

Hypothesis: intracellular acidification contributes to infertility in varicocele

Kamyar Ghabili, M.D.,^a Mohammadali M. Shoja, M.D.,^a Paul S. Agutter, M.D.,^b and Ashok Agarwal, Ph.D., H.C.L.D.^c

^a Tuberculosis and Lung Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran; ^b Theoretical and Cell Biology Consultancy, Glossop, United Kingdom; and ^c Reproductive Research Center, Glickman Urological and Kidney Institute, Cleveland Clinic, Cleveland, Ohio

We suggest that varicocele leads to male factor infertility by a mechanism involving underperfusion of the testis, a shortfall in glucose supply to the tissue, decreased flux through the pentose phosphate pathway, lowering of the reduced nicotinamide–adenine dinucleotide phosphate/oxidized nicotinamide–adenine dinucleotide phosphate ratio and the supply of glutathione to the antioxidant systems, increased levels of reactive oxygen species, peroxidation of spermatozoon membrane lipids, and the consequent generation of acidic degradation products and sequestering of spermine. Acidification of the seminal plasma impairs sperm motility and also inhibits most antioxidant enzymes, exacerbating the accumulation of reactive oxygen species and the resultant lowering of pH. (Fertil Steril® 2008; ■: ■–■. ©2008 by American Society for Reproductive Medicine.)

Key Words: Infertility, varicocele, reactive oxygen species, pH

Varicocele, which is characterized by pathologic dilatation of the venous pampiniform plexus of the spermatic cord, accounts for nearly a third of all cases of male factor infertility (1, 2). The precise mechanism by which varicocele impairs male factor fertility remains uncertain; hypotheses implicating scrotal hyperthermia, retrograde flow of adrenal or renal metabolites, reactive oxygen species (ROS), and venous obstruction delineate current understanding of the pathophysiology (1, 3). Either a left or a right varicocele could cause bilateral testicular dysfunction (4). Various treatment options such as surgical or laparoscopic ligation of the spermatic veins (varicocelectomy) and different embolization techniques have been used to correct these lesions (5). Nonetheless, the management of varicocele continues to stimulate controversy among reproductive experts (6). A meta-analysis by Evers and Collins (7) of seven prospective randomized trials that evaluated varicocelectomy and pregnancy outcomes reported insufficient evidence to show that treatment of clinical varicocele improved the likelihood of conception for couples with male factor infertility; a recent study by Marmar et al. (8) casts doubt on this and indeed proves the opposite in certain cases. The continuing debate over the management of varicocele is probably fueled by the inadequacy of current etiologic hypotheses (6). There is a need for more molecular and genetic studies to clarify the pathophysiology of this condition. Novel treatment plans may be developed from such studies.

In this article, we propose that the impairment of fertility in varicocele may result from decreased pH in the spermatozoal

cytosol and the seminal plasma incident on local underperfusion. We briefly consider the implications of this proposal.

VARICOCELE AND RENAL/ADRENAL REFLUX

Because both the left testicular vein and left adrenal vein insert into the left renal vein, reflux from the kidney and/or adrenal gland may increase the concentrations of metabolic products from these organs in the testes of men with varicoceles. This may lead initially to chronic testicular vasoconstriction and ultimately to testicular toxicity (9, 10). Elevated catecholamine levels in refluxing testicular venous blood may lead to vasoconstriction of the intratesticular arterioles, contributing, along with impaired venous return caused by valve insufficiency (4, 9), to persistent testicular underperfusion (9, 11).

Clearly, such vascular changes diminish the oxygen supply to the cells (9, 11). Therefore, it might be expected that intracellular energy production would become increasingly dependent on anaerobic glycolysis, resulting in the accumulation of lactic acid and inorganic phosphate, hence reducing intracellular pH (12). However, laboratory studies failed to confirm the hypothesis that lactic acid accumulates in the testicular veins of patients with varicocele (13). Indeed, lactate produced by the somatic Sertoli cells is the central energy metabolite for the germ cells (14). Hence, although testicular energy metabolism is altered by the local ischemic effect of varicocele, lactate or pyruvate accumulation is not a factor in infertility. Nevertheless, experimentally induced varicocele does result in the acidification of testicular and epididymal fluids and a decrease in bicarbonate concentration (15). How is this to be explained, and is it clinically important?

The glycolytic pathway appears to be impaired in varicocele (13). The most plausible reason is that the local circulatory impairment diminishes the supply of glucose to the

Received March 30, 2008; revised May 15, 2008; accepted May 16, 2008. K.G. has nothing to disclose. M.M.S. has nothing to disclose. P.S.A. has nothing to disclose. A.A. has nothing to disclose.

