

## Increased levels of oxidants and reduced antioxidants in semen of infertile men with varicocele

Significantly higher levels of oxidants (malonaldehyde and nitric oxide) and reduced levels of antioxidants (superoxide dismutase, glutathione peroxidase, catalase, and ascorbic acid) are seen in semen of infertile men with varicocele. Seminal oxidative stress (OS) seen in men with varicocele is associated with sperm motility and grade of varicocele. (*Fertil Steril*® 2010;94:1531–4. ©2010 by American Society for Reproductive Medicine.)

Varicocele is found in ~15% of the general population and in 19%–41% of men presenting for infertility investigations. The incidence of varicocele in men with secondary infertility is ~70%–80% (1). Defective spermatogenesis in some patients with varicocele has been attributed to many factors, including reflux of toxic metabolites from an adrenal or renal origin, disturbed hormone status, spermatic venous hypertension, testicular hypoxia secondary to stasis, and abnormal temperature regulation (1). Recent studies have elucidated the effects of increased oxidative stress (OS) in the serum, seminal ejaculates, and testicular tissues of patients with varicocele (2–7). Increased levels of seminal OS have been correlated with sperm dysfunction through different mechanisms which include lipid peroxidation of the sperm plasma membrane and impairment of sperm motility and fertilizing capacity (8, 9) and high frequencies of single and double DNA strand breaks reported to negatively correlate with natural as well as assisted fertility (7–9).

Nitric oxide (NO) originates from different sources in the male genital tract, including phagocytes, endothelial cells, and smooth muscle cells (10). Nitric oxide is derived from oxidative deamination of the amino acid L-arginine by the effect of the enzyme NO synthase (11–13). The reaction of NO with superoxide anion results in formation of the more noxious oxidant peroxynitrite, which is highly toxic to sperm (14). Recent reports indicated an

excessive release of NO within the dilated spermatic veins in subfertile patients with varicocele (2, 15–18).

The objectives of the present study were to: 1) compare levels of seminal oxidants [malondialdehyde (MDA) and NO] and antioxidants [superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT), and ascorbic acid] in infertile men with varicocele and in a control group of fertile men with normal genital examination; and 2) determine the relationship between levels of seminal oxidants and antioxidants in infertile men with the varicocele grade and sperm parameters.

After institutional approval, this prospective study was conducted between January 2007 and January 2008. The study included 36 infertile men with varicocele and a control group of 18 fertile men with normal genital examination. Clinical diagnosis of varicocele was confirmed by scrotal Doppler ultrasonography. A radiologic diagnosis of varicocele was made when one or more veins had a maximal diameter of >3 mm and retrograde flow was seen either at rest or under Valsalva maneuver. Patients with a history of smoking or recent genital tract infection were excluded. Patients who had received medical treatment for infertility during the 6 months before enrolment in the study were also excluded.

Sperm parameters (sperm concentration, percent motility, and sperm morphology) were determined according to the World Health Organization (WHO) guidelines (19). Patients who had leukocytospermia (peroxidase-positive leukocytes  $>1 \times 10^6$ /mL semen) were excluded. Liquefied semen aliquots were centrifuged at 300g for 7 minutes. The seminal plasma was aliquoted and frozen at  $-20^\circ\text{C}$ . Colorimetric method was used to measure levels of seminal plasma lipid peroxidation, nitric oxide, ascorbic acid, CAT, GPX, and SOD (10, 20–25).

Continuous variables among the groups were compared using Kruskal-Wallis tests. Correlation between variables was calculated using the Spearman nonparametric method. All analyses were calculated with SPSS statistical software package (version 8.0). Values are presented as mean  $\pm$  SD. Statistical significance was considered to be at  $P < .05$ .

Out of the 36 infertile men, eight had grade 1, 14 grade 2, and 14 grade 3 varicocele. Compared with control subjects, the infertile men with varicocele had a significantly lower sperm concentration ( $14 \pm 2\%$  vs.  $85 \pm 19\%$ ;  $P = .001$ ), motility ( $30 \pm 7\%$  vs.

