group (Lenzi et al, 1993; Geva et al, 1996). In our study, TAS was significantly decreased in subfertile men with abnormal semen parameters, but not in the idiopathic subfertile group.

PON-1 is an antioxidant enzyme that is highly effective in preventing lipid peroxidation of LDL (Mackness et al, 1993). It is principally responsible for the breakdown of lipid peroxides before they accumulate on LDL (Mackness et al, 1993). PON-1 can also destroy H₂O₂, a major ROS produced under oxidative stress during atherogenesis (Aviram et al, 1998), and increase the LDL clearance (Shih et al, 2000).

PON-1 also protects HDL against lipid peroxidation (Mackness et al, 1993; Aviram et al, 1998; Rozenberg et al, 2003). Inhibition of HDL oxidation by PON-1 preserves the antiatherogenic effects of HDL in reverse cholesterol transport (Aviram et al, 1998). The antioxidant effect of HDL is also assumed by PON-1 (Aviram and Rosenblat, 2004).

The association between PON-1 activity and male infertility is unknown. PON-1 activity was significantly lower in male-factor subfertile patients compared with idiopathic subfertile men and fertile donors in the present study. There were also significant positive correlations between PON-1 activity and semen parameters such as concentration, motility, and morphology. We suggest that decreased PON-1 activity must be related to enhanced production of ROS. In addition, it has been previously shown that PON-1 activity was decreased in some diseases because of ROS pathogenesis under oxidative stress and inflammation conditions such as diabetes, coronary artery disease, and endometriosis (Ayub et al, 1999; Durrington et al, 2001; Verit et al, 2008).

The most widely used methods for measuring ROS are colorimetry, fluorescence, chemiluminescence, and electron spin resonance (ESR) spectroscopy (Tarpey et al, 2004). We measured total oxidant levels in seminal plasma by a colorimetric method that was developed by Erel (2005) in this study. This technique has many advantages. Various other methods that have been developed for measuring TOS had no accepted reference method. In addition, this method led to a certain decision concerning the standardizations, the terms, and

the units (Erel, 2005). Moreover, the fluorescence, chemiluminescence, and ESR methods need sophisticated techniques, and in most routine clinical biochemistry laboratories these improved systems are not available.

Measurement of TAS within semen can be conducted in a variety of ways. The ability of seminal plasma to inhibit chemiluminescence elicited by a constant source of ROS (horseradish peroxidase) is a commonly used technique. The TAS is usually quantified against a Vitamin E analogue (Trolox) and expressed as a ROS-TAS score (Sharma et al, 1999). However, colorimetry techniques based on the color change of 2,2'-azinobis(3-ethylbenzo-thiazoline-6-sulphate) (ABTS) are now becoming more popular because they are cheaper and easier to perform (Said et al, 2003; Erel, 2004). The reduced ABTS molecule is oxidized to ABTS⁺ using H₂O₂ and a peroxidase to form a relatively stable blue-green color measured at 600 nm with a standard spectrophotometer. Antioxidants present within seminal plasma suppress this color change to a degree that is proportional to their concentrations. Again the antioxidant activity is quantified using Trolox.

In conclusion, our results showed that TOS was significantly higher and TAS and PON-1 activity were significantly lower in patients with male-factor subfertility, but not in an idiopathic subfertile group. Reduced PON-1 activity may play a role in the pathogenesis of male subfertility. Therefore, both protection from oxidative stress and increases in PON-1 activity could be used as a powerful tool for the prevention of subfertility.

References

- Agarwal A, Prabakaran S, Allamaneni SS. Relationship between oxidative stress, varicocele and infertility: a meta-analysis. *Reprod Biomed Online*. 2006;12:630–633.
- Aitken RJ, Buckingham D, West K, Wu FC, Zikopoulos K, Richardson DW. Differential contribution of leukocytes and spermatozoa to the generation of reactive oxygen species in the ejaculates of oligozoospermic patients and fertile donors. *J Reprod Fertil*. 1992;94:451–462.
- Aitken RJ, Fisher H. Reactive oxygen species generation and human spermatozoa: the balance of benefit and risk. *Bioessays*. 1994;16:259–267.
- Aitken RJ, West KM. Analysis of the relationship between reactive oxygen species production and leukocyte infiltration in fractions of human semen separated on Percoll gradients. *Int J Androl*. 1990;3:433–451.
- Alkan I, Simsek F, Haklar G, Kervancioglu E, Ozveri H, Yalcin S, Akdaş A. Reactive oxygen species production by the spermatozoa of patients with idiopathic infertility: relationship to seminal plasma antioxidants. *J Urol*. 1997;157:140–143.
- Alvarez JG, Storey BT. Differential incorporation of fatty acids into and peroxidative loss of fatty acids from phospholipids of human spermatozoa. *Mol Reprod Dev*. 1995;42:334–336.