Reprint requests: Kamyar Ghabili, M.D., Tuberculosis and Lung Diseases Research Center, Tabriz University of Medical Sciences, Daneshgah St., Tabriz, Iran (FAX: 984113363846; E-mail: kghabili@gmail.com).

metabolically active tissues; if the glucose supply becomes sufficiently low, then lactate and pyruvate will not accumulate despite the significant hypoxia. There is indirect support for this proposal: limited gluconeogenesis occurs in the testis (16), and varicocele results in an increased local production of urea (17), suggesting an increase in protein catabolism. Under conditions of low glucose supply, the flux through the pentose phosphate pathway, which is significant in normal germ cells as well as Sertoli cells (18), will be markedly decreased. Therefore, the reduced nicotinamide–adenine dinucleotide phosphate/oxidized nicotinamide–adenine dinucleotide phosphate (NADPH/NADP⁺) ratio will fall drastically. We suggest that this change in NADPH/NADP⁺ ratio is indirectly responsible for the tissue acidification that we believe provides the mechanistic link between varicocele and infertility.

VARICOCELE AND OXIDATIVE STRESS

Scrotal varicocele is associated with elevated spermatozoal ROS production and diminished seminal plasma antioxidant activity (19–21). Reactive oxygen species are metabolites of oxygen, including hydrogen peroxide and the superoxide and hydroxyl radicals (3). The human spermatozoon can generate ROS under physiologic conditions to mediate signal transduction, as well as to regulate the sperm function (10). On the other hand, like most cells, spermatozoa are equipped with antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, and catalase to detoxify harmful excessive ROS and to prevent cell injury (22). The antioxidant-rich secretions of the seminal vesicles, including both enzymatic and nonenzymatic antioxidant systems, also assist in protecting the spermatozoa from ROS (23).

Reduced glutathione (GSH) is an important component of antioxidant systems. When the NADPH/NADP⁺ ratio falls, as described above, GSH regeneration is retarded and the antioxidant capacity of the tissue is impaired. Under such pathologic circumstances, ROS production surpasses the antioxidant capacity and causes increased oxidative stress (10). In support of this account, exogenous glutathione is known to ameliorate the effects of varicocele and partially to restore fertility (24). Owing to the low amount of cytoplasm and abundance of polyunsaturated fatty acids in the plasma membrane of spermatozoa, spermatozoa are susceptible to damage by oxidative stress through ROS, especially lipid peroxidation (2, 25, 26). This may result in acidification by some or all of the following mechanisms.

Malondialdehyde levels increase markedly with varicocele (19, 21), indicating that lipid peroxidation is extensive, as predicted by the foregoing argument. Lipid peroxidation may lead to the production of free short-chain fatty acids. Malondialdehyde, also produced by the peroxidation of polyunsaturated fatty acids, may be oxidized to malonic acid, which potentially can be decarboxylated to acetate, as in the liver (27, 28). Polyamines, particularly spermine, are essential for spermatozoal activity (29). Malondialdehyde will react with spermine and other amines, forming Schiff bases.

This will further decrease the intracellular pH, as well as directly impairing spermine-dependent cellular functions. The optimum pH for ROS scavenging by the enzymatic antioxidant systems ranges between neutral and slightly alkaline (30). Thus, lowering of the pH markedly depresses the antioxidant enzyme activities, which indeed are significantly impaired in varicocele cases (31). Therefore, there is a directly proportional relationship between acidification and increasing ROS levels.

VARICOCELE AND SPERM FUNCTION

Pasqualotto et al. (1) reported that infertile patients with varicoceles have lower sperm concentration and motility than control subjects. A proposed mechanism by which sperm function becomes defective in men with varicocele is peroxidation of the unsaturated fatty acids in the sperm plasma membrane (10). In addition, the acrosome reaction that occurs during zona pellucida binding is thought to be impaired by the same ROS-induced defect (32). These are probably significant mechanisms, but we believe that the lowering of pH is a particularly important factor in male factor infertility.

It has long been known that sperm activity is enhanced in neutral and slightly alkaline media, for example, in ejaculated semen, but is markedly depressed in mildly acidic media (33). Surprisingly, Olmsted et al. (33) noted that spermatozoa are irreversibly immobilized after 15 minutes in mild acidity. Masters and Johnson (34) revealed that ejaculated semen acts as a potent alkaline buffer, abrogates vaginal acidity (pH 4.0–4.5) within seconds, and keeps the vagina neutral for several hours after intercourse. Moreover, they found that sperm in the vagina remained motile until the vagina became fully reacidified. In contrast, sperm in unusually nonalkaline semen will not be motile and therefore cannot evade the spermicidal activity of the vagina after ejaculation.

