

Role of Oxidative Stress in Pathogenesis of Varicocele and Infertility

Ashok Agarwal, Rakesh K. Sharma, Nisarg R. Desai, Sushil Prabakaran, Antonio Tavares, and Edmund Sabanegh

This review summarizes the published literature about the role of oxidative stress in the pathophysiology of varicocele and the beneficial effects of varicocele repair on oxidative stress. Literature survey was performed using the Medline, EMBASE, BIOSIS, and Cochrane databases between 1993 and 2008 that were relevant to oxidative stress and varicocele. Varicocele treatment can reduce reactive oxygen species levels and improve sperm parameters and pregnancy rates, although it is still controversial with Assisted Reproductive Techniques outcomes. We conclude that spermatozoal dysfunction in varicocele patients could be multifactorial, and oxidative stress-induced injury appears to be one of the main causes. *UROLOGY* 73: 461–469, 2009. © 2009 Elsevier Inc.

Varicocele is characterized by abnormal tortuosity and dilation of the veins of the pampiniform plexus within the spermatic cord and is the most common surgically correctible cause of male infertility.^{1,2} The prevalence of varicocele in the general population is 15%-20%, but it is notably greater (25%-40%) in infertile couples with male factor infertility.^{1,2}

Varicoceles are progressive and often appear at puberty; they are more commonly (90%) found on the left side.³ However, it has long been recognized that left-sided varicoceles can have bilateral effects.² Varicoceles naturally occur in humans but not in other mammalian species, and many proposed theories basically argue from an anatomic standpoint. Varicoceles are theorized to result from incompetent valves in the spermatic veins resulting in increased pressure transmitted to the testicular vein and causing retrograde blood flow down the internal spermatic veins and cremasteric veins. Anatomically, the left testicular vein is longer than the right testicular vein and enters the left renal vein at a right angle. In addition, more rarely, it can be compressed between the descending aorta and the superior mesenteric artery, causing a “nutcracker” effect. The resultant increase in renal vein pressure would then be transmitted to the testicular vein, explaining the reason left-sided varicocele is more common than right-sided varicocele.

However, we believe that no single physiologic mechanism has satisfactorily accounted for the development of a varicocele, and it seems likely that the etiology is multifactorial.^{1,2}

Clinical varicocele is divided into 3 grades: grade 1, palpable only during a Valsalva maneuver; grade 2, palpable distension with the patient standing upright; and grade 3, visible distension.⁴ Clinical findings can be confirmed by noninvasive testing (power Doppler ultrasonography) to demonstrate retrograde blood flow during the Valsalva maneuver.¹

Varicocele-associated infertility can be related to many factors, including histologic alterations, molecular/genetic changes such as microdeletion of a Y chromosome,⁵ glutathione-S-transferase M1 gene polymorphisms, high levels of testicular apoptosis defects in fibroblast-associated (Fas)-Fas ligand activity that regulates apoptosis at the level of plasma membrane, caspase activity at the cytoplasmic level or gene (Bax/Bcl-2) expression at the nuclear level, and expression of heme oxygenase isoenzyme-1 (HO-1) on Leydig cell.^{6,7} Increased expression of HO-1 in a varicocele testis could lead to greater carbon monoxide levels, which is a co-factor in apoptosis.⁷

MALE INFERTILITY AND OXIDATIVE STRESS

A relationship between infertility and the generation of reactive oxygen species (ROS) has been established and extensively studied.⁸ ROS include hydrogen peroxide and unstable free radicals with unpaired electrons in their outer orbits (eg, the hydroxyl radical and superoxide anion). Mitochondria and plasma membranes of morphologically abnormal spermatozoa produce ROS through the nicotinamide adenine dinucleotide phosphate-dependent and nicotinamide adenine dinucleotide-dependent oxidoreductase systems, respectively. Extracellular

This study was supported by funds from grants 5549 and 6209 from the Cleveland Clinic and a fellowship from the American Foundation of Urological Diseases to R. K. Sharma.

From the Center for Reproductive Medicine, Glickman Urological and Kidney Institute and Obstetrics-Gynecology and Women's Health Institute, Cleveland Clinic, Cleveland, Ohio

Reprint requests: Ashok Agarwal, Ph.D., H.C.L.D., Department of Surgery, Cleveland Clinic Lerner College of Medicine, and Center for Reproductive Medicine, Glickman Urological and Kidney Institute and Obstetrics-Gynecology and Women's Health Institute, Cleveland Clinic, 9500 Euclid Avenue, Desk A19.1, Cleveland, OH 44195. E-mail: Agarwaa@ccf.org

Submitted: July 1, 2008, accepted (with revisions): July 29, 2008

ROS are produced by leukocytes, mainly neutrophils. Leukocytes are normally present in prostatic and seminal vesicle secretions. During inflammation or infection, they are activated by way of cytokines and interleukins, increasing their production of ROS. In addition, lifestyle and environmental factors (smoking, pollution), medical conditions such as varicocele and infection, and environmental factors (pollution, radiation) increase ROS production.¹

Low levels of ROS are essential for normal fertilization, capacitation, hyperactivation, motility, and acrosome reaction. Excess production of ROS or decreased antioxidant defenses in the seminal plasma induces oxidative stress (OS), which damages spermatozoa. Sperm plasma membranes are particularly susceptible to OS owing to their high levels of polyunsaturated fatty acids that readily undergo lipid peroxidation. Lipid peroxidation of the plasma membrane affects the fluidity of the sperm plasma membrane and causes functional defects in sperm-oocyte fusion. In addition, 4-hydroxy-2-nonenal—an aldehyde end product of lipid peroxidation—is an alkylating agent that damages DNA and forms adducts with proteins, inducing apoptosis.⁹ Thus, OS ultimately affects the genomic and mitochondrial spermatozoa DNA, causing fragmentation and base degradation. This could contribute to the molecular or genetic changes that are responsible for several different pathologic features, including infertility. It has been shown that sperm DNA fragmentation inhibits normal activation of paternal gene expression at the 4-cell stage of the developing embryo.

