Current Opinions on Varicocele and Oxidative Stress and its Role in Male Infertility

a report by

Ashok Agarwal, PhD,1 Sushil Prabakaran, MD,1 Suresh C Sikka, PhD2

1. Center for Advanced Research in Human Reproduction, Infertility, and Sexual Function, Glickman Urological Institute and Department of Obstetrics-Gynecology, Cleveland Clinic, and 2. Department of Urology, Tulane University Health Sciences Center

Varicocele is a pathobiological condition associated with abnormal tortuosity and dilatation of the veins of the pampiniform plexus within the spermatic cord; it is one of the leading causes of male infertility (see Figure 1). Although 15% of adult men are believed to have either clinical or subclinical varicocele, its prevalence may be as high as 40% among infertile men.1 It is interesting to find that, anatomically, varicocele is left-sided in almost 85% of men, whereas only 15% of men have right-sided varicocele. Amongst these, approximately 20% of men have bilateral varicocele. Only one out of every five infertile varicocele patients seeks treatment for infertility.

Etiology of Varicocele

The etiology of varicocele is multifactorial.2 Many theories have been proposed for its development. The most common are:

- differences in structural anatomy of left and right internal spermatic vein;
- incompetent or absent valves in the spermatic vessels, causing retrograde blood flow; and
- the nutcracker effect—compression of the left renal vein between aorta and superior mesenteric artery, with high pressure transmitted to the left internal spermatic vein.

Pathophysiology of Varicocele

The exact mechanism of impaired testicular function in patients with varicocele is not known. The most widely accepted concept is currently a varicocele-related increase of testicular temperature (see Figure 2). Normally, the difference between the intra-abdominal and scrotal temperature averages 2.2°C. Varicocele can cause an increase in scrotal temperature by 2.6°C, neutralizing the required temperature gradient. However, there is considerable overlap between the range of scrotal temperatures in infertile men with varicoceles and in normal fertile men.3

The varicocele-associated pathology mainly includes changes in testicular size, histology, impaired Leydig cell function (steroidogenesis), and sperm characteristics.4 Subfertile men with varicocele have disrupted spermatogenesis, which is manifested by low sperm count, decreased sperm motility, and a low percentage of normal sperm morphology. The World Health Organization (WHO) reported that varicocele (mostly left-sided) was associated with relative left testicular atrophy compared with the contralateral testis. In addition, decrease in testicular volume was associated with increasing varicocele grade.

Pathological changes in testicular histology associated with varicocele vary from normal to Sertoli cell only pattern, but hypospermatogenesis and premature sloughing of germ cells are found to be the most common causes of infertility. Stasis of blood in veins could decrease in oxygen supply. Reflux of blood in the testicular vein could expose the testes to accumulated renal and adrenal breakdown products.7 Another possibility could be androgen deprivation as a result of Leydig cell pathology or androgen receptor defects.5,7

Many varicocele patients have documented hyperplasia of Leydig cell dysfunction. Additionally, the mean testosterone concentration of men with varicocele older than 30 years of age was found to be significantly lower than that of younger patients with varicocele. Conversely, significant increase in mean testosterone levels was documented after varicocelectomy without concomitant increase in follicle-stimulating hormone (FSH) and leutinizing hormone (LH) levels. These findings suggest a direct detrimental time-dependent effect of varicocele on Leydig cells.

Varicocele and Oxidative Stress

Varicocele has been associated with increased oxidative stress, especially in the gonads.8-10 Alterations in the testicular hemodynamics and microenvironment due to varicocele probably increases reactive oxygen species (ROS) production in association with decreased antioxidant capacity.11,12 An increase in oxidative stress markers (e.g. superoxide and nitric oxide anions,
peroxinitrite), has been observed in the serum, semen and testicular tissues of varicocele patients. This is probably due to induction of xanthine oxidase (a source of superoxide anion from the substrate xanthine) and nitric oxide synthase (NOS), a source of nitric oxide (NO) in the spermatic veins of patients with varicocele. Elevated levels of NO in dilated varicocele veins by itself may be responsible for the spermatozoa dysfunction seen in varicocele patients.

