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Varicocele and male infertility: Part II

Pathophysiology of varicoceles in male infertility

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Varicoceles are found in 19 to 41% of infertile men, and is one treatable form of male infertility. The mechanism by which varicoceles cause the variable effect on male infertility and spermatogenesis is still unknown. Experimental animal models play a useful (but limited) role due to the sudden and variable iatrogenic nature of the varicoceles and the duration of the studies. Much of the human data are derived by the characterization of associated differences in measurable parameters between men with and without varicoceles. The role of hyperthermia, testicular blood flow and venous pressure changes, reflux of renal/adrenal products, hormonal dysfunction, autoimmunity, defects in acrosome reaction, and oxidative stress, in the pathophysiology of varicocele will be discussed.

Key words: infertility/pathophysiology/spermatozoa/varicocele

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Introduction

Varicoceles are pathological dilations of the venous pampiniform plexus of the spermatic cord, and occur more frequently on the left side. The aetiology of varicoceles is controversial, with theories ranging from anatomical variations, to venous reflux secondary to congenital and/or acquired valve dysfunction, to venous obstruction. There are three accepted theories on the causes of varicoceles. First, that there are anatomical differences between the left and right testicular veins; specifically, that the right testicular vein inserts directly into the inferior vena cava, while the left testicular vein inserts into the left renal vein. The different insertion of the left testicular vein is believed to result in an increase in hydrostatic pressure, which is subsequently transmitted to the pampiniform plexus, causing dilatation and tortuosity of the veins. Second, that there is an absence of competent venous valves, resulting in reflux of venous blood. The venographic pattern of 659 consecutive men with varicoceles showed that 73% of these men had absent venous valves (Braedel *et al.*, 1994). Third, that there is partial obstruction of the testicular vein caused by compression of the left renal vein between the aorta and the superior mesenteric artery, or the 'nutcracker effect'. It is likely that the aetiology is multifactorial.

The incidence of varicoceles in the general population is approximately 15%, while 19 to 41% of men presenting for infertility investigation demonstrate varicoceles (Hendry *et al.*, 1973; Cockett *et al.*, 1984). Correction of varicoceles in infertile men has demonstrated improved semen parameters in 50–80% of patients (Lome and Ross, 1977; Marks *et al.*, 1986), pregnancy rates of 31–71% (Scott and Young, 1962; Madgar *et al.*, 1995) and, most recently, increased per cycle pregnancy and live birth rates with intrauterine insemination (Daitch *et al.*, 2000). Nonetheless, the treatment of varicoceles is highly controversial, as not all men with varicoceles are infertile. Interestingly, the varicocele incidence in that subset of men with secondary infertility (previously fertile, but now infertile) is about 70–80% (Dubin and Amelar, 1977; Gorelick and Goldstein, 1993; Witt and Lipshultz, 1993). These data suggest that men with prior fertility may suffer from varicocele-mediated secondary inferti-

lity, and that the presence of a varicocele may cause a progressive decline in fertility. No varicoceles were detected in 188 boys aged 6–9 years, but they were detected with increasing incidence in boys aged 10–14 years (Oster, 1971), which suggests that varicoceles develop at puberty. The mechanisms by which the spectrum of varicocele grade and age of onset correspond to the variable and progressive spermatogenic dysfunction are still unknown.

This review will outline the proposed hypotheses and supporting data, including hyperthermia, testicular blood flow and venous pressure changes, reflux of renal/adrenal products, hormonal dysfunction, autoimmunity, defects in acrosome reaction, and oxidative stress, that shapes our present understanding of the pathophysiology of the varicocele.

Materials and methods

For each section of this review, a systematic Medline search was conducted inclusive of all dates using the keywords 'control' and 'varicocele' and section heading, i.e. 'hyperthermia'. Since controlled, animal studies and prospective, randomized clinical trials are regarded as the most reliable method of proving the effectiveness of diagnostic and therapeutic strategies, this review will focus on presentation of these selected papers. Although every section of this review will focus on the data from controlled investigations, it is important to stress from the onset, that even controlled studies from animal models carry limitations. Specifically, due to the sudden iatrogenic nature of the varicoceles created, these animal reports may not truly represent the gradual and continuous effect of varicoceles on human spermatogenesis. Despite these limitations, controlled animal studies are useful, in that they allow direct studies targeted at the mechanism(s) of varicocele pathophysiology. Whilst all animal studies worth mentioning should be controlled, unfortunately it is more difficult to conduct prospective controlled studies involving humans. If no data from controlled trials are available, then data from uncontrolled trials will be mentioned. Each section will begin with perspective on the theory behind each mechanism, followed by a discussion of the pertinent animal studies germane to the topic, and conclude with the human clinical investigations, that have contributed to our present understanding of the pathophysiology of varicoceles in male infertility.