PATHOPHYSIOLOGY OF MALE INFERTILITY IN VARICOCELE

The exact mechanism of impaired testicular function in patients with varicocele has not yet been found.¹ Elevated testicular and scrotal temperature, venous stasis and the resultant hypoxia, reflux of adrenal breakdown products in the testicular vein, lower intratesticular testosterone, and androgen receptor defects are possible causes. The greater resistance index and pulsatility index of the capsular branches of the testicular artery indicate impaired microcirculation in patients with clinical varicocele.²

Spermatogenesis is temperature-sensitive and proceeds optimally in men at 35°C. The internal spermatic artery (surrounded by the pampiniform plexus) maintains the testes at 35°C, 2.2°C lower than the core body temperature. Varicocele causes an average increase of 2.6°C in the scrotal temperature. The results from experimental studies of elevated scrotal temperature have been conflicting. Considerable overlap exists between the range of scrotal temperatures in infertile men with varicoceles and the temperature range in fertile men. Furthermore, infertile men with and without varicocele showed no difference in scrotal temperature. Nonetheless, it is well established that varicocele repair in humans lowers the testicular temperature.²

The presence of a varicocele is associated with decreased testicular volume and correlates with varicocele grade. Grade 1 varicocele has less significant testicular

volume loss than other grades as measured by ultrasonography. The predominant testicular pathologic feature of varicoceles is decreased spermatogenesis because of premature sloughing of immature germ cells into the lumen of the seminiferous epithelium, sometimes in conjunction with maturation arrest. Not all men with varicocele have poor sperm quality, and pregnancies have been reported in the female partners of these patients.¹⁰ The lack of consistent effects could be related to the heterogeneous expression of molecular/genetic defects.⁷ Morphologically, abnormal spermatozoa demonstrate disruption of the sperm head actin by cadmium, which is present in high concentrations in men with varicoceles. Furthermore, microdeletions of the alpha-1 subunit of the sperm calcium channels in men with varicoceles suggest a genetic defect that leads to abnormal acrosomal function. This could explain why all men with varicoceles are not infertile.

A body of data has indicated that important sperm functions can also be impaired in infertile patients with varicoceles. Defects in sperm-cervical mucus interaction, sperm survival in the female genital tract, sperm zona-binding ability, acrosin activity, a decreased response to the capacitating challenge, and a lower incidence and intensity of tyrosine phosphorylation and acrosome exocytosis have also been reported.^{2,7}

VARICOCELE AND OS

Alterations in the testicular microenvironment and hemodynamics can increase production of ROS and/or decrease the local antioxidant capacity, resulting in OS. A large number of studies have elucidated the effects of increased OS in the serum, semen, and testicular tissues of patients with varicocele. Although it has been established that OS is an important pathophysiologic factor that causes impairment of sperm parameters in varicocele,¹ the etiology of OS elevation in association with varicoceles is unclear. It could be the result of various compensatory mechanisms that operate in patients with varicocele and help to maintain spermatogenesis. Some of them are detrimental to spermatozoa, because these mechanisms can lead to upregulation or downregulation of various pathways/molecular mechanisms involved in generation of free radicals.¹¹⁻²¹

Possible Mechanisms

Some of the possible mechanisms include cytokines (interleukins [ILs]), leptin receptors, nitric oxide (NO), glial cell line-derived neurotrophic factor receptor-alpha-1, voltage-dependent calcium channel, and HO-1.

Cytokines (ILs). ILs play a regulatory role in testicular function. Normal levels of IL-1 modulate function of Sertoli cells and Leydig cells and participate in spermatogenesis and steroidogenesis. The expression of IL-1, a potent pro-inflammatory activator, is upregulated during experimental varicocele. This compensatory upregula-

tion can contribute to the survival of highly immunogenic germ cells in testicular tissue. IL-1 generates free radicals in many tissues, and the role of cytokines as a mediator of OS is well known. This compensatory overexpression can cause increased production of ROS in varicocele testes, causing an inflammatory response that is detrimental to testicular tissue. The levels of IL-6 and ROS in semen have also been shown to correlate significantly with varicocele.¹¹

Leptin Receptors. In the male reproductive tract, leptin and leptin receptors are expressed mainly on spermatocytes and Leydig cells. They regulate proliferation and differentiation of testicular germ cells.¹² In patients with varicocele with oligospermia, leptin expression on germ cells was significantly increased compared with fertile patients and patients with obstructive azoospermia. This could be a compensatory mechanism to maintain spermatogenesis.¹² The antioxidant role of leptin has been well reported. Leptin induces mitochondrial superoxide production in aortic endothelial cells and also increase lipid peroxidation in obese people.^{13,14} Although no direct evidence has been demonstrated that leptin is associated with OS in the presence of a varicocele, increased expression of leptin on Leydig and germ cells in patients with varicocele suggests a possible role for leptin in inducing OS in those with varicocele.

