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Varicocele and male infertility: Part II

Varicocele: effect on sperm functions

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Despite the numerous studies published over the past decade, the role of varicocele in male infertility is still controversial. Although more frequent in infertile men, its influence on sperm production or function has not, as yet, been determined. Moreover, the exact mechanism of varicocele action is not clear. We have surveyed the literature, the correlation of varicocele to sperm parameters and to sperm function tests, such as binding capacity, hypo-osmotic swelling test, presence of reactive oxygen species, and in particular, the correlation to fertility potential. Almost every subject examined had contradictory results. Larger control studies may possibly elucidate and clarify the cases in which varicocele is associated to sperm function, and where treatment may improve fertility.

Key words: acrosome reaction/binding tests/hypo osmotic swelling test/reactive oxygen species/sperm function

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Introduction

Many studies have been published concerning the role of varicocele in male infertility, but this issue is still controversial. The concept that the presence of a varicocele has a detrimental effect on fertility was supported by the existence of a relatively higher frequency of men with varicocele among the infertile population (25.4%) than men with normal sperm quality (11.7%) (World Health Organization, 1992). Despite the high frequency, it is also quite surprising and even disconcerting that the exact action mechanism of the varicocele's negative effect on sperm function and fertility is not resolved. The different methods of measuring the severity of varicocele (clinical evaluation, isotope mapping, thermography or ultrasound measurement of vein diameter) and the variety of treatment options used, such as surgical ligation of the spermatic veins (high or low), laparoscopic procedures, or different embolization techniques, make comparison and evaluation of the results far more complicated.

In the present survey, the different aspects of sperm function were examined in relation to varicocele presence, prior to and after treatment of varicocele. This survey was based on more than 400 studies published since 1966 as screened by Medline Express® of Silver Platter information (WinSPIR version 2.0). In most cases the better-designed studies were selected, such as prospective and large-scale studies. Yet, in some subjects, due to a paucity of publications, all available studies were included. Therefore, the size of the patient groups, as well as the design of the study was emphasized.

Sperm analysis

Correlation of varicocele with sperm parameters

Several studies have shown a significant correlation between varicocele and poor sperm quality. Some show a correlation with all parameters, and others to a variety of different parameters only. A study conducted by the WHO in 1992 which included 9034 men from 34 centres in 24 countries who presented as a partner of the infertile couple, showed a decreased testis volume and lower total sperm count per ejaculate in those with varicocele (WHO, 1992). However, other sperm characteristics of motility and morphology were not influenced by the presence of

varicocele. The rate of spontaneous pregnancies was similarly frequent in couples in which the men did or did not have a varicocele. Similar results were presented in a prospective, controlled comparison of adolescents with or without varicocele (Haans *et al*., 1991). Sperm characteristics of motility and morphology did not differ, and only total sperm count and volume of the left testis were significantly lower among those with varicocele.

Recently, all sperm parameters were found to be significantly lower in 40 patients with varicocele than 40 fertile subjects (Villanueva-Diaz *et al*., 1999), but no correlation was found with the varicocele grade. Others (Chehval and Purcell, 1992) found a statistically significant deterioration of all sperm parameters of 13 men who presented initially with infertility, varicocele and normal spermiogram. Clearly, these types of study suffer from small participant numbers, which might limit the conclusions.

In a retrospective comparison (Naftulin *et al*., 1991), the sperm characteristics of 100 infertile patients with and without varicocele differed only in the percentage of tapered cells, which were observed significantly more frequently in cases with varicocele. Nevertheless, others disagreed with this finding. A prospective study was conducted where the sperm morphology of 56 men with varicocele, and 126 men without varicocele, was compared (Rodrigues-Rigau *et al*., 1981). No significant differences in any pattern of morphology, including tapered cells, was observed. Other studies have totally contradictory results. For example, in a prospective, longitudinal study (Lund and Larsen, 1998), men with untreated varicocele and a control group without varicocele, were reinvestigated 8 years later. There was a decline in sperm count in the control group, but almost no change in the men with varicocele. Moreover, there was no difference in sperm motility between the groups. It was suggested by others (Benoff *et al*., 1997) that infertility in patients with varicocele might be a manifestation of underlying defects in sperm structure and molecular function, such as defects in transition metal regulation or excessive cadmium exposure.

Changes in sperm parameters after varicocele treatment

Several studies reported an improvement in sperm parameters after varicocele treatment. In one study (Parikh *et al*., 1996), computer-assisted semen analysis (CASA) was used in 49 men before and after varicocelectomy; all sperm parameters of count, motility and morphology were improved significantly. Others (Pierik *et al*., 1998) reported both sperm count and progressive motility to be significantly improved after varicocele ligation in a retrospective study of 139 patients.

In a retrospective review of sperm motion changes measured by CASA in 34 men who underwent varicocele operation, only some of the parameters improved after treatment (Ismail *et al*., 1999). An increase in various sperm parameters was noted, but only progressive sperm velocity was increased to a statistically significant level after varicocele ligation.