Varicoceles and hyperthermia

The relationship of increased intrascrotal temperature and varicoceles, in association with decreased sperm parameters, has been investigated since the 1960s. Scrotal temperature is physiologically maintained at less than body temperature, and is crucial for the normal countercurrent heat-exchange system first postulated in 1959 (Dahl and Herrick, 1959). The thinking then was—and still is now—that the inflowing core temperature of spermatic arterial blood is cooled by the outflowing cooler venous blood of the pampiniform plexus. Varicosities of the plexus may decrease the effectiveness of testicular cooling. In reviewing the literature on varicoceles and hyperthermia, it is necessary to discern whether investigators are measuring intrascrotal or intratesticular temperatures.

To our knowledge, no animal studies have been performed to determine the relationship between experimental varicocele and intrascrotal temperatures.

In humans, the data are conflicting. In one study, a 0.4–0.5°C increase in scrotal temperatures in both the right and left hemiscrotum of fertile versus infertile non-azoospermic men was noted and, as expected, the increased scrotal temperature was associated with compromised sperm characteristics. However, when comparing infertile men with and without varicocele, there was no difference in scrotal temperature (Mieusset *et al.*, 1987). Similarly, in another study, although varicocele was associated with impaired sperm quality compared with control subjects, there was no difference in scrotal skin temperature in men with or without varicocele (Lund and Nielsen, 1996).

In contrast, the bilateral scrotal surface temperatures of anaesthetized infertile men with varicocele were found to be elevated compared with those in control subjects when using a surface probe (35–36°C versus 33°C) (Goldstein and Eid, 1989). These results were remarkably similar to those obtained in a study conducted more than 15 years earlier, which also showed a bilateral increase in scrotal temperature using a water-bath thermometer placed against the anterior surface of the scrotum (Zorgniotti and MacLeod, 1973). Using a miniaturized portable digital data recorder for the continuous determination of scrotal temperatures, two studies reported very wide variation in scrotal temperature during a 24 h period in normal men, depending upon their activity. Minimal scrotal temperatures were increased in some men with varicocele compared with normal fertile men (Jockenhovel *et al.*, 1990). However, although sperm counts increased after varicocele ligation or embolization, there was no change in scrotal temperature (Lerchl *et al.*, 1993).

In the adolescent population, patients with a palpable grade II–III varicocele had significant bilateral elevation of the scrotal temperatures compared with the control subjects in both supine and standing positions. Curiously, in adults, standing caused a decrease in scrotal temperatures bilaterally in both the varicocele and control populations (Zorgniotti and MacLeod, 1973; Goldstein and Eid, 1989), while in adolescents, standing increased the scrotal temperature only unilaterally, and only in varicocele patients compared with controls. Those adolescent patients who, in the standing position, could not maintain a left scrotal temperature at least 1.4°C cooler than axillary temperature were noted to have significant left testicular volume loss. Following successful varicocele surgery, left scrotal temperatures were significantly cooler, and left testicular volumes significantly improved (Salisz *et al.*, 1991).

Unlike intrascrotal temperature studies, several animal studies have been conducted to investigate intratesticular temperatures, using both dogs and rats. These studies demonstrate a clear increase in bilateral intratesticular temperature from unilateral iatrogenic varicocele formation by partial ligation of the renal vein or surgical valve destruction of the testicular vein (Saypol *et al.*, 1981), or by excision of a longitudinal strip of the fasciomuscular tube of the spermatic cord (Shafik *et al.*, 1989). More convincing evidence of the causal relationship between varicocele and temperature is the normalization of elevated intratesticular temperature following varicocele repair in rats (Green *et al.*, 1984) and rabbits (Sofikitis and Miyagawa, 1992). Increased intratesticular temperature is associated with abnorm-

alities in spermatogenesis by testicular histology in some animals (Saypol *et al.*, 1981; Shafik *et al.*, 1989), and with decreased ejaculated sperm quality (Shafik *et al.*, 1989; Sofikitis and Miyagawa, 1992). Unfortunately, these end-points were not examined in the varicocele repair experiments.