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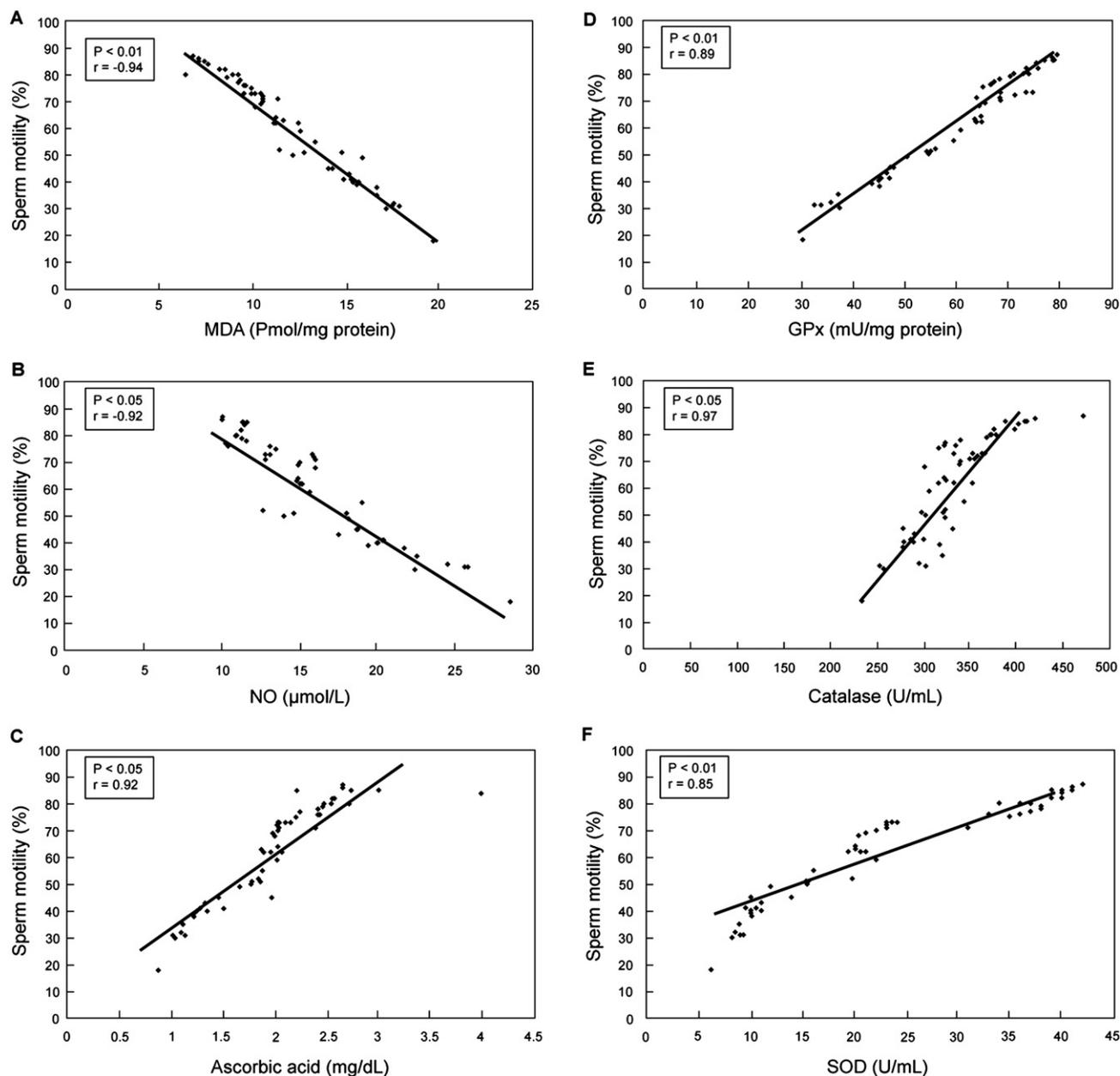
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**FIGURE 1**