The variability of these results might be explained on the basis of differences in group size, and also because of the variety of diagnostic evaluation testing of varicocele described in the studies. Another factor that might influence the results is clear from the outcome of another study where patients with varicocele were treated by three different methods (either embolization or two different operative techniques) (Yavetz *et al.*, 1992). With regard to sperm quality and pregnancy outcome, high ligation of the internal spermatic vein (the Ivanissevich technique) resulted in better results than low ligation or embolization.

A beneficial effect of early correction of varicocele in adolescents was demonstrated in a study comparing sperm parameters after 2–8 years of follow-up. All 19 patients selected for the study underwent left high ligation of the spermatic vein by the same team and technique. Sperm parameters of motility and morphology (but not concentration) were significantly higher in those who were operated on, and similar to a control group of healthy patients (Lenzi *et al.*, 1998).

Treatment of cases with subclinical varicocele is controversial. A partial beneficial effect on sperm parameters was demonstrated in infertile patients with subclinical varicocele in a randomized, prospective, controlled study (Yamamoto *et al*., 1994). A significant increase in sperm density and total motile count, but not in other sperm parameters, was recorded 1 year after operation.

In a unique study (Abdelrahim *et al*., 1993), bilateral testicular biopsies were taken from 30 men during and 3–6 months after operative treatment of varicocele. Reversal of various pathologies in testicular histology, such as sloughing of spermatogenic epithelium and increased prominence of the Leydig cells, were observed after the operation, together with an increase in spermatogenesis.

Frequently, only cases with impaired sperm parameters were considered as candidates for the treatment of varicocele. Recently, one group (Kim *et al*., 1999) reported their experience with azoospermic men who underwent varicocele repair. Some 43% of men had spermatozoa in their ejaculates after operative treatment. Since testicular biopsies were also performed in all cases, these results could be correlated with the results of testicular histology. Spermatozoa in the ejaculate appeared only in those presenting with hypospermatogenesis, or spermatogenic arrest at the spermatid stage, and not in cases with Sertoli cell-only or maturational arrest up to the spermatocyte stage.

In contrast, several studies showed there to be no benefit to sperm parameters after varicocele treatment. In one randomized, controlled study (Nilsson *et al*., 1979) there were no changes in any sperm parameters among 96 men with varicocele, and between operated and non-operated groups.

A comparison of three groups of infertile men showed that there was significantly better morphology in the group without varicocele (Gerhard *et al*., 1992). Nevertheless, when only those with varicocele were analysed, morphology characteristics were lower in the operated group. These results should be considered with caution, since the study contained no fertile control group, and the groups were not randomly chosen, while those with highly abnormal spermiogram were usually chosen for surgery.

Binding to human zona pellucida

A comparison was also made of the number of sperm cells which bind to human zona pellucida (ZP), among three groups: fertile; infertile without varicocele; and infertile with varicocele (Vigil *et al*., 1994). These authors reported that the varicocele group had a significantly lower number of sperm cells bound to the ZP than did the fertile group ($P < 0.05$), and a significantly higher number than the infertile group without varicocele ($P < 0.03$). These

results suggest that binding was impaired in infertile patients, while the presence of varicocele did not alter the mechanism of sperm binding to ZP.

The hemizona assay (HZA) also evaluates sperm binding capacity to ZP, compared with a control sperm sample taken from individuals with proven fertility, using the sibling hemizona of the same oocyte. HZA results were found to have a higher correlation with pregnancy achievement than that of sperm cell parameters, after varicocele treatment (Hauser *et al*., 1997). While sperm parameters improved in all patients, the HZA index improved only in those who achieved pregnancy. These results may explain why improvement in sperm parameters is not always correlated with improvement in fertility. It is possible that a larger-scale study might establish whether the HZA could be used as a predictive test for those who may benefit from varicocele repair.

Penetration into zona-free hamster oocytes

In a comparison of three patient groups (Vigil *et al*., 1994), the mean percentage of hamster zona-free oocytes penetrated by sperm cells was significantly lower in patients with varicocele than in infertile patients without varicocele ($P < 0.05$), or in a fertile control group ($P < 0.001$). These results suggest that the presence of varicocele alters gamete membrane fusion.

Reactive oxygen species (ROS)

The peroxidative damage of unsaturated fatty acids in sperm plasma membrane, induced by ROS, has been proposed as a major cause of defective sperm function in cases of male infertility. A correlation of varicocele with ROS level has been suggested by some authors. For example, significantly elevated ROS levels and depressed total antioxidant capacity levels were described in 21 infertile patients with varicocele, when compared with 17 control fertile patients (Hendin *et al*., 1999). However, a group of 15 fertile patients with incidental varicocele had similar ROS levels to those with varicocele-related infertility, and significantly lower total antioxidant levels compared with fertile controls without varicocele. These results suggest that ROS is associated with varicocele, but may not correlate with fertility.