- Aslan M, Kosecik M, Horoz M, Selek S, Celik H, Erel O. Assessment of paraoxonase and arylesterase activities in patients with iron deficiency anemia. *Atherosclerosis*. 2007;191:397–402.
- Aviram M, Rosenblat M. Paraoxonases 1, 2, and 3, oxidative stress, and macrophage foam cell formation during atherosclerosis development. *Free Radical Biol Med*. 2004;37:1304–1316.
- Aviram M, Rosenblat M, Bisgaier CL, Newton RS, Primo-Parmo SL, La Du BN. Paraoxonase inhibits high density lipoprotein (HDL) oxidation and preserves its functions: a possible peroxidative role for paraoxonase. *J Clin Invest*. 1998;101:1581–1590.
- Ayub A, Mackness MI, Arrol S, Mackness B, Patel J, Durrington PN. Serum paraoxonase after myocardial infarction. *Arterioscler Thromb Vasc Biol*. 1999;19:330–335.
- De Lamirande E, Gagnon C. Human sperm hyperactivation in whole semen and its association with low superoxide scavenging capacity in seminal plasma. *Fertil Steril*. 1993;59:1291–1295.
- Durrington PN, Mackness B, Mackness MI. Paraoxonase and atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2001;21:473–480.
- Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clin Biochem*. 2004;37:277–285.
- Erel O. A new automated colorimetric method for measuring total oxidant status. *Clin Biochem*. 2005;38:1103–1111.
- Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA (J Am Med Assoc)*. 2001;285:2486–2497.
- Geva E, Bartoov B, Zabludovski N, Lessing JB, Lerner-Geva L, Amit A. The effect of antioxidant treatment on human spermatozoa and fertilization rate in an in vitro fertilization program. *Fertil Steril*. 1996;66:430–434.
- Gomez E, Irvine DS, Aitken RJ. Evaluation of a spectrophotometric assay for the measurement of malondialdehyde and 4-hydroxyalkenals in human spermatozoa: relationships with semen quality and sperm function. *Int J Androl*. 1998;21:81–94.
- Griveau JF, de Lannou D. Reactive oxygen species and human spermatozoa. *Int J Androl*. 1997;20:61–69.
- Hughes CM, Lewis SEM, McKelvey-Martin VJ, Thompson W. A comparison of baseline and induced DNA damage in human spermatozoa from fertile and infertile men using a modified comet assay. *Mol Hum Reprod*. 1996;2:613–620.
- Khosrowbeygi A, Zarghami N. Levels of oxidative stress biomarkers in seminal plasma and their relationship with seminal parameters. *BMC Clin Pathol*. 2007;1:6.
- Kodoma H, Yamaguchi R, Fukuda J, Kasai H, Tanaka T. Increased oxidative deoxyribonucleic acid damage in the spermatozoa of infertile male patients. *Fertil Steril*. 1997;68:519–524.
- Kruger TF, Menkveld R, Stander FS, Lombard CJ, Van der Merwe JP, van Zyl JA, Smith K. Sperm morphologic features as a prognostic factor in in vitro fertilization. *Fertil Steril*. 1986;46:1118–1123.
- Lenzi A, Culasso F, Gandini L, Lombardo F, Dondero F. Placebo controlled, double blind, cross-over trial of glutathione therapy in male infertility. *Hum Reprod*. 1993;10:1657–1662.
- Lewis SE, Boyle PM, McKinney KA, Young IS, Thompson W. Total antioxidant capacity of seminal plasma is different in fertile and infertile men. *Fertil Steril*. 1995;64:868–870.
- Lewis SE, Sterling ES, Young IS, Thompson W. Comparison of individual antioxidants of sperm and seminal plasma in fertile and infertile men. *Fertil Steril*. 1997;67:142–147.
- Mackness MI, Arrol S, Abbott C, Durrington PN. Protection of low-density lipoprotein against oxidative modification by high-density lipoprotein associated paraoxonase. *Atherosclerosis*. 1993;104:129–135.
- Mackness MI, Harty D, Bhatnagar D, Winocour PH, Arrol S, Ishola M. Serum paraoxonase activity in familial hypercholesterolaemia and insulin dependent diabetes mellitus. *Atherosclerosis*. 1991;86:193–199.
- Ochsendorf FR, Buhl R, Bastlein A, Beschmann H. Glutathione in spermatozoa and seminal plasma of infertile men. *Hum Reprod*. 1998;13:353–359.