Nitric Oxide. Locally produced NO is involved in the regulation of testicular vasculature. NO is synthesized from L-arginine by the catalytic activity of 3 different isoforms of nitric oxide synthase: constitutive neuronal, endothelial, and inducible NO synthase (NOS). Endothelial NOS forms are normally expressed in Leydig cells and testicular vasculature. In varicocele testes, the expression of inducible NOS is upregulated to maintain testicular arterial blood flow as a compensatory mechanism to increase blood flow resulting from the hypoxia induced by venous stasis, which may be detrimental to spermatogenesis. In some adolescent patients with varicocele, increased malondialdehyde (MDA) levels occurred together with elevated NO levels, indicating excessive lipid peroxidation.¹⁵ NO reacts with the superoxide anion to yield the active metabolites peroxynitrite and peroxy nitrous acid, both of which are strong oxidants. Peroxy nitrous acid interacts with cysteine and with glutathione, and peroxynitrite produces nitrotyrosine.¹⁶ A causal relationship between elevated intratesticular temperatures with increased apoptosis in varicocele owing to increased NO levels may exist (similar to that seen in cryptorchidism). Studies have suggested a role for NO in heat-induced apoptosis. The NOS inhibitor decreases apoptosis and improves spermatogenesis in cryptorchidism, and the “knock out” of NOS in mice leads to an increase in testicular weight and sperm output, as well as resistance to heat-induced apoptosis.

Glial Cell Line-derived Neurotrophic Factor Receptor- α_1 . Glial cell line-derived neurotrophic factor is a growth factor in the brain that has a critical role in

spermatogenesis. It plays a role in spermatogenesis and DNA synthesis. Untreated varicocele has been shown to cause decreased expression of glial cell line-derived neurotrophic factor receptor- α_1 in perinuclear spermatids and in the cytoplasm of Leydig cells.¹⁷ Glial cell line-derived neurotrophic factor receptor- α_1 protects the neuron from OS,¹⁸ and its reduced expression, reported in various parts of varicocele testes, may account for the OS in testes.

Voltage-dependent Calcium Channel. Cadmium enters cells of seminiferous epithelium by way of the L-type of the voltage-dependent calcium channel, containing the α_{1c} subunit. Deletion in the exon of this subunit causes loss of ion selection properties and voltage-dependent channel inactivation. Patients with varicocele have increased levels of cadmium in their seminal plasma and testes.¹⁹ The negative effects of cadmium can be mediated by induction of the Fas ligand to trigger apoptosis; indirect generation of the hydroxyl radical, superoxide anion, hydrogen peroxide, and/or NO; and/or by reducing the zinc concentration (antioxidant). It does so by 3 different mechanisms: (a) protection of the protein sulfhydryl groups, (b) reduction of hydroxyl radical formation from hydrogen peroxide by antagonism of iron and copper, which are redox-active transition metals, and (c) serving as a co-factor in antioxidant enzymes.²⁰ Cadmium has a direct antagonist effect on zinc by competing with its binding sites. Hence, increased cadmium levels in patients with varicocele suggest that poor semen quality in varicocele could be due to the reduced antioxidant effect of zinc.

HO-Isoenzyme 1. Greater HO-1 expression may stimulate apoptosis; however, the expression level of HO-1 is inversely correlated with 4-hydroxy-2-nonenal protein concentrations. This mechanism may be in place to protect the testicular germ cell from OS in the presence of varicocele.²¹ Greater HO-1 production can increase the carbon monoxide level and cause apoptosis of Leydig cells.

OS in Serum

OS in patients with varicocele is also induced by excessive xanthine oxidase (a source of superoxide anion from the substrate xanthine) and excessive NO (a reactive nitrogen species) present in the spermatic veins of patients with varicocele.²² In some adolescent patients, increased MDA levels occurred together with elevated NO levels, indicating excessive lipid peroxidation.¹⁵ Elevated OS levels were limited to the spermatic vein in adolescents, but in adults, systemic OS was evident.¹⁶ These observations support the hypothesis that the negative effects of varicoceles on the testis are progressive, reinforcing the argument for early varicocele repair.

Increased levels of NO in dilated varicocele veins may be responsible for the spermatozoal dysfunction seen in patients with varicocele.¹⁵ Consistent with the discussion in the preceding section, oxidative damage to blood

proteins, as evidenced by the accumulation of protein carbonyls, is more extensive in patients with varicocele in blood drawn from the spermatic veins than in blood from the peripheral veins. This correlates with decreased antioxidant capacity (catalase, superoxide dismutase [SOD], glutathione peroxidase, protein thiols, and ascorbic acid levels) in seminal plasma^{16,23,24}

In addition, a body of data has suggested that increased OS in serum is responsible for increased DNA damage and apoptosis with varicoceles. One of the most abundant oxidative products of DNA induced by ROS is 8-hydroxydeoxyguanosine. Increased levels of 8-hydroxydeoxyguanosine in leukocytic DNA in the spermatic vs peripheral veins of infertile men with varicoceles correlated positively with sperm mitochondrial DNA damage and negatively with the standard parameters of semen analysis.²⁵

OS in Semen

Our group found that in patients with varicocele, the level of seminal ROS correlated with the varicocele grade^{6,9,15,16,19,20,22-24,26-44} (Table 1). Greater levels of ROS were seen in the seminal plasma of patients with grade 2 and 3 varicocele than in the seminal plasma from men with grade 1 varicocele.³⁵ Patients with varicocele and infection produced the greatest amount of ROS, followed by patients with varicocele alone.³¹ A meta-analysis of 4 studies published until 2006 showed that patients with varicocele had significantly greater ROS and lower total antioxidant capacity (TAC) levels. Similar to the findings in the spermatic vein, the finding of increased MDA and NO levels in the semen of patients with varicocele is also suggestive of OS.²⁶

Varicocele has been associated with increased numbers of spermatozoa with abnormal morphology and residual cytoplasm, and these numbers decrease after repair.²⁹ In subjects without varicocele undergoing fertility screening, a strong association has been reported between the relative ROS levels of immature, morphologically abnormal spermatozoa and the TAC levels in the seminal plasma. This suggests that as the relative ROS levels in morphologically abnormal (ie, with excessive presence of cytoplasm) spermatozoa increase, the TAC levels decrease. This also indicates an association with an increased consumption of soluble, nonenzymatic antioxidants in the semen.