Association of oxidative stress markers and antioxidants with sperm motility. (A) Linear correlation between seminal malondialdehyde (MDA) and sperm motility. (B) Linear correlation between seminal nitric oxide (NO) and sperm motility. (C) Linear correlation between seminal ascorbic acid and sperm motility. (D) Linear correlation between seminal glutathione peroxidase (GPx) and sperm motility. (E) Linear correlation between seminal catalase (CAT) and sperm motility. (F) Linear correlation between seminal superoxide dismutase (SOD) and sperm motility.



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$70 \pm 10\%$ ;  $P = .01$ ), and normal sperm forms ( $30 \pm 7\%$  vs.  $72 \pm 12\%$ ;  $P = .001$ ). Similarly compared with control subjects, infertile men with varicocele had a significantly higher percentage of sperm with head ( $16 \pm 4\%$  vs.  $8 \pm 2\%$ ;  $P = .001$ ), midpiece ( $10 \pm 3\%$  vs.  $6 \pm 2\%$ ;  $P = .001$ ), and tail ( $22 \pm 4\%$  vs.  $14 \pm 4\%$ ;  $P = .001$ ) defects. Similarly compared with fertile control subjects, infertile men with varicoceles showed increased levels of MDA ( $13.5 \pm 2.8$

pmol/mg vs.  $8.4 \pm 1.3$  pmol/mg;  $P = .01$ ) and NO ( $17.9 \pm 4.1$  vs.  $11.3 \pm 1.0$   $\mu\text{mol/L}$ ;  $P < .05$ ). CAT, SOD, GPX, and ascorbic acid were significantly lower in infertile men with varicocele compared with fertile men ( $P$  values  $< .05$ ,  $.01$ ,  $.01$ , and  $.05$ , respectively). A comparison of seminal plasma concentrations of MDA, NO, CAT, SOD, GPX, and ascorbic acid in the three groups of infertile men with varicocele (grades 1, 2 and 3) showed that

only SOD and ascorbic acid levels were significantly reduced ( $P < .05$ ) between grades 1 and 2. Significantly higher levels of MDA ( $P < .001$ ) and NO ( $P < .001$ ) were seen and antioxidant levels were significantly lower in grade 1 versus grade 3 varicocele (CAT:  $P < .01$ ; SOD:  $P < .01$ ; GPX:  $P = .01$ ; and ascorbic acid:  $P < .01$ ). Similarly, comparison between grade 2 and grade 3 showed significantly higher levels of MDA ( $P < .01$ ) and NO ( $P < .01$ ) and lower antioxidant levels (CAT:  $P < .05$ ; SOD:  $P = .01$ ; GPX:  $P = .01$ ; and ascorbic acid:  $P < .05$ ).

A significant negative correlation was seen between sperm motility and seminal plasma concentrations of MDA ( $P < .01$ ;  $r = -.94$ ; Fig. 1A) and NO ( $P < .05$ ;  $r = -.92$ ; Fig. 1B). Sperm motility correlated with seminal plasma concentrations of ascorbic acid ( $P < .01$ ;  $r = .85$ ; Fig. 1C), GPX ( $P < .01$ ;  $r = .89$ ; Fig. 1D), CAT ( $P < .05$ ;  $r = .97$ ; Fig. 1E), and SOD ( $P < .05$ ;  $r = .91$ ; Fig. 1F).