In humans, direct intratesticular temperatures were measured in one study using a 29-gauge needle thermistor inserted 1 cm into the testicular substance on the anterior surface; temperatures were found to be significantly elevated bilaterally in association with unilateral varicocele (34–35°C versus 32°C) (Goldstein and Eid, 1989). In this same study, the investigators measured both intrascrotal and intratesticular temperatures, and found that indeed these correlated. Hence, at least in this study, the intrascrotal temperature was reflective of intratesticular temperature. Remarkably similar to animal experiments, bilaterally elevated intratesticular temperatures prior to varicocele repair were restored in control patient levels following unilateral or bilateral varicocelectomy (Wright *et al.*, 1997).

Although the data concerning the direct correlation between varicoceles and scrotal hyperthermia are conflicting, both animal models and most human studies clearly demonstrate elevated intratesticular temperatures associated with varicoceles. The power of this correlation rests on the observation that varicocele repair successfully decreased intratesticular temperatures to control levels in both animal and human studies. The mechanism by which heat affects spermatogenesis is unclear. Direct thermal damage to nuclear RNA binding proteins and DNA at the level of the seminiferous tubules and/or Leydig cells is suspected (Nakamura *et al.*, 1987; Fujisawa *et al.*, 1989; Nishiyama *et al.*, 1998). However, high temperature does not appear to disturb Leydig and Sertoli cell function, at least in the short term, as measured by LH and FSH binding sites and testosterone production in human testis *in vitro* (Namiki *et al.*, 1987). The specific activities of DNA polymerases α, β and γ have been reported to be reduced bilaterally by 50% in the testes of infertile men with unilateral varicoceles versus normal testes of control patients without varicoceles (Fujisawa *et al.*, 1989).

Varicoceles and testicular blood flow

The role of testicular blood flow in varicocele pathophysiology is closely integrated with the theories regarding hyperthermia and is, therefore, equally controversial.

Animal studies report both increases and decreases in testicular blood flow in association with varicocele. Testicular blood flow was increased in experimentally induced varicocele in adult rats and dogs (Saypol *et al.*, 1981), and returned to control levels following varicocele repair in rats in the short term (Green *et al.*, 1984) and long term (Hurt *et al.*, 1986). Several investigators have shown that a bilateral increase in testicular blood flow occurs in the presence of a unilateral varicocele (Green *et al.*, 1984; Turner and Lopez, 1990; Turner *et al.*, 1993). The aetiology for this remains elusive. Contralateral organs may respond to an ipsilateral insult due to either hormonal or neural mechanisms. An elegant study looked at the effect of right blood flow after left varicocele formation and left orchiectomy. Right testicular blood flow, or contralateral testicular blood flow, still increased despite left orchiectomy. Therefore, it does not appear that the bilateral effect is due to any hormonal signal from the left testicle in the case of left varicocele formation (Hurt *et al.*, 1986). The role of baroreceptors or stretch receptors in the left spermatic vein or another type of neural/non-neural feedback mechanism is unknown. Interestingly, the bilateral increased blood flow was greater in younger rats versus older ones with unilateral varicoceles; however, how this correlates with age of onset of varicoceles and the infertile state remains unknown (Turner and Lopez, 1990).

Studies demonstrating decreased testicular blood flow in rats following experimental varicocele by partial ligation of the left renal vein measured at 30 min (Li *et al.*, 1999), and at 4 weeks (Hsu *et al.*, 1994) are also available. Experimental varicoceles in primates resulted in a decrease in blood flow at 4–5 months (Harrison *et al.*, 1983), but this returned to normal over a 2-year period (Harrison *et al.*, 1986).

Overall, documented changes in testicular blood flow with experimental varicocele in animal studies are conflicting. The method of measuring blood flow, variable effects on the types of animals used, and the duration of the created varicocele studied may partly explain the controversy.

Studies in humans are less controversial in that the testicular blood flow of men with varicoceles was shown, using colour duplex ultrasonography, to be not significantly different from that in control subjects without varicoceles (Ross *et al.*, 1994; Grasso *et al.*, 1997). However, these techniques cannot be used to study the testicular microcirculation, despite an ability to assess the spermatic cord vessels. Improved approaches to study the microcirculation using power Doppler or other more sophisticated technologies may soon be possible.