CONCLUSIONS

We propose that chronic vasoconstriction of the intratesticular arterioles and impaired venous return flow, resulting from varicocele, impairs glycolysis by limiting the glucose supply to the tissue. This also decreases the pentose phosphate pathway flux and therefore the provision of reductants to the antioxidant systems. Reactive oxygen species levels increase within the spermatozoa, and the polyunsaturated fatty acyl residues in the membrane lipid are peroxidized. This damages membrane function, including head and midpiece function, altering sperm morphology and impairing motility, but it also leads to a lower intracellular pH, partly though not exclusively because of malondialdehyde-mediated reactions. This acidification further impairs antioxidant activity and further diminishes sperm motility (33). Reduced sperm motility and impairment of zona pellucida binding may account for infertility in men with varicocele (35). Our proposal also seems consistent with the concomitant progressive effect of associated smoking and varicocele (36).

Because of individual differences, the mechanism we propose is not deterministic. If, for example, the glucose supply is less restricted or the accessory glands are particularly efficient, the accumulation of ROS and acidification of the seminal plasma could be ameliorated. Thus, fertility may be maintained in some cases of varicocele, even grade III. Nevertheless, the speculation offered in this article may elucidate the controversy over the outcomes of varicocelectomy. Ultimately, a surgical approach coupled with mild alkalization of both the intratesticular environment and the semen may lead to more successful outcomes regarding male fertility.