Despite a high incidence of varicocele among the infertile population, the exact mechanism(s) of varicocele-mediated sperm dysfunction has not yet been resolved (1). A recent review indicated that free radicals in semen, including reactive oxygen and nitrogen species, play a crucial role in the pathogenesis of varicocele-mediated male infertility (7). The present results showed significantly increased levels of oxidants (MDA and NO) and reduced levels of enzymatic (CAT, SOD, GPX) and non-enzymatic (ascorbic acid) antioxidants in the semen of infertile men with varicocele. Similar results have been recently reported by Mostafa et al. (3), who compared MDA, hydrogen peroxide, and five antioxidants (CAT, SOD, GPX, and vitamins C and E) in fertile and infertile men with and without varicocele. Those authors concluded that varicocele is associated with OS even in fertile normozoospermic men. In addition, the authors speculated that men with varicocele may have a threshold value of OS over which male fertility may be impaired.

The present findings are in agreement with the results of earlier studies using the chemiluminescence technique which allowed collective measurement of seminal reactive oxygen species (ROS) and total antioxidant capacity (TAC) (4) and, more recently, the use of ROS/TAC score as an index for OS (26, 27). The present study also found that infertile men with grade 3 varicocele had significantly higher levels of seminal oxidants and significantly lower levels of seminal antioxidants than those with grade 1. These observations agree with the findings of earlier studies (28, 29). However, one recent study did not find a relationship between levels of OS and the grade of varicocele in a fertile population (30).

The present study also found a significant reduction in sperm concentration, motility, and normal sperm forms in infertile men with varicocele compared with fertile men with normal genital examination. In 1992, WHO reported data from 9,034 infertile men from 34 centers in 24 countries and showed that those with varicocele had a lower total sperm count per ejaculate (31). In a more

recent study, all sperm parameters were found to be significantly lower in 40 patients with varicocele than in 40 fertile subjects, and no correlation was found between varicocele grades (32). This is contrary to a report by Zargooshi (33) in which the majority of patients with high-grade varicocele had increased normal sperm output. In addition, higher grades of varicocele were not associated with more pronounced deleterious effects on sperm concentration and percentage motility.

Interestingly, we found that the infertile men with varicocele had a significantly higher percentage of sperm with midpiece defects than the normal fertile men. It has been indicated that spermatozoa with midpiece defects and excess residual cytoplasm are major sources of ROS production in infertile men (34). Retention of residual cytoplasm by spermatozoa is positively correlated with ROS generation via mechanisms that may be mediated by the cytosolic enzyme glucose 6-phosphate dehydrogenase (35).

The finding in the present study of increased levels of NO in seminal plasma in infertile patients with varicocele and its negative correlation with sperm quality is in agreement with earlier reports showing the harmful effects of NO mediated by biologically active molecules produced by reaction of NO with oxidant molecules (36–38). Activation of the inducible isoform of NO causes injury, because its toxicity is greatly enhanced when it combines with  $O_2^-$  to generate peroxynitrite. These are strong oxidant molecules that can cause molecular damage to a variety of tissues. The peroxynitrous acid produced reacts with the cysteine residues of proteins or glutathione, forming S-nitrosothiols. Both increased oxidants and reduced antioxidants, together with increased peroxynitrites, overwhelm the repair mechanisms in men with varicocele (37).

In the present study, levels of seminal plasma antioxidants were significantly lower in the patients with varicocele than in the normal fertile control subjects. We found that reduced sperm motility in the infertile men with varicocele was correlated with low levels of seminal antioxidants. An earlier study indicated that varicolectomy reduces ROS levels and increases antioxidant activity of the seminal plasma in infertile men with varicocele (39).

In conclusion, the present results confirm the findings of earlier studies showing the presence of significantly increased amounts of seminal OS in patients with varicocele. This increase was evident regardless of the method used to assess seminal OS. We also found a significant negative relationship between seminal OS and sperm parameters, which indicates that seminal OS plays an important role in the pathogenesis of varicocele-mediated male infertility. Finally, seminal OS levels were higher in the patients with high grades of varicocele, which may have important clinical implications. Infertile men should be counseled regarding the importance of early diagnosis and treatment of varicocele as a way to reduce the negative effects of seminal OS on their fertility potential.

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