

Increased seminal reactive oxygen species levels in patients with varicoceles correlate with varicocele grade but not with testis size

Increased levels of reactive oxygen species (ROS) are associated with clinical varicoceles; however, its correlation with varicocele grade and testis size is unknown. In our study, seminal ROS levels showed significant correlation with left varicocele grade and significantly elevated seminal ROS levels were seen in men with left varicocele grade 2 and 3 compared to grade 1. (*Fertil Steril*® 2004;82:1684–6. ©2004 by American Society for Reproductive Medicine.)

Oxidative stress is implicated as a mediator of sperm dysfunction and male infertility (1). Spermatozoa produce baseline low levels of reactive oxygen species (ROS) for physiological functions like capacitation, and fertilization (1). Spermatozoal membrane is rich in polyunsaturated fatty acids, and is susceptible to lipid peroxidation in the presence of elevated seminal ROS levels (2). High levels of seminal ROS also impair the sperm fertilizing capacity by DNA damage and apoptosis (1, 3).

The incidence of varicocele in men presenting for an infertility evaluation ranges between 19% and 41% (4, 5). The pathophysiology of varicoceles is not clearly understood. Several proposed mechanisms include testicular blood flow and venous pressure changes, oxidative stress, retrograde flow of renal or adrenal products, Leydig cell dysfunction, and hyperthermia (6). Increased seminal ROS levels are associated with clinical varicoceles (7–9). The presence of a varicocele is sometimes associated with loss of ipsilateral testicular volume (10, 11). Furthermore, the degree of testicular atrophy may be proportional to the clinical grade of the varicocele (12). Ipsilateral testicular atrophy may be associated with decreased total motile sperm counts in infertile men with clinical varicoceles (13).

Although the presence of a varicocele is associated with both increased ROS levels and testicular atrophy, the impact of the varicocele's clinical grade on seminal ROS levels is unknown. The objective of this study was [1] to determine the association of seminal ROS with varicocele grade and testis size in men with varicocele, and [2] to determine whether increased seminal ROS levels could predict testicular atrophy in men with varicocele.

The Institutional Review Board approved this study. We reviewed the medical charts of 46 men diagnosed with a unilateral left varicocele during an infertility evaluation and underwent seminal ROS analysis. All men were evaluated by physical examination in the standing position with and without Valsalva maneuver by an infertility specialist (A.J.T.). Varicoceles were clinically graded as grade 1 (palpable with Valsalva), grade 2 (palpable without Valsalva), and grade 3 (visible through the scrotal skin). Testicular dimensions were measured by caliper by a single examiner (A.J.T.). Testicular volume (in milliliters) was calculated by $(0.53) \times (\text{length}) \times (\text{width}) \times (\text{depth})$ (12). A testicular volume of <19 mL was considered hypotrophic. A testicular volume difference between the right and left testicles of 3 mL or more was considered asymmetric (13).

Levels of ROS were measured by a chemiluminescence assay. Briefly, liquefied semen specimens were centrifuged at $300 \times g$ for 7 minutes, and seminal plasma was removed. The pellet was washed with phosphate buffered saline (PBS) and resuspended in the same media and 400- μL aliquot was used for assessment of ROS levels. Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione; Sigma Chemical Co., St. Louis, MO) was used as a probe. Ten microliters of luminol, prepared as 5 mM stock in dimethyl sulfoxide (DMSO), was added. A negative control was prepared by adding 10 μL of 5 mM luminol to 400 μL of PBS.

The ROS levels were assessed by measuring chemiluminescence with an Autolumat LB 953 luminometer (Berthold technologies, Bad-Wildbad, Germany) in the integrated mode for 15 minutes. The results were expressed as $\times 10^4$ counted photons per minute (cpm) per 20×10^6 spermatozoa.

Statistical computations were performed with SAS version 8.1 (SAS institute Inc., Cary, NC), and statistical significance was assessed using two-tailed tests. A *P* value of <.05 was considered statistically significant. Pair-wise comparisons between the groups were performed with Wilcoxon rank-sum tests. Correlation between variables was calculated using Spearman's nonparametric method.

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TABLE 1

Correlation of varicocele grade and testis volume with reactive oxygen species (ROS) levels in 46 men with unilateral left varicocele.