- Pasqualotto FF, Sharma RK, Nelson DR, Thomas AJ, Agarwal A. Relationship between oxidative stress, semen characteristics, and clinical diagnosis in men undergoing infertility investigation. *Fertil Steril*. 2000;73:459–464.
- Pasqualotto FF, Sundaram A, Sharma RK, Borges E Jr, Pasqualotto EB, Agarwal A. Semen quality and oxidative stress scores in fertile and infertile patients with varicocele. *Fertil Steril*. 2008;89:602–607.
- Raijmakers MT, Roelofs HM, Steegers EA, Steegers-Theunissen RR, Mulder TP, Knapen MF, Wong WY, Peters WH. Glutathione and glutathione S-transferases A1-1 and P1-1 in seminal plasma may play a role in protecting against oxidative damage to spermatozoa. *Fertil Steril*. 2003;79:169–172.
- Rozenberg O, Rosenblat M, Coleman R, Shih DM, Aviram M. Paraoxonase (PON-1) deficiency is associated with increased macrophage oxidative stress: studies in PON1-knock out mice. *Free Radical Biol Med*. 2003;34:774–784.
- Said TM, Kattal N, Sharma RK, Sikka SC, Thomas AJ Jr, Mascha E, Agarwal A. Enhanced chemiluminescence assay vs colorimetric assay for measurement of the total antioxidant capacity of human seminal plasma. *J Androl*. 2003;24:676–680.
- Saleh RA, Agarwal A, Nada EA, El-Tonsy MH, Sharma RK, Meyer A, Nelson DR, Thomas AJ. Negative effects of increased sperm DNA damage in relation to seminal oxidative stress in men with idiopathic and male factor infertility. *Fertil Steril*. 2003;79:1597–1605.
- Sharma RK, Agarwal A. Reactive oxygen species and male infertility. *Urology*. 1996;48:835–850.
- Sharma RK, Pasqualotto FF, Nelson DR, Thomas AJ Jr, Agarwal A. The reactive oxygen species-total antioxidant capacity score is a new measure of oxidative stress to predict male infertility. *Hum Reprod*. 1999;14:2801–2807.
- Shih DM, Xia YR, Wang XP, Miller E, Castellani LW, Subbanagounder G, Cheroute H, Faull KF, Berliner JA, Witztum JL, Lusis AJ. Combined serum paraoxonase knockout/apolipoprotein E knockout mice exhibit increased lipoprotein oxidation and atherosclerosis. *J Biol Chem*. 2000;275:17527–17535.
- Sikka SC. Relative impact of oxidative stress on male reproductive function. *Curr Med Chem*. 2001;8:851–862.
- Smith R, Kaune H, Parodi D, Madariaga M, Rios R, Morales I, Castro A. Increased sperm DNA damage in patients with varicocele: relationship with seminal oxidative stress. *Hum Reprod*. 2006;21:986–993.
- Smith R, Vantman D, Ponce J, Escobar J, Lissi E. Total antioxidant capacity of human seminal plasma. *Hum Reprod*. 1996;11:1655–1660.
- Snick HK, Snick TS, Evers JL, Collins JA. The spontaneous pregnancy prognosis in untreated subfertile couples: the Walcheren primary care study. *Hum Reprod*. 1997;12:1582–1588.
- Storey BT. Biochemistry of the induction and prevention of lipoperoxidative damage in human spermatozoa. *Mol Hum Reprod*. 1997;3:203–214.
- Tarpey MM, Wink DA, Grisham MB. Methods for detection of reactive metabolites of oxygen and nitrogen: in vitro and in vivo considerations. *Am J Physiol Regul Integr Comp Physiol*. 2004;286:R431–R444.

- Tavilani H, Doosti M, Saeidi H. Malondialdehyde levels in sperm and seminal plasma of asthenozoospermic and its relationship with semen parameters. *Clin Chim Acta*. 2005;356:199–203.
- Verit FF, Erel O, Celik N. Serum paraoxonase-1 activity in women with endometriosis and its relationship with the stage of the disease. *Hum Reprod*. 2008;23:100–104.
- Verit FF, Verit A, Kocyigit A, Ciftci H, Celik H, Koksak M. No increase in sperm DNA damage, seminal oxidative stress in patients with idiopathic infertility. *Arch Gynecol Obstet*. 2006;274:339–344.
- World Health Organization. *WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interactions*. 4th ed. Cambridge, United Kingdom: Cambridge University Press; 1999.