Sharma et al.²⁸ developed the ROS-TAC score using a principal component analysis. This score is a better predictor of infertility than the individual measurements of ROS or TAC. A ROS-TAC score of <30 indicates that a patient has elevated OS in his serum.²⁸ The ROS-TAC score in the seminal plasma of patients with varicocele was found to be significantly lower than that in normal, healthy men.³³

Thus, it is the balance between ROS production and the antioxidant status that appears to be important. In addition to elevated ROS levels, the antioxidant status appears to be decreased in men with varicocele. Our

results showed decreased levels of seminal antioxidants in patients with varicocele, in addition to poor motility and morphology. A low total reactive antioxidant potential was reported in patients with varicocele, and a gradual decline was seen in the total reactive antioxidant potential value among normozoospermic, oligoasthenozoospermic, and asthenozoospermic patients.

Co-enzyme Q10 (CoQ10) is mainly associated with intact spermatozoa. CoQ10 and its reduced and oxidized forms (ubiquinol and ubiquinone) were low in the sperm from patients with varicocele plus asthenozoospermia.³⁴ These compounds are lipid-soluble, chain-breaking antioxidants that protect sperm against the deleterious effects of ROS. In the presence of varicocele, the amount of CoQ10 in the seminal plasma is markedly increased, suggesting the loss of intracellular buffering against free radical damage. In one study, the CoQ10 levels in seminal plasma declined after surgical varicocele correction and the concentrations of other antioxidants such as SOD, catalase, glutathione peroxidase, and vitamin C in the seminal plasma increased, along with an improvement in sperm quality within 3 months after the procedure.³² Glutathione-S-transferase M1 gene polymorphism is responsible for increased 8-hydroxydeoxyguanosine content in sperm DNA and lower antioxidant levels in seminal plasma in some patients.²⁵

Effect of Varicolectomy on Various Markers of OS

Varicolectomy is a cost-effective treatment.^{1,10} It has been shown to normalize various biomarkers of OS. Varicolectomy both decreases and normalizes the incidence of 4977-bp mitochondrial DNA deletion, 8-hydroxy-2'-deoxyguanosine content in sperm DNA, oxidative DNA damage, thiobarbituric acid reactive substance (in seminal, as well as peripheral, plasma), and nitrate plus nitrite content.^{6,26,32,41} In addition, varicocele repair also normalizes the levels of nonenzymatic antioxidants (both hydrosoluble, lipid soluble, and minerals, such as α -tocopherol, ascorbate, retinol, selenium, and zinc, as well as TAC).⁶ However, the findings from studies of varicocele and enzymatic antioxidants have been conflicting and are discussed later. The levels of 4-hydroxy-2-nonenal modified proteins in the testis were reported to be significantly greater in patients with varicocele who responded to varicocele repair. Therefore, ROS are not only involved in the pathophysiology of varicocele, but also patients with high levels of ROS should be considered as favorable candidates for varicolectomy.⁴⁵ A discussion about the effects of varicolectomy on other parameters such as semen parameters and pregnancy is beyond the scope of this report.

OS in Testes

In a study by Koksai et al.,³⁰ it was found that lipid peroxidation is increased in patients with varicocele and that the level of lipid peroxidation depended on the varicocele grade, with the greatest level seen in patients

with grade 3 varicocele. However, an animal study conducted by the same group showed that OS might not explain testicular dysfunction in rats with experimentally induced varicocele. In 2003, the same group reported elevated levels of MDA in the testicular tissue of infertile patients with varicocele and implicated ROS as the cause of the testicular dysfunction.⁴⁶ Consistent with this, the expression of 4-hydroxy-2-nonenal modified proteins in testicular biopsy increases with the grade of varicocele and also correlates with increasing patient age, again providing evidence that the effects of varicocele are progressive.²¹ 4-Hydroxy-2-nonenal modified p53 expression in testes was also greater in patients with varicocele compared with that in fertile adults.⁹ 8-Hydroxy-2'-deoxyguanosine is a marker of OS. Its expression has also been reported to increase in the testicular tissue of patients with varicocele.⁴⁰

Cadmium deposition in the testis induces OS, and human studies have reported greater cadmium levels in patients with varicocele. These could affect sperm quality directly by affecting spermatogenesis or indirectly by increasing OS. In terms of a direct effect, increased cadmium could disrupt Sertoli cell actin, block spermiation, disrupt membrane integrity by causing loss of sperm head actin, and damage the actin cytoskeleton. Changes in sperm head actin have been implicated in the acrosome-reaction deficits associated with varicoceles. The indirect effects of cadmium in inducing OS could be responsible for the association detected between testicular cadmium levels and germ cell apoptosis.¹⁹

Controversies in Role of OS in Pathophysiology of Varicocele

Despite the above-mentioned findings, the role of OS and semen antioxidants in the etiology of varicocele-associated infertility is not without controversy. The OS levels in fertile men with varicoceles and infertile men with varicoceles are similar.^{26,27} In addition, a recent study reported no significant difference in ROS levels between fertile men with and without varicocele and a lack of any correlation of ROS level with varicocele grade.³⁸ Furthermore, it has been suggested that the effects of OS are time dependent and are not yet manifested in fertile men with varicoceles.²⁷

Some studies have indicated that the concentration of antioxidants in seminal plasma is greater in subjects with varicoceles—both before and after varicocelectomy—compared with normozoospermic and oligozoospermic controls.⁴⁷ Another recent study reported greater levels of SOD and catalase in the erythrocytes of patients with varicocele compared with the levels in age-matched fertile men. These levels normalized after varicocelectomy.⁶ Ozbek et al.⁴³ demonstrated greater SOD and glutathione peroxidase activity in the internal spermatic vein compared with the brachial veins of the same patients. Greater SOD activity in the semen was reported among patients with varicocele and was normalized after varico-

celectomy.⁴⁴ Thus, the use of antioxidants might be inefficient in infertile men with varicocele or a compensatory increase in antioxidant enzyme activity might occur in patients with varicocele. That OS is a known inducer of apoptosis suggests that OS could be a contributing factor to infertility in these patients and explains why antioxidants can help.