Although the direction of testicular blood flow change in association with varicocele is unclear, it is important to recognize that an increase in testicular blood flow is more consistent with an increase in testicular temperature.

Varicoceles and venous pressure

The existence and mechanism of venous pressure changes associated with varicoceles has long been debated, and reflects the controversy that exists regarding the pathogenesis of varicoceles. Increased venous tension may affect the blood supply and microvasculature of the testis, by down-regulating arterial inflow, to maintain the homeostasis of the intratesticular pressure (Sweeney *et al.*, 1991, 1995).

Direct measurements of intravascular pressures in microvessels present on the subcapsular surface of the hamster testis disclosed that testicular capillary pressure is very low, and is regulated by the arterial side of the vascular network. The distribution of vascular resistance indicates that capillary pressure may be extremely sensitive to increases in venous pressure in the hamster model (Sweeney *et al.*, 1991). Over 90% of venous pressure elevation caused by ligating collateral routes of venous outflow and partially occluding the main venous outflow distal to the pampiniform plexus was transmitted to the postcapillary venules. The chronic precapillary vasoconstriction may have a deleterious effect on the 'nutrient supply' to the testicle, and may consequently affect spermatogenesis. Additionally, the increased venous pressure may lead to changes in intratesticular oncotic and hydrostatic pressures and change the transport/paracrine environment of key hormones, and may also alter microvascular fluid

exchange. Evidence that this notion may be correct is the decreased adenine nucleotide concentrations (Hsu *et al.*, 1994) and reduced nicotinamide adenine dinucleotide-cytochrome *c* reductase activities in varicocele-bearing rat testicles compared with those in sham-operated rats (Hsu *et al.*, 1995). These studies suggest defective mitochondrial oxidative phosphorylation, or defective energy metabolism, in varicocele-induced testicles.

The normal resting venous tension of a spermatic cord vein was measured by introducing a needle attached to a saline manometer into the anterolateral aspect of the scrotum directly into a pampiniform vein in humans (Shafik and Bedeir, 1980). Patients with varicoceles demonstrated a mean increase in ipsilateral venous pressure of 19.7 mmHg within the pampiniform plexus compared with control subjects. The pressure difference further increased with the Valsalva manoeuvre to 22 mmHg. Despite this, 18 of 32 patients with varicoceles had normal semen parameters, which questions the direct association between increased venous pressure, varicoceles and impaired spermatogenesis.

Nevertheless, the natural subsequent investigation of measuring the pressure following varicocelectomy was performed in a follow-up study by the same investigators. Indeed, 88% of 60 patients after varicocelectomy demonstrated decreased venous tension following surgery, though the duration of the follow-up period was not mentioned. Forty-two (70%) of the original patients demonstrated both decreased venous tension and improved semen parameters, but only 32% of the original group showed decreased venous tension, improved semen parameters, and produced a natural pregnancy. In the subgroup of patients who could demonstrate a decrease in venous tension, there was a significant difference in the improvement in sperm motility when comparing the group that was able naturally to conceive compared with the group that could not conceive (Shafik, 1983).

Varicoceles and renal/adrenal reflux

Some 50% of men have retrograde flow in the left spermatic vein (Ahlberg *et al.*, 1966). In patients with varicoceles, an increase in venous reflux, documented by venography, is observed (Comhaire and Kunnun, 1976; Comhaire *et al.*, 1981). Here, the hypothesis is that reflux of metabolic products from the kidney and/or adrenal gland (e.g. catecholamines) are present in higher concentrations in men with varicoceles, and that this may lead initially to chronic testicular vasoconstriction and ultimately be toxic to testicular function.

This hypothesis has not been confirmed in animal models. When labelled microspheres were infused into the left renal vein, they did not appear in either the left or right testes of animals which had undergone experimental left varicocele formation (Turner and Lopez, 1989). Further, left adrenalectomy did not rescue the increased testicular temperature, reduction in fertility, decreased epididymal spermatozoal content and motility or reduced testicular weight of Wistar rats 12 weeks following varicocele formation (Sofikitis and Miyagawa, 1993).