REFERENCES

- Pasqualotto FF, Lucon AM, de Góes PM, Sobreiro BP, Hallak J, Pasqualotto EB, et al. Semen profile, testicular volume, and hormonal levels in infertile patients with varicoceles compared with fertile men with and without varicoceles. *Fertil Steril* 2005;83:74–7.
- Chen SS, Huang WJ, Chang LS, Wei YH. Attenuation of oxidative stress after varicocelectomy in subfertile patients with varicocele. *J Urol* 2008;179:639–42.
- Mostafa T, Anis TH, Ghazi S, El-Nashar AR, Imam H, Osman IA. Reactive oxygen species and antioxidants relationship in the internal spermatic vein blood of infertile men with varicocele. *Asian J Androl* 2006;8:451–4.
- Gat Y, Zukerman Z, Chakraborty J, Gornish M. Varicocele, hypoxia and male infertility. Fluid mechanics analysis of the impaired testicular venous drainage system. *Hum Reprod* 2005;20:2614–9.
- Hauser R, Paz G, Yogev L, Yavetz H. Varicocele: effect on sperm functions. *Hum Reprod Update* 2001;7:482–5.
- Marmar JL. The pathophysiology of varicoceles in the light of current molecular and genetic information. *Hum Reprod Update* 2001;7:461–72.
- Evers JL, Collins JA. Assessment of efficacy of varicocele repair for subfertility: a systemic review. *Lancet* 2003;361:1849–52.
- Marmar JL, Agarwal A, Prabakaran S, Agarwal R, Short RA, Benoff S, et al. Reassessing the value of varicocelectomy as a treatment for male subfertility with a new meta-analysis. *Fertil Steril* 2007;88:639–48.
- Gat Y, Gornish M, Navon U, Chakraborty J, Bachar GN, Ben-Shlomo I. Right varicocele and hypoxia, crucial factors in male infertility: fluid mechanics analysis of the impaired testicular drainage system. *Reprod Biomed Online* 2006;13:510–5.
- Naughton CK, Nangia AK, Agarwal A. Pathophysiology of varicoceles in male infertility. *Hum Reprod Update* 2001;7:473–81.
- Forti G, Krausz C, Cilotti A, Maggi M. Varicocele and infertility. *J Endocrinol Invest* 2003;26:564–9.
- Hou ST, MacManus JP. Molecular mechanisms of cerebral ischemia-induced neuronal death. *Int Rev Cytol* 2002;221:93–148.
- Girgis SM, Abd el-Rahman Y, Awad H, Eisa I, Younan N, Mittawy B, et al. Lactate and pyruvate levels in the testicular vein of subfertile males with varicocele as a test for the theory of underlying hypoxia. *Andrologia* 1981;13:16–9.
- Boussouar F, Benahmed M. Lactate and energy metabolism in male germ cells. *Trends Endocrinol Metab* 2004;15:345–50.
- Caffisch CR. Acidification of testicular and epididymal fluids in the rat after surgically-induced varicocele. *Int J Androl* 1992;15:238–45.
- Yáñez AJ, Bustamante X, Bertinat R, Werner E, Rauch MC, Concha II, et al. Expression of key substrate cycle enzymes in rat spermatogenic cells: fructose 1,6 bisphosphatase and 6 phosphofructose 1-kinase. *J Cell Physiol* 2007;212:807–16.
- Adamopoulos DA, Kontogeorgos L, Abrahamian-Michalakis A, Terzis T, Vassilopoulos P. Raised sodium, potassium, and urea concentrations in spermatic venous blood: an additional causative factor in the testicular dysfunction of varicocele? *Fertil Steril* 1987;48:331–3.
- Ito M, Nakaoka Y, Watanabe H, Amano H. Presence of dehydrogenases of the pentose phosphate pathway in boar spermatozoa and testis. *Nippon Sanka Fujinka Gakkai Zasshi* 1987;39:195–201.
- Mostafa T, Anis TH, El-Nashar A, Imam H, Othman IA. Varicocelectomy reduces reactive oxygen species levels and increases antioxidant activity of seminal plasma from infertile men with varicocele. *Int J Androl* 2001;24:261–5.
- Yeşilli C, Mungan G, Seçkiner I, Akduman B, Açıkgöz S, Altan K, et al. Effect of varicocelectomy on sperm creatine kinase, HspA2 chaperone protein (creatine kinase–M type), LDH, LDH-X, and lipid peroxidation product levels in infertile men with varicocele. *Urology* 2005;66:610–5.
- Köksal IT, Tefekli A, Usta M, Erol H, Abbasoglu S, Kadioglu A. The role of reactive oxygen species in testicular dysfunction associated with varicocele. *BJU Int* 2000;86:549–52.
- Suleiman SA, Ali ME, Zaiu ZMS, El-Malik EMA, Nasr MA. Lipid peroxidation and human sperm motility: protective role of vitamin E. *J Androl* 1996;17:530–7.
- Werthman P, Wixon R, Kasperon K, Evenson DP. Significant decrease in sperm deoxyribonucleic acid fragmentation after varicocelectomy. *Fertil Steril*. Published online June 11, 2007.
- Lenzi A, Picardo M, Gandini L, Lombardo F, Terminali O, Passi S, et al. Glutathione treatment of dyspermia: effect on the lipoperoxidation process. *Hum Reprod* 1994;9:2044–50.
- Gonzales GF. Function of seminal vesicles and their role on male fertility. *Asian J Androl* 2001;3:251–8.
- Zalata AA, Ahmed AH, Allamaneni SS, Comhaire FH, Agarwal A. Relationship between acrosin activity of human spermatozoa and oxidative stress. *Asian J Androl* 2004;6:313–8.
- Siu GM, Draper HH. Metabolism of malonaldehyde in vivo and in vitro. *Lipids* 1982;17:349–55.
- Davydov VV. Pathways of endogenous malonate formation in the rat liver. *Ukr Biokhim Zh* 1993;65:85–8.
- Atmar VJ, Kuehn GD, Casillas ER. A polyamine-dependent protein kinase from bovine epididymal spermatozoa. *J Biol Chem* 1981;256:8275–8.
- BRENDA, The Comprehensive Enzyme Information System, <http://www.brenda-enzymes.org/>. Accessed 14 June 2008.
- Barbieri ER, Hidalgo ME, Venegas A, Smith R, Lissi EA. Varicocele-associated decrease in antioxidant defenses. *J Androl* 1999;20:713–7.
- Vigil P, Wöhler C, Bustos-Obregón E, Comhaire F, Morales P. Assessment of sperm function in fertile and infertile men. *Andrologia* 1994;26:55–60.
- Olmsted SS, Dubin NH, Cone RA, Moench TR. The rate at which human sperm are immobilized and killed by mild acidity. *Fertil Steril* 2000;73:687–93.
- Masters WH, Johnson VE. The physiology of the vaginal reproductive function. *Western J Surg Obstet Gynecol* 1961;69:105–20.
- Cedenho AP, Spaine DM, Barradas V, Srougi M, Oehninger S. Adolescents with varicocele have an impaired sperm–zona pellucida binding capacity. *Fertil Steril* 2002;78:1339–40.
- Klaiber EL, Broverman DM, Pokoly TB, Albert AJ, Howard PJ Jr, Sherer JF Jr. Interrelationships of cigarette smoking, testicular varicoceles, and seminal fluid indexes. *Fertil Steril* 1987;47:481–6.