STRATEGIES

Oral administration of the antioxidant vitamins C, D, and E reduces the sperm DNA damage attributable to ROS and has improved sperm function and conception rates in vivo in men without varicoceles.⁴⁸ However, scavenger therapies have focused primarily on the use of glutathione to decrease lipid peroxidation damage to sperm.² Limited clinical experience has suggested that the administration of reduced glutathione is therapeutically efficacious in reducing lipid peroxidation and increasing sperm motility in subfertile men with varicoceles.² The data from infertile men with varicoceles showed seminal zinc levels decreased with a concomitant increase in seminal plasma cadmium levels. In addition, it has been reported that men who remained infertile after varicocelectomy have low levels of seminal plasma zinc.⁴⁹ Among these patients, the semen parameters and pregnancy rates have improved with oral zinc therapy.⁷

Although some reports have been published on the benefits of antioxidative therapies on infertility, most of the studies were not randomized, or controlled with placebo. Also, only seldom were the pregnancy rates reported, and if they did, these were with a small number of patients. A study from China reported that the administration of an herbal remedy Jingling Oral Remedy to men after varicocele surgery reduced the seminal cadmium levels and increased the seminal zinc and SOD levels.⁵⁰ It is believed to increase the antioxidant levels. Jingling Oral Remedy has been reported to improve and regulate the reproductive hormone disturbances in infertile patients treated for varicocele after varicocelectomy. It was able to enhance the semen quality and increase the pregnancy rates. The pregnancy rate in the treated group was 76.6% compared with 40.0% in the control group, a significant difference ($P < .05$). The reproductive hormones were also improved in both groups ($P < .01$). In the treated group, the SOD and zinc levels in semen increased, and that of cadmium decreased, after treatment compared with those before treatment; these differences were significant ($P < .05$). From these findings, patients with varicocele may benefit from a regimen of antioxidant supplementation, although this needs to be validated by large multicenter clinical trials.

CONCLUSIONS

The negative effects of varicocele on fertility are widely recognized. Researchers have shown that varicocele-mediated spermatozoa damage might be the major cause of

Table 1. Oxidative stress in treated and untreated patients with varicocele

Investigator	Patients (n)	Controls (n)	Comment
Weese et al. ²⁶	Fertile patients with palpable varicocele (n = 7)	Fertile patients without varicocele (n = 14)	Increase in chemoattractant stimulated ROS
Hendin et al. ²⁷	Infertile patients with varicocele (n = 21) Incidental patients with varicocele (n = 15)	Normal donors (n = 17)	Low levels of seminal total antioxidant levels in varicocele ($P = .02$) and incidental patients with varicocele ($P = .05$) compared with controls
Sharma et al. ²⁸	Patients with varicocele (n = 55)	Healthy donors (n = 24)	Higher log (ROS + 1) levels in patients with varicocele ($2.10 \pm 1.21 \times 10^6$ cpm) compared with controls ($1.39 \pm 0.73 \times 10^6$ cpm); lower ROS-TAC levels in patients with varicocele (50.0 ± 10.0) compared with controls (34.87 ± 13.54)
Zini et al. ²⁹	Infertile males with varicocele (preoperative, n = 43)	Patients after varicocelectomy (n = 43)	Decrease in % of spermatozoa with residual cytoplasm
Koksal et al. ³⁰	Patients with varicocele (n = 15)	Normal males (n = 10)	Increasing MDA levels in men with subclinical, grades 1-3 varicocele (15.7, 32.9, 37.1, 86.9 pmol/mg)
Pasqualotto et al. ³¹	Patients with varicocele (n = 77)	Normal donors (n = 11)	Mean ROS levels in patients with varicocele ($2.2 \pm 0.13 \times 10^6$ cpm) greater than in controls ($1.3 \pm 0.3 \times 10^6$ cpm); lower levels of TAC in patients with varicocele (1173.05 ± 58.07 trolox equivalents) compared with controls (1653.98 ± 115.29 trolox equivalents)
Chen et al. ²³	Patients with varicocele (n = 30) Patients with subclinical varicocele (n = 25)	Normal males (n = 15)	Greater concentrations of protein carbonyls in spermatic vein of patients with varicocele (3.72 ± 0.56 nmol/mg protein) and subclinical varicocele (3.50 ± 0.30 nmol/mg protein) compared with control group (2.35 ± 0.33 nmol/mg protein)
Mostafa et al. ³²	Infertile males with varicocele (Preoperative, n = 68)	Postoperative infertile males (n = 68)	Greater levels of preoperative MDA (23.2 ± 2.7 nmol/mL), hydrogen peroxide (45.2 ± 10.2 μ mol/mL), nitric oxide (5.8 ± 10 nmol/L) compared with 3-mo postoperative MDA (15.3 ± 4.6 nmol/L), hydrogen peroxide (39.4 ± 7.9 μ mol/mL), and nitric oxide (5.5 ± 0.9 nmol/L) levels
Pasqualotto et al. ³³	Patients with varicocele (n = 16)	Treated male factor patients (n = 52) Healthy donors (n = 19)	Elevated log (ROS + 1) levels in patients with varicocele ($1.46 \pm 0.22 \times 10^6$ cpm) compared with controls ($1.39 \pm 0.16 \times 10^6$ cpm); lower ROS-TAC levels in patients with varicocele (50.5 ± 2.1) compared with controls (39.0 ± 3.1)
Romeo et al. ²²	Adolescent patients with idiopathic varicocele grade 2 and 3 (n = 10)	Self controls (n = 10)	Significantly increased serum L-NHA levels in spermatic veins compared with levels in peripheral veins (176.8 ± 32.3 mmol/L vs 3.38 ± 0.64 mmol/L; $P < .0004$)
Balercia et al. ³⁴	varicocele-associated asthenozoospermic patients (n = 12)	Controls (n = 5)	Low level of CoQ10 in patients with varicocele (3.08 ± 0.14 ng/ 10^6 sperm) compared with controls (4.63 ± 0.26 ng/ 10^6 sperm)
Romeo et al. ¹⁶	Adolescents with varicoceles (n = 10), median age 13 y	Nitrotyrosine in plasma of controls	NO-mediated OS limited to testis of adolescents with varicocele; systemic stress, as described in adults, was not observed
Allamaneni et al. ³⁵	Infertile men with unilateral varicocele (n = 46)	Grade 1 varicocele (n = 15)	Increased ROS levels in seminal plasma in patients with grade 2 and 3 varicocele compared with patients with grade 1 ($P = .02$)
Benoff et al. ¹⁹	Infertile men with left varicocele (n = 47)	Men with obstructive azoospermia (n = 16)	Increased apoptotic nuclei in men with varicocele (1.3%-53.9%) compared with controls (7.3%-13.9%)