The role of refluxing adrenal steroids as a cause of decreased spermatogenesis in men with varicoceles has been suggested (MacCleod, 1965). In humans, the mean concentration of catecholamines in refluxing testicular venous blood obtained during surgery compared with peripheral blood was about 3-fold higher, while in control subjects the difference was only about 1.5-fold higher (Comhaire and Vermeulen, 1974). Elevated catecholamine concentrations may be exchanged from the veins to the testicular artery at the level of the pampiniform plexus via the countercurrent exchange mechanism, resulting in increased testicular artery noradrenaline concentrations, and causing vasoconstriction of the intratesticular arterioles; contributing to testicular hypoxia. However, measurement of other adrenal products such as cortisol and dihydroepiandrosterone in the spermatic vein compared with peripheral blood in infertile patients with varicoceles have not revealed any differences (Steeno *et al.*, 1976; Sayfan and Adam, 1978).

Varicoceles and hormonal dysfunction

The finding of reduced serum testosterone concentrations in infertile patients with varicoceles has led to the hypothesis that varicoceles result in Leydig cell dysfunction, and subsequently to decreased testosterone production.

Experimental varicocele in dogs caused decreased serum testosterone and increased prolactin concentrations, as assessed by radioimmunoassay, at 8 weeks (Shafik *et al.*, 1989). Bilateral reductions in intratesticular testosterone concentrations were seen following left varicocele induction in rats (Turner *et al.*, 1990), while another study demonstrated only ipsilateral intratesticular testosterone reduction (Ghosh and York, 1994). Using a rat unilateral varicocele model, an equal and bilateral decrease in intratesticular testosterone, as well as two enzymes involved in testosterone biosynthesis (17,20-desmolase and 17α-hydroxylase), was observed (Rajfer *et al.*, 1987). These data suggest that decreased serum testosterone concentrations observed in varicocelized animals may be explained by defective testosterone synthesis. Alternatively, the mechanism of decreased intratesticular testosterone in varicocele may be partly explained by the attenuated response of serum testosterone after human chorionic gonadotrophin (HCG) stimulation in rabbits (Sofikitis and Miyagawa, 1994) and the decreased binding of HCG to Leydig cells in varicocelized testes in rats (Kazama, 1995).

A World Health Organization (WHO) multicentre study on the influence of varicocele on fertility parameters demonstrated that the mean testosterone concentration of men aged over 30 years and with varicoceles was significantly lower than that of younger patients with varicoceles, whereas this trend was not observed in men without varicoceles (WHO, 1992). These findings suggest a detrimental, time-dependent effect of varicocele on Leydig cell function.

Normally, peripheral testosterone concentrations show a biphasic response to HCG, with an initial peak at 1–4 h and a second peak at 36–96 h. One study showed that the early peak of testosterone may be blunted in men with varicocele, suggesting that there is an enzymatic impairment in testosterone biosynthesis, possibly caused by a block at the level of 17,20-lyase, based on accumulation of upstream concentrations of 17α-hydroxyprogesterone in varicocele patients following stimulation with HCG (Scholler *et al.*, 1984). Lower concentrations of circulating free testosterone, higher oestradiol, and higher steroid-binding globulin levels are observed in varicocele patients compared with control subjects (Hudson, 1996). Taken together, these data suggest that altered free sex steroid concentrations may be the result of a subtle, intrinsic defect in the testes of some men with

varicoceles. However, whether this endocrinopathy is the cause or the effect of decreased spermatogenesis in varicocele patients is unclear. Despite the statistically significant decrease in testosterone production in some varicocele patients reported by many investigators, the actual values are within normal limits. This finding may be due to Leydig cell hyperplasia which compensates for decreased testosterone production per Leydig cell (Sirvent *et al.*, 1990; Su *et al.*, 1995). Other investigators have reported no significant difference in FSH, LH, testosterone and oestradiol concentrations in both peripheral and testicular venous blood in men with and without varicoceles (Swerdloff and Walsh, 1975; Schiff *et al.*, 1976; Hudson and McKay, 1980; Hudson *et al.*, 1981). Furthermore, reversibility of the hormonal dysfunction by varicocelectomy remains controversial. No significant difference in pre- and postoperative testosterone concentrations have been shown by several investigators (Hudson *et al.*, 1985; Segenreich *et al.*, 1986). Others have reported a significant change in serum testosterone concentrations, especially in patients with low preoperative values (Comhaire and Vermeulen, 1975; Su *et al.*, 1995).