Others (Weese *et al*., 1993) assessed spermatozoal ROS production with various stimulants, and also found an association with varicocele presence: fertile patients with varicocele showed significantly enhanced ROS generation compared with fertile donors without varicocele. Conversely, these authors also reported markedly increased ROS generation in infertile patients, compared with fertile men, suggesting a possible association of ROS with fertility.

Acrosome reaction

The acrosome reaction consists of fusion of the spermatozoon plasma membrane with the outer acrosomal membrane, and is an important indicator of the ability of the spermatozoon to penetrate the ZP. Human follicular fluid was found to induce the acrosome reaction significantly less frequently in infertile patients when compared with a control fertile group (Vigil *et al*., 1994). Nevertheless, the presence of varicocele in infertile patients did not influence the frequency of this process, suggesting the lack of any association of varicocele as a direct factor with the acrosome reaction.

Others (El Mulla *et al*., 1995) obtained similar results, having found no difference in the percentage of acrosome-reacted spermatozoa (either spontaneous or induced by ionophore) between cases with varicocele and fertile men. On the other hand, significantly better semen characteristics (concentration, motility and morphology) as well as enhanced acrosin activity were detected in the fertile group. Acrosin activity was determined by the percentage of spermatozoa with halo formations measured by gelatinolysis.

Hypo-osmotic swelling test (HOST)

The hypo-osmotic swelling test measures the functional competence of the sperm membrane. The percentage of swollen sperm cells was lower in 35 patients with varicocele, than in the controls (Fuse *et al*., 1991), suggesting that varicocele may cause injury to the sperm membrane. Others (Villanueva-Diaz *et al*., 1999) studied potentially functional spermatozoa per ejaculate and, in addition to HOST, evaluated progressive motility, acrosome integrity and the viability of sperm cells in 40 subfertile patients with varicocele, compared with 40 fertile men. The quality of spermatozoa in patients with varicocele was lower in all the above variables. A positive correlation was established between HOST and progressive motility ($r = 0.71$), and between HOST and total potentially functional spermatozoa per ejaculate (TPFS; an index of total count $\times$ % motility $\times$ normal morphology) ($r = 0.69$) in patients with varicocele, but not in fertile men. The authors concluded that varicocele causes deleterious effects by affecting sperm penetration and motility in the female tract, which in turn may affect gamete interaction.

In another study (Fuse *et al*., 1995), in which sperm swelling was measured before and after treatment for varicocele, a significant improvement was detected in a group of patients who achieved pregnancies, compared with those who failed to impregnate their partners.

Conclusions

Despite intensive studies carried out on varicocele in recent years, no conclusive evidence of the aetiology or the pathophysiology of the condition could be reached. Only recently, a theory was presented (Graif *et al.*, 2000) which proposed a 'nutcracker' phenomenon, caused by the narrow aortomesenteric angle in association with dilated left testicular veins, as a possible cause of varicocele.

As is evident from our review, it is difficult to compare the results of the studies dealing with varicocele for several reasons. First, the diagnostic tests of varicocele differ from one study to another, ranging from a physical examination based on palpation only (Nilsson *et al.*, 1979; Rodriguez-Rigau *et al*., 1981; Chehval and Purcell, 1992; Madgar *et al*., 1995) to isotope scanning (Yavetz *et al*., 1992) or ultrasound measurement of the dilated vessels (Parikh *et al*., 1996; Hauser *et al*, 1997; Pierik *et al*., 1998; Villanueva-Diaz *et al*., 1999).

Varicocele may be presented in various degrees of severity, ranging from subclinical to externally observed dilated vessels. The presence of retrograde flow during the Valsalva manoeuvre

was examined in only some of the studies. Furthermore, correction of varicocele was made using several different operative techniques, or by embolization (Yavetz *et al*., 1992; Comhaire *et al*., 1995).

Perhaps additional clues regarding the variability of results can be drawn from one study where only a short-term benefit of operative treatment on fertility was demonstrated (Madgar *et al.*, 1995).

In the current review we have focused our attention on the effect of varicocele on the functions of human sperm cells, and sperm characteristics. Most studies showed decreased sperm quality in patients with varicocele, and in some also of functions of the sperm cells. Nonetheless, the results in many of the aspects surveyed were contradictory. It is possible that, based on the studies published, HOST may serve as a predictive test for the beneficial effects of varicocele repair. However, these findings should be treated with caution until they can be confirmed by further studies.

These variable results, in addition to the finding that varicocele might be present in men with no apparent problems of infertility, raise the possibility that varicocele is a syndrome comprising of several pathogeneses. Another option is that an additional, underlying, pathology might be present and react with varicocele, thereby having a deleterious effect on either spermatogenesis or fertility potential. There is no doubt that large-scale, carefully designed and controlled studies must be conducted to elucidate the role of varicocele in infertility, and to clarify which sperm function tests are associated with reduced fertility potential so that those who may benefit from the correction of varicocele can be identified.

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