In animal models, the sperm plasma membrane proteins undergo oxidative damage and protein carbonyls is accumulated leading to decreased fertilizing ability. Varicocele patients demonstrated elevated protein carbonyls in blood drawn from their spermatic veins than in blood from peripheral veins. It was also found to be correlated with decreased antioxidant capacity (protein thols and ascorbic acid levels) in seminal plasma.

Levels of ROS were also significantly higher in the seminal plasma of patients with grade II and III varicocele than in seminal plasma from men with grade I varicocele. Another major source of ROS in seminal plasma is leukocytes, which is also increased in some varicocele patients. The seminal plasma of men with varicocele also contains elevated NO levels that induce oxidative stress (OS). Varicocele repair reduces both ROS and NO.

Some of the parameters of oxidative stress (ROS and lipid peroxidation) were significantly increased in infertile varicocele patients in comparison with normal, healthy donors. Varicocele-induced OS can also be demonstrated by the increased presence of 4-hydroxy-2-nonenal (HNE) modified proteins. As a protective measure, increased HO-1 expression is seen in their Leydig cells.

Elevated OS parameters are reflected by many abnormal spermatozoa found in these patients. Varicocele has been associated with increased numbers of spermatozoa with abnormal morphology and residual cytoplasm; these numbers fall after repair.

**Varicocele and Antioxidants**

Varicocele reduces both the seminal plasma and blood antioxidant defenses. In the authors’ meta-analysis, they showed that markers of total antioxidant capacity (TAC) were significantly lower in the infertile varicocele patients when compared with the controls. In comparison with the individual measurements of ROS or TAC, a combined ROS-TAC score is a better predictor of infertility. A ROS-TAC score below 30 indicates that a patient has high OS in his serum. This score was found to be significantly lower in seminal plasma of normospermic varicocele patients than that in normal, healthy men. Low levels of the antioxidant Coenzyme Q10 has been reported in spermatozoa of varicocele patients, probably leading to higher sensitivity to oxidative damage. Administration of reduced glutathione is therapeutically efficacious in reducing lipid peroxidation and increasing sperm motility in subfertile men with varicoceles. Low levels of certain antioxidants therefore seem to contribute to the development of varicocele.

Varicocele and DNA Damage

It is well known that increased OS is responsible for increased DNA damage leading to high levels of its byproduct 8-hydroxydeoxyguanosine (8-OHdG) and apoptosis. Its level is increased in leukocyte DNA of spermatic veins in comparison with peripheral veins of infertile men with varicocele. A polymorphic gene of glutathione-S-transferase could be responsible for increased 8-OHdG content in sperm DNA with lower anti-oxidant levels in seminal plasma. Animal studies show that Cd-induced testicular toxicity is the result of increased lipid peroxidation with markedly decreased SOD activity, associated with significant single-strand DNA breaks. Infertile patients with varicocele had a higher percentage of sperm cells with damaged nuclear DNA, compared with other infertile patients with no apparent varicocele, even in the presence of normal seminal parameters. In normal men without
Varicocele, administration of anti-oxidants such as vitamins C, D, and E reduces sperm DNA damage due to decrease in ROS and improvement in sperm function and pregnancy rates.43–45

Varicocele, Heat, and Apoptosis

Testicular tissue apoptosis is increased in experimental varicocele that can be reversed by the administration of an antioxidant, such as vitamin E.46,47 Several studies suggest that varicocele influence increases intra-testicular temperature.48,49 Transient testicular heating and experimental cryptorchidism in adult animal models induce apoptosis, which explains the relationship between elevated intra-testicular temperature and increased apoptosis as observed in varicoceles.50,51 Nitric oxide seems to play a major role in apoptosis due to varicocele. Administration of a NOS inhibitor attenuated apoptosis and improved spermatogenesis in cryptorchidism.52 The role of NO in elevating apoptosis in response to heat was confirmed through the study of NOS-deficient knockout mice.53 Apoptosis is also affected by deletions of normal testicular L-type, voltage-dependent calcium channel sequence by altering calcium channel function.54