Other studies suggest that the gonadotrophin response to gonadotrophin-releasing hormone (GnRH) stimulation is a more sensitive test of Leydig cell function than HCG stimulation. Men with varicoceles were shown to have an excessive response, in terms of LH and FSH release, to a 4 h infusion of GnRH (Hudson and McKay, 1980). The magnitude of the response was also greater in severely oligozoospermic men than in those with sperm concentrations between 11 and 30×10^6/ml. More importantly, the men with exaggerated gonadotrophin response to GnRH were most likely to show improvement in semen parameters following varicocelectomy, regardless of the degree of oligozoospermia. Others (Fujisawa *et al.*, 1994) demonstrated that a normalization of the LH response to GnRH stimulation after varicocelectomy was predictive of improved fertility postoperatively; that is, the normalization correlated with higher pregnancy rates.

These data suggest that varicoceles affect the hypothalamic–pituitary–gonadal axis, and that men with varicocele and abnormal Leydig cell function may be the subgroup most likely to benefit from varicocelectomy.

Varicoceles and autoimmunity

The blood–testis barrier and immunoregulatory proteins at the level of the Sertoli cells, rete testis and efferent ductules, provide immunological protection of sperm antigens and inhibit lymphocyte proliferation and complement-mediated cell lysis (Furuya *et al.*, 1978). Disruption of this blood–testis barrier is believed to result in the production of antisperm antibodies. The proposed aetiologies for such disruption include ductal obstruction, testicular torsion, infection/epididymitis, prostatitis, testicular trauma and varicocele (Jarrow and Sanzone, 1992). Although testicular biopsies of healthy men with varicoceles revealed intact Sertoli cell–Sertoli cell junctions, fertility status was not addressed (Cameron and Snyder, 1980). The mechanism by which varicoceles induces an antisperm antibody effect without damage to the blood–testis barrier remains unclear (Turner *et al.*, 1987). Nevertheless, in a rat model, an experimentally induced varicocele demonstrated significantly higher antisperm antibody levels than in sham and non-operated rats (Shook *et al.*, 1988).

The remaining available literature on varicocele and autoimmunity rely upon data from human observations. The prevalence of antisperm antibodies in the general male population has been reported to be 0–2% (Haas *et al.*, 1980; Knudson *et al.*, 1994), while the prevalence among unselected infertile men is reported to be 3–12% (Hendry *et al.*, 1977; Moghissi and Thomas, 1990). Antisperm antibodies have been shown to cause agglutination and immobilization of spermatozoa, sperm cytotoxicity, impairment of sperm penetration into the cervical mucus, prevention of capacitation or the acrosome reaction in response to zona pellucida, and enhanced phagocytosis of spermatozoa by macrophages (Bronson *et al.*, 1984; Haas, 1986). The association between varicoceles and antisperm antibodies is conflicting, and depends largely upon the parameters and methodology used by investigators, i.e. a direct immunobead technique to evaluate antibodies directed against the spermatozoon itself, or the enzyme-linked immunosorbent assay (ELISA) technique to evaluate antibodies directed against the seminal plasma (Table I). If the antibody levels are analysed on spermatozoa and in seminal plasma separately, there is no significant difference

Table I. Incidence of antisperm antibodies in varicocele patients versus controls

Reference	Fluid type	Method	Positive antibody (%)		Antibody type (%)
			Control	Varicocele	
Witkin and Toth (1983)	SP	ELISA	5[a]	30	30 IgG; 10 IgA; 20 IgM
Golomb *et al.* (1986)	'Total'	ELISA	41[a]	91	–
	Serum only	–	14	13	41 IgG; 47 IgA; 31 IgM
	Sperm only	–	5	3	6 IgG; 34 IgA; 31 IgM
Gilbert *et al.* (1989)	Sperm	ELISA	–	32	67 IgG; 85 IgA; 74 IgM
Jarrow *et al.* (1992)	Sperm	Immunobead	9[a]	13	–
Oshinsky *et al.* (1993)	Sperm	Immunobead	11[a]	17	18 IgG; 10 IgA
Knudson *et al.* (1994)	Sperm	Immunobead	0	28	100 IgG; 86 IgA; 43 IgM

[a]Controls are infertile men without varicoceles.
ELISA = enzyme-linked immunosorbent assay; SP = seminal plasma.

between infertile men with or without varicoceles; however, if all antisperm antibody levels in these fractions are combined, then 91% of infertile men with varicoceles had antibodies compared with 41% of infertile men without varicoceles (Golomb *et al.*, 1986) (Table I). The significance of antibody levels directly on the spermatozoa versus those levels in seminal plasma, remains unknown. These data suggest that infertile men have a higher incidence of antisperm antibodies than the fertile male population, and that infertile men with varicoceles have a similar percentage of antisperm antibodies as infertile men without varicoceles.