Continued

Table 1. *Continued*

Investigator	Patients (n)	Controls (n)	Comment
Turkyilmaz et al. ¹⁵	Blood specimens obtained from dilated spermatic and peripheral veins of adolescents with left idiopathic varicocele, grade 2-3 (n = 13)	13 Healthy children as controls	Increased NO(x) levels with lipid oxidation occur locally in adolescent varicocele, implying that these events could be reversed by early treatment
Benoff et al. ²⁰	Infertile men with varicocele (n = 44)	Men with obstructive azoospermia (n = 7)	Expression of undeleted L-type calcium channel mRNAs correlates with normal testicular cadmium and increased sperm count after varicocelectomy
Agarwal et al. ³⁶	Meta-analysis OS, varicocele and infertility (23 studies)		Significant increase in OS (high ROS, $P = .00001$ and low TAC concentrations ($P < .00001$) in infertile patients with varicocele
Cervellione et al. ³⁷	Children with left-sided varicocele (n = 11; age 10-16 y)	1 y After varicocelectomy	TBARS level significantly reduced in pampiniform plexus 1 y after varicocelectomy
Mostafa et al. ²⁴	Blood sample from internal spermatic vein (n = 68, oligoasthenozoospermic patients undergoing varicocelectomy)	Blood sample from median cubital vein of same patients (n = 68)	Significantly greater ROS markers and lower antioxidant in internal spermatic vein compared with median cubital vein
Coccuza et al. ³⁸	33 fertile men with varicocele (grade 1, n = 14; grade 2, n = 11; grade 3, n = 8)	81 Normal fertile men and 30 infertile men without varicocele	No significant difference in ROS level between fertile men with and without varicocele; ROS levels did not significantly correlate with varicocele grade (neat and washed semen)
El Karakasy et al. ³⁹	Asthenozoospermia with scrotal varicocele (n = 32)	Normozoospermic fertile controls (n = 46)	Significantly greater mast cells in semen of asthenozoospermic patients with varicocele
Hurtado de Catalfo et al. ⁶	Infertile varicocele with unilateral varicocele (n = 36)	Age-paired proved fertile men (n = 33); postoperative infertile men	Increase in markers of OS in patients' semen, as well in peripheral blood, compared with controls; decreased in lipid and water soluble nonenzymatic antioxidants (ascorbate, alpha-tocopherol, retinol) in sperm; increased activity of SOD and catalase in erythrocytes; normalization of all parameters within 3-6 mo
Ishikawa et al. ⁴⁰	Varicocele (n = 36)	Normal volunteers (n = 5)	Increased expression of 8 OH-dG in testicular tissues of patients with varicocele; it is associated with deficient spermatogenesis
Shiraishi et al. ⁹	Varicocele (n = 20)	Fertile adults (controls, n = 8)	Increased expression of 4-hydroxy-2-nonenal modified p53 in patients with varicocele patients
Chen et al. ⁴¹	Subfertile men with varicocele (n = 30)	6 mo After varicocelectomy (same patients)	Significant decrease of 4977-bp deletion of mitochondrial DNA in sperm and level of 8-OH-dG in sperm DNA, and increase in seminal plasma protein thiols and ascorbic acid
Pasqualotto et al. ⁴²	Infertile patients (n = 21) with varicoceles	17 Healthy fertile men	Increase in ROS, decrease in TAC, and decrease in ROS-TAC score in infertile patients with varicocele
Ozbek E et al. ⁴³	Primary infertile varicocele (n = 15)	Normal subjects (n = 10)	Activity of SOD and GSH-PX was greater in internal spermatic vein of patients with varicocele compared with brachial veins of same patients; activity of SOD and GSH-PX in internal spermatic vein of patients also greater compared with normal subjects
Sakamoto et al. ⁴⁴	Oligozoospermic patients with varicocele (n = 15); normozoospermic varicocele patient (n = 15)	Oligospermic (n = 15) and normozoospermic patients without varicocele and after varicocelectomy	Significantly greater NO, HEL, SOD activity in seminal plasma of patients with varicocele compared with infertile patients with normal semen parameters; greater IL-6 level in oligospermic patients with varicocele and reduction in NO, HEL, 8-OH-dG, IL-6 level, and SOD activity after varicocelectomy

MDA = malondialdehyde; 8-OH-2'-dG = 8-hydroxy 2-deoxyguanosine; dG = deoxyguanosine; TRAP = total reactive antioxidant potential; ROS = reactive oxygen species; TAC = total antioxidant capacity; L-NHA = *N*-hydroxyl *L*-arginine; NO = nitric oxide; NOS = NO synthase; XO = xanthine oxidase; TBARS = basal thiobarbituric acid reactive substance; HEL = hexanoyl-lysine; CoQ10 = coenzyme Q10; OS = oxidative stress; SOD = superoxide dismutase; IL = interleukin; GSH-PX = glutathione peroxidase.

infertility in this group of patients. Although many different mechanisms have been proposed as the cause of spermatozoal injury, varicocele-induced OS could be the leading etiologic factor.