Management of Varicocele

A variety of surgical approaches have been recommended for varicocelectomy. These include minimally invasive procedures such as laparoscopic varicocelectomy and transvenous percutaneous embolization ligation, and the traditional open surgical approach (retroperitoneal,inguinal, and subinguinal). Microsurgical varicocelectomy is an effective procedure for treatment of recurrent painful varicocele.55 It also improves sperm DNA integrity in infertile men with varicocele.56 It has been shown to improve semen parameters, as well as pregnancy outcome in varicocele patients.57 An improvement in serum testosterone levels and testicular volume is also noted after varicocelectomy.58 Open varicocelectomy is more effective in higher grade varicocele patients, with marked improvement in semen parameters than those having lower grade varicoceles.59 Microsurgical shunting also led to normalization of sperm count, motility and morphology in infertile patients with severely distorted seminal parameters.60 Embolization of the internal spermatic vein also improved semen parameters and blood testosterone levels in infertile patients with varicocele.61 Bilateral varicocelectomy is advised as it reverses testicular dysfunction to a greater extent, and improve spermatogenesis.62–64 Any correlation between improvement in semen parameters with pregnancy outcome after varicocele repair is a topic of on-going debate.65–67 A recent Cochrane meta-analysis of randomized clinical trials by Evers et al. concluded that surgical or radiological treatment of varicocele in patients with unexplained subfertility should not be recommended.65 However, several authors have questioned this meta-analysis.68,69 Marmar et al. pointed out the exclusion of microsurgical procedures in that study and emphasized the need to assess the molecular and genetic defects in this patient population.68 Improved testicular histology in varicocele patients appears to correlate with successful outcome of varicocele repair. Varicocele patients with both germ-cell aplasia or sperm maturation arrest had a larger increase in testicular size and higher post-operative pregnancy rates after varicocelectomy.69 In this regard, it is important to note that many factors, including patient selection, genetic defects, and the presence of untreated female factors,57,66,71 can all impact study findings. Sperm count is an important predictive factor,72 and patients with a sperm count of less than five million/ml have a very low chance of initiating spontaneous pregnancy following varicocelectomy. Such couples should be offered assisted reproductive techniques (ART).62,64,74 If semen parameters can be improved, less invasive methods of assisted reproduction may be able to overcome the male factor infertility.75,76

A Molecular Approach to Varicocele

The major problem associated with varicocele is to understand why some men with varicocele are fertile, and others fail to improve their fertility after varicocelectomy. New approaches based on molecular and genetic markers are being employed to identify the patients at risk of infertility. Levels of cadmium in the tissue and calcium channel mRNA sequence are some of the markers that can predict the outcome of varicocelectomy.77 Genetic

![Figure 2: Possible Mechanisms by which the Varicocele Induces Infertility](image_url)
factors such as chromosome abnormalities and Yq microdeletions are possible causes of spermatogenic impairment in males as shown by their higher frequency in infertile men than in the general male population. These factors can affect germ-cell development, differentiation, and function. It is interesting to find that up to 54% of infertile men with palpable varicocele had Yq microdeletions in the sequence of the L-type, voltage dependent calcium channel.

Conclusion

Varicocele is an important cause of male infertility. Owing to its unknown etiology and relative conflicting pathophysiology, it is imperative that varicocele is managed on an individual basis. Oxidative stress is increased in these patients, and, hence, adequate intake of antioxidants is recommended. Many studies have shown improved semen parameters and pregnancy outcome after varicocelectomy. Evaluating molecular and genetic composition of subjects with varicocele may allow the prediction of chances of success in the people at risk for infertility and prognosis after varicocelectomy. This would alleviate the emotional trauma associated with infertility to a larger extent.

References