Varicoceles and the acrosome reaction

The role of varicoceles in unexplained normospermic (idiopathic) male infertility has been investigated. The defect is believed to exist in sperm function rather than morphology or quantity, and is also thought to involve the acrosome reaction during zona pellucida binding (Rogers *et al.*, 1985; Vigil *et al.*, 1994). The mannose binding assay may be used to determine acrosomal activity (Benoff *et al.*, 1993). Polyvalent mannose ligands act as zona pellucida glycoprotein agonists in the presence of free mannose, which rapidly induce acrosome exocytosis in fertile motile spermatozoa after in-vitro capacitation (Benoff *et al.*, 1996). To our knowledge, no animal studies regarding varicoceles and the acrosome reaction have been published.

Patients with varicoceles and idiopathic infertility express mannose ligand receptors equivalent to fertile donors, but spermatozoa fail to undergo the acrosome reaction in response to mannose treatment, and are subsequently unable to penetrate the zona pellucida. In the same study, no improvement in the acrosome reaction was noted post-varicocelectomy; however, the number of varicocelectomy patients studied was too small to draw any definitive conclusions and the postoperative follow-up period prior to performing the mannose binding assay was not mentioned (Benoff *et al.*, 1996).

A relationship exists between the distribution of sperm surface mannose binding sites and anti-myosin antibody binding, as demonstrated by immunohistochemistry. Anti-myosin antibody reactivity also correlates with the state of the acrosome (Benoff *et al.*, 1995, 1996). Spermatozoa from varicocele patients demonstrate a full complement of myosin-like epitopes, which suggests that the defect in patients with varicoceles and idiopathic infertility occurs in the mechanisms regulating signal transduction or calcium ion influx (Benoff *et al.*, 1994, 1995). Co-factors, such as trace metals, may contribute to the infertile state associated with varicoceles, and the existence of a candidate gene for the defect in trace metal regulation has been postulated (Benoff *et al.*, 1996, 1997). The variable effect on fertility reported in patients with varicoceles may represent a quantitative and qualitative molecular difference in the expression of important sperm plasma proteins, ultimately affecting the acrosome reaction.

Varicoceles and oxidative stress

Reactive oxygen species (ROS) include superoxide anions, the hydroxyl radical, nitrous oxide, hypochlorous acid and hydrogen peroxide (Sharma and Agarwal, 1996). The human spermatozoon represents a member in a growing list of cell types, including leukocytes and macrophages, that exhibit a capacity to generate ROS when incubated under aerobic conditions (Babior *et al.*, 1973; Klebanoff, 1980; Holland *et al.*, 1982). The production of ROS by spermatozoa is a normal physiological process which serves as an important mediator in signal transduction mechanisms (Schreck *et al.*, 1991), regulation of sperm hyperactivation/capacitation, and facilitation of the acrosome reaction and spermatozoon–oocyte attachment (de Lamirande and Gagnon, 1993; de Lamirande *et al.*, 1993; Aitken and Fisher, 1994).

In normal healthy men, the seminal plasma contains natural scavengers or antioxidants to neutralize the effects of excessive ROS generation. Under pathological conditions, however, ROS production overwhelms the antioxidant capacity and causes increased oxidative stress (Aitken *et al.*, 1989; Iwasaki and Gagnon, 1992; de Lamirande and Gagnon, 1995; Alkan *et al.*, 1997). ROS may cause defective sperm function as a result of lipid peroxidation of the polyunsaturated fatty acids in the sperm head and mid-piece, alter sperm morphology and lead to decreased motility and ineffective spermatozoon–oocyte fusion reaction (Aitken and Clarkson, 1987; Alvarez *et al.*, 1987). ROS also cause a high rate of double- and single-stranded DNA damage (Lopes *et al.*, 1998; Twigg *et al.*, 1998). To our knowledge, no animal models have been utilized in the study of infertility and varicoceles and ROS.