Several investigators have demonstrated that ROS production is elevated in patients with varicocele. Other investigators have reported a low level of nonenzymatic antioxidants in patients with varicocele, lowering their ability to counter the increased ROS production. We have reported the pathologic mechanisms of ROS production through various metabolic pathways in patients with varicocele. Some compensatory molecular mechanisms lead to OS, and some might protect from it.

Studies have consistently reported that OS damages sperm DNA and leads to spermatozoal dysfunction. OS is one of the important factors leading to sperm damage and subsequent consecutive infertility in patients with varicocele. Future clinical trials in this area are needed to provide a conclusive answer by allowing the determination of the ROS type that is elevated, allowing for the selection of appropriate antioxidants to treat the OS.

References

- French DB, Desai NR, Agarwal A. Varicocele repair: Does it still have a role in infertility treatment? *Curr Opin Obstet Gynecol.* 2008;20:269-274.
- Naughton CK, Nangia AK, Agarwal A. Pathophysiology of varicoceles in male infertility. *Hum Reprod Update.* 2001;7:473-481.
- Skog SJ, Roberts KP, Goldstein M, et al. The adolescent varicocele: What's new with an old problem in young patients? *Pediatrics.* 1997;100:112-121.
- Jarow JP. Effects of varicocele on male fertility. *Hum Reprod Update.* 2001;7:59-64.
- Dada R, Kumar R, Shamsi MB, et al. Azoospermia factor deletions in varicocele cases with severe oligozoospermia. *Indian J Med Sci.* 2007;61:505-510.
- Hurtado de Catalfo GE, Ranieri-Casilla A, Marra FA, et al. Oxidative stress biomarkers and hormonal profile in human patients undergoing varicocelectomy. *Int J Androl.* 2007;30:519-530.
- Marmar JL. The pathophysiology of varicoceles in the light of current molecular and genetic information. *Hum Reprod Update.* 2001;7:461-472.
- Agarwal A, Makker K, Sharma R. Clinical relevance of oxidative stress in male factor infertility: An update. *Am J Reprod Immunol.* 2008;59:2-11.
- Shiraishi K, Naito K. Effects of 4-hydroxy-2-nonenal, a marker of oxidative stress, on spermatogenesis and expression of p53 protein in male infertility. *J Urol.* 2007;178:1012-1017.
- Richardson I, Grotas AB, Nagler HM. Outcomes of varicocelectomy treatment: An updated critical analysis. *Urol Clin North Am.* 2008;35:191-209.
- Nallella KP, Allamaneni SS, Pasqualotto FF, et al. Relationship of interleukin-6 with semen characteristics and oxidative stress in patients with varicocele. *Urology.* 2004;64:1010-1013.
- Ishikawa T, Fujioka H, Ishimura T, et al. Expression of leptin and leptin receptor in the testis of fertile and infertile patients. *Andrologia.* 2007;39:22-27.
- Konukoglu D, Serin O, Turhan MS. Plasma leptin and its relationship with lipid peroxidation and nitric oxide in obese female patients with or without hypertension. *Arch Med Res.* 2006;37:602-606.
- Yamagishi SI, Edelstein D, Du XL, et al. Leptin induces mitochondrial superoxide production and monocyte chemoattractant protein-1 expression in aortic endothelial cells by increasing fatty acid oxidation via protein kinase A. *J Biol Chem.* 2001;276:25096-100.
- Turkyilmaz Z, Gulen S, Sonmez K, et al. Increased nitric oxide is accompanied by lipid oxidation in adolescent varicocele. *Int J Androl.* 2004;27:183-187.
- Romeo C, Ientile R, Impellizzeri P, et al. Preliminary report on nitric oxide-mediated oxidative damage in adolescent varicocele. *Hum Reprod.* 2003;18:26-29.
- Akkoyunlu G, Erdogru T, Seval Y, et al. Immunolocalization of glial cell-derived neurotrophic factor (GDNF) and its receptor GFR-alpha1 in varicocele-induced rat testis. *Acta Histochem.* 2007;109:130-137.
- Chen Z, Chai Y, Cao L, et al. Glial cell line-derived neurotrophic factor promotes survival and induces differentiation through the phosphatidylinositol 3-kinase and mitogen-activated protein kinase pathway respectively in PC12 cells. *Neurosciences.* 2001;104:593-598.
- Benoff SH, Millan C, Hurley IR, et al. Bilateral increased apoptosis and bilateral accumulation of cadmium in infertile men with left varicocele. *Hum Reprod.* 2004;19:616-627.
- Benoff S, Goodwin LO, Millan C, et al. Deletions in L-type calcium channel alpha1 subunit testicular transcripts correlate with testicular cadmium and apoptosis in infertile men with varicoceles. *Fertil Steril.* 2005;83:622-634.
- Shiraishi K, Naito K. Increased expression of Leydig cell haeme oxygenase-1 preserves spermatogenesis in varicocele. *Hum Reprod.* 2005;20:2608-2613.
- Romeo C, Ientile R, Santoro G, et al. Nitric oxide production is increased in the spermatic veins of adolescents with left idiopathic varicocele. *J Pediatr Surg.* 2001;36:389-393.
- Chen SS, Chang LS, Wei YH. Oxidative damage to proteins and decrease of antioxidant capacity in patients with varicocele. *Free Radic Biol Med.* 2001;30:1328-1334.
- Mostafa T, Anis TH, Ghazi S, et al. Reactive oxygen species and antioxidants relationship in the internal spermatic vein blood of infertile men with varicocele. *Asian J Androl.* 2006;8:451-454.
- Chen SS, Huang WJ, Chang LS, et al. 8-Hydroxy-2'-deoxyguanosine in leukocyte DNA of spermatic vein as a biomarker of oxidative stress in patients with varicocele. *J Urol.* 2004;172:1418-1421.
- Weese DL, Peaster ML, Himsl KK, et al. Stimulated reactive oxygen species generation in the spermatozoa of infertile men. *J Urol.* 1993;149:64-67.
- Hendin BN, Kolettis PN, Sharma RK, et al. Varicocele is associated with elevated spermatozoal reactive oxygen species production and diminished seminal plasma antioxidant capacity. *J Urol.* 1999;161:1831-1834.
- Sharma RK, Pasqualotto FF, Nelson DR, et al. 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.
- Zini A, Buckspan M, Jamal M, Jarvi K. Effect of varicocelectomy on the abnormal retention of residual cytoplasm by human spermatozoa. *Hum Reprod.* 1999;14:1791-1793.
- Koksal IT, Tefekli A, Usta M, et al. The role of reactive oxygen species in testicular dysfunction associated with varicocele. *BJU Int.* 2000;86:549-552.
- Pasqualotto FF, Sharma RK, Nelson DR, et al. Relationship between oxidative stress, semen characteristics, and clinical diagnosis in men undergoing infertility investigation. *Fertil Steril.* 2000;73:459-464.
- Mostafa T, Anis TH, El-Nashar A, et al. Varicocelectomy reduces reactive oxygen species levels and increases antioxidant activity of seminal plasma from infertile men with varicocele. *Int J Androl.* 2001;24:261-265.
- Pasqualotto FF, Sharma RK, Kobayashi H, et al. Oxidative stress in normospermic men undergoing infertility evaluation. *J Androl.* 2001;22:316-322.
- Balercia G, Arnaldi G, Fazioli F, et al. Coenzyme Q10 levels in idiopathic and varicocele-associated asthenozoospermia. *Andrologia.* 2002;34:107-111.