Variable	Median (25th, 75th percentile)	Correlation value (P value) ^a
ROS levels ($\times 10^4$ cpm)	119 (13, 2475)	—
Left varicocele grade (1–3)	2.0 (1.0, 2.0)	0.36 (0.01)
Right testis volume (mL)	19.8 (14.9, 23.8)	−0.06 (0.72)
Left testis volume (mL)	17.5 (13.6, 22.2)	0.03 (0.87)
Total volume (mL)	38.6 (28.5, 44.4)	0.01 (0.94)

^a Correlation with ROS levels by Spearman nonparametric test; $P < .05$ was considered significant.

Allamaneni. ROS and varicocele grade and testis size. *Fertil Steril* 2004.

The mean age of subjects was 32 ± 5 years. Fifteen men presented with grade 1, 23 men with grade 2, and 8 with grade 3 unilateral left varicocele. The median ROS levels were 119×10^4 cpm. There was a statistically significant correlation between ROS levels and varicocele grade, but no correlation between ROS levels and testicular volume (Table 1).

The ROS levels were significantly greater in patients with grades 2 and 3 varicoceles compared to men with grade 1 varicoceles ($P = .02$). No significant differences were observed in ROS levels between men with left testis volume <19 mL compared to men with testis volume of ≥ 19 mL ($P = 0.912$). Similarly, testicular asymmetry of ≥ 3 mL between the right and left testicles did not correlate with higher ROS levels ($P = .58$).

Our study results indicate that in men with varicoceles, seminal ROS is increased with the severity of the varicocele grade. Although the association of varicocele and infertility has long been recognized, the underlying pathophysiology has yet to be clearly elucidated. Many studies implicate oxidative stress in infertility associated with varicocele, evidenced by increased ROS generation or decreased total antioxidant capacity of semen (7–9, 14–16). Chemostimulant-induced ROS levels are higher in patients with varicocele compared to those without varicocele (8). Both ROS and antioxidant capacity levels are lower in patients with varicocele compared to those of control groups (7).

Testicular biopsy levels of malondialdehyde (end-product of lipid peroxidation) concentrations are higher in patients with higher grade varicocele compared with lower grade varicocele (14). One recent study found ineffective utilization of antioxidants in varicocele patients with oligozoospermia (15). Varicocelectomy results in a significant decrease in ROS levels and increase in antioxidant activity of seminal plasma (16).

Our results demonstrate no association between the seminal ROS levels and left testicular volume. But there is evidence of progressive testicular damage in some men with varicoceles manifested by the relative atrophy of the ipsilateral testis (10, 11). The lack of ROS level correlation with testicular atrophy may be a reflection of the time required to cause testicular size changes, and the multifactorial nature of varicocele pathophysiology. Infertility associated with varicocele may be due to the direct effect of ROS

on spermatozoa by impairing their fertilizing capacity rather than affecting testicular volume. The testicular atrophy associated with varicocele may be due to other pathological mechanisms associated with a varicocele and may contribute to abnormal semen parameters at a later stage.

The mechanisms by which the varicocele grade corresponds to the variable spermatogenic function are unknown. Some studies demonstrated that the quality of semen is inversely associated with the severity of varicocele (10, 17). In contrast, another study found no correlation with semen parameters and the varicocele grade (18). This may be attributed to increased ROS levels in infertile normospermic men with varicoceles but with normal semen parameters. The lack of correlation between sperm parameters and severity of varicocele in infertile patients in some studies may be due to direct effects of increased ROS impairing the sperm-fertilizing capacity without affecting the routine semen parameters.

The fertility potential of men with varicocele is not predictable with existing markers. Pinto et al. (11) studied the use of varicocele-associated testicular atrophy as a predictor of fertility in patients with varicocele. They reported that testicular size was not predictive of fertility potential of varicocele patient and emphasized the need for better predictors. The use of semen parameters to predict the fertility potential in varicocele patients is controversial. The significant increase in pregnancy rate for couples who underwent intrauterine insemination (IUI) with husband's semen after varicocelectomy was not associated with improvement in standard semen parameters in all cases (19). The improved fertility after varicocelectomy associated with decreased levels of ROS still requires investigation. Further studies will help to explore the possible use of ROS levels as a tool to predict the fertility potential in patients with varicocele. The limitation of our study is its retrospective design.

In conclusion our study demonstrates that the elevated levels of ROS in infertile men with a unilateral left varicocele are associated with higher varicocele grade, but not testicular atrophy.

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