ROS have been implicated in reduced fertility in patients with varicoceles. ROS concentrations are higher in semen samples

Table II. Reactive oxygen species (ROS) and total antioxidant capacity (TAC) in varicocele patients versus controls

	Control patients[a]	Incidental varicocele	Infertile varicocele	Control versus. all varicocele	Control versus varicocele	
					Incidental	Infertile
	($n = 17$)	($n = 15$)	($n = 21$)	*P*	*P*	*P*
Age (years)	31.1 ± 2.1	29.9 ± 1.7	33.6 ± 1.0	NS	NS	NS
Log (ROS + 1)	1.30 ± 0.33	1.99 ± 0.26	2.1 ± 0.25	0.007	0.02	0.02
TAC (trolox equivalents)	1443.0 ± 105.0	939.0 ± 107.0	1186.0 ± 96.9	0.02	0.05	0.05

[a]Controls are normal donors without varicoceles
NS = not significant.
Table derived from Hendin *et al.* (1999) *J. Urol.*, **161**, 1831–1834.
Trolox equivalents = amount of trolox (μmol/l) needed to give a 10% recovery of the initial light output.

from both fertile and infertile men with varicoceles compared with controls, when using chemostimulants to induce ROS generation (Weese *et al.*, 1993). If ROS production is the active cause of infertility in patients with varicoceles, then the fact that many of these patients are still fertile suggests that a spectrum of ROS-induced damage exists; moreover, it is also possible that the level of antioxidant also varies between the fertile and infertile varicocele patients. The increased sensitivity to oxidative damage of spermatozoa from varicocele patients may, in part, be due to a deficiency in the intracellular concentration of certain antioxidants (Mancini *et al.*, 1998). Indeed, among 56 varicocele patients, ROS concentrations were higher and the total antioxidant capacity (TAC) lower than those of the control group (Sharma *et al.*, 1999). Elevated concentrations of ROS were found in 80% of infertile varicocele patients, 77% with incidental varicoceles, but in only 20% of normal donors. The incidental varicocele group consisted of men who had a clinically apparent varicocele, but who were not infertile. Normal donors had significantly higher total antioxidant concentrations than either the incidental varicocele group or infertile men with varicoceles (Table II) (Hendin *et al.*, 1999). The same research group is presently conducting a prospective study of ROS and TAC measurements before and after varicocele repair to confirm that varicoceles are indeed associated with oxidative stress.

The increased sensitivity to oxidative stress may be partly explained by the differences in composition of fatty acids in the sperm plasma membrane. In a preliminary study, the polyunsaturated fatty acid content of sperm plasma membranes was significantly decreased in the majority of oligoasthenospermic varicocele patients compared with normospermic varicocele patients (Lenzi *et al.*, 1996). At testicular biopsy, patients with varicoceles had significantly greater malondialdehyde (MDA) concentrations than patients without varicoceles. MDA concentration is an indirect indicator of ROS-induced lipid peroxidation, and provides further support of ROS as a potential mechanism of varicocele pathophysiology (Koksal *et al.*, 2000). Furthermore, varicocele-related male infertility has been associated with impaired disposal of residual sperm cytoplasm (Zini *et al.*, 2000)—a morphological characteristic that is itself correlated with defective sperm function and excessive elaboration of ROS (Aitken *et al.*, 1994; Gomez *et al.*, 1996; Keating *et al.*, 1997).

Summary

Unfortunately, it is not possible to pinpoint one mechanism responsible for the pathophysiology of the varicocele. In considering the available literature, it must be remembered that experimental animal models play a useful, albeit limited, role due to the sudden and variable iatrogenic nature of the varicoceles, and also to the limited duration of the studies. The sudden creation of these varicoceles may not represent the gradual and continuous effect on spermatogenesis, nor represent any underlying testicular pathology that may pre-exist in humans.

Much of the human data studying the underlying mechanisms of varicocele-related infertility are derived by measuring parameters between men with and without varicoceles and between men pre and post-varicocelectomy. However, none of these associations is able clearly to explain the variable effect of varicoceles on spermatogenesis, or directly explain the mechanism(s) involved, especially for the bilateral effect. The aetiology may be multifactorial, and may even include a pre-existing genetic disposition, which may account for the variable effect of varicoceles on spermatogenesis and infertility seen in humans. At present, most of the data are observational, but they do provide a starting point for investigations. The associations made with increased temperature, increased testicular blood flow, increased venous tension, and increased oxidative stress in men with varicoceles, provide the scaffold upon which future investigations should be built, to delineate better the pathophysiology of varicoceles.

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