35. Allamaneni SS, Naughton CK, Sharma RK, et al. Increased seminal reactive oxygen species levels in patients with varicoceles correlate with varicocele grade but not with testis size. *Fertil Steril.* 2004;82:1684-1686.
36. Agarwal A, Prabakaran S, Allamaneni SS. Relationship between oxidative stress, varicocele and infertility: A meta-analysis. *Reprod Biomed Online.* 2006;12:630-633.
37. Cervellione RM, Cervato G, Zampieri N, et al. Effect of varicocelectomy on the plasma oxidative stress parameters. *J Pediatr Surg.* 2006;41:403-406.
38. Cocuzza M, Athayde KS, Agarwal A, et al. Impact of clinical varicocele and testis size on seminal reactive oxygen species levels in a fertile population: A prospective controlled study. *Fertil Steril.* 2008;90:1103-1108.
39. El-Karakasy A, Mostafa T, Shaeer OK, et al. Seminal mast cells in infertile asthenozoospermic males. *Andrologia.* 2007;39:244-247.
40. Ishikawa T, Fujioka H, Ishimura T, et al. Increased testicular 8-hydroxy-2'-deoxyguanosine in patients with varicocele. *BJU Int.* 2007;100:863-866.
41. Chen SS, Huang WJ, Chang LS, et al. Attenuation of oxidative stress after varicocelectomy in subfertile patients with varicocele. *J Urol.* 2008;179:639-642.
42. Pasqualotto FF, Sundaram A, Sharma RK, et al. Semen quality and oxidative stress scores in fertile and infertile patients with varicocele. *Fertil Steril.* 2008;89:602-607.
43. Ozbek E, Cekmen M, Simsek A, et al. Comparison of antioxidant enzyme activity in the internal spermatic vein and brachial veins of patients with infertile varicocele. *Int Urol Nephrol.* 2008;40:679-683.
44. Sakamoto Y, Ishikawa T, Kondo Y, et al. The assessment of oxidative stress in infertile patients with varicocele. *BJU Int.* 2008;101:623-628.
45. Shiraishi K, Naito K. Generation of 4-hydroxy-2-nonenal modified proteins in testes predicts improvement in spermatogenesis after varicocelectomy. *Fertil Steril.* 2006;86:233-235.
46. Koksal IT, Usta M, Orhan I, et al. Potential role of reactive oxygen species on testicular pathology associated with infertility. *Asian J Androl.* 2003;5:95-99.
47. Mancini A, Meucci E, Milardi D, et al. Seminal antioxidant capacity in pre- and postoperative varicocele. *J Androl.* 2004;25:44-49.
48. Greco E, Iacobelli M, Rienzi L, et al. Reduction of the incidence of sperm DNA fragmentation by oral antioxidant treatment. *J Androl.* 2005;26:349-353.
49. Takihara H, Cosentino MJ, Cockett AT. Zinc sulfate therapy for infertile male with or without varicocelectomy. *Urology.* 1987;29:638-641.
50. Yan LF, Jiang MF, Shao RY. Clinical observation on effect of jingling oral liquid in treating infertile patients with varicocele after varicocelectomy. *Zhongguo Zhong Xi Yi Jie He Za Zhi.* 2004;24:220-222.