The study of varicocele through the use of animal models

T.T. Turner

Departments of Urology and Cell Biology, University of Virginia School of Medicine, Charlottesville, VA 22908, USA

Address for correspondence: Department of Urology, Box 422, University of Virginia, School of Medicine, P.O. Box 800422
Charlottesville, VA 22908, USA. E-mail: ttt@virginia.edu

The pathophysiology of the varicocele has received considerable study, both in humans and in animal models. Mechanistic information is difficult to obtain from human subjects because study designs must not be invasive and the subject population is variable in the status of the varicocele, patient age, fertility or other health-related issues. Because of these limitations, animal models of varicocele have been developed in several species, the most common being the rat. Surgery to establish the varicocele involves partial obstruction of the left renal vein, causing a varicosity of the left spermatic vein, including the pampiniform plexus. Studies using this model have shown that experimental left varicocele induces bilateral increases in testicular blood flow and temperature contemporaneous with decreases in intratesticular testosterone and testicular sperm output. Spermatic vein reflux is not related to the pathophysiological consequences of experimental varicocele. Many questions remain regarding the mechanism by which varicocele induces testicular dysfunction, chief among them being how the unilateral varicocele causes a bilateral testicular response in the first place.

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Introduction

It has been estimated that, each year, between 20,000 and 40,000 men undergo surgery for repair of varicocele (Saypol et al., 1981; Mali, 1984). Despite this fact—and despite several decades with attention being paid to the association between varicocele and infertility—many questions remain regarding how varicocele develops, whether varicocele actually causes testicular dysfunction and, if varicocele does cause testicular dysfunction, how does it cause that dysfunction? Even these general questions are difficult to approach by investigation of human patients. Issues such as establishing appropriate controls, accounting for the interactions of age, environment, and health, and dealing with the appropriate ethical concerns of human research have meant that many studies of the human population are either incomplete or open to varied interpretation. Most of these reports simply add themselves to a stack of competing ‘pro- or con-’ reports on some particular feature of varicocele, and understanding of the lesion is little advanced.

Specific questions on the physiological or cell biological consequences of varicocele are even more difficult to address in the human population. Acquisition of tissue is limited, surgical invasion for experimental purposes is forbidden, and availability of appropriate numbers of control patients and varicocele patients of desired ages, durations of varicocele, or other characteristics required by specific protocols is inevitably limited. Thus, the use of animal models has been important in the development of the present understanding of this lesion. Since, with rare exception, varicocele is only known to develop in the human, animal models for varicocele have the lesion induced surgically. The spermatic varicosity is induced by partial occlusion of the left renal vein medial to the insertion of the left spermatic vein, about which further comment will be made later.

Early reports on the effects of unilateral, experimental varicocele used monkeys (Kay et al., 1979; Fussell et al., 1981) and dogs (Al-Juburi et al., 1979) to demonstrate bilateral increases in testicular temperature, bilateral morphological damage to the testes, and decreases in ejaculate sperm concentrations. These early studies tended to support the existing, albeit controversial, proposition that unilateral varicocele in humans induces a bilateral elevation in testicular temperature and bilateral testicular dysfunction. Specific mechanisms were not investigated. Our laboratory observed these early reports with attention since our own interest in testicular blood flow could perhaps bring insight into this vascular lesion. Subsequently, we developed the experimental varicocele model in our own laboratory, using both dogs and rats.
Surgery to induce experimental left varicocele (ELV)

This surgical model for varicocele has been essentially the same in a variety of laboratories whether the animals used were monkeys, dogs, rats or rabbits. In the following discussion the procedure used in our laboratory is described, and some important cautions are given regarding quality control. Because most of our experience is with the rat model, much of the subsequent discussion will focus on that species.

The upper left abdominal quadrant is approached through a midline laparotomy incision. The abdominal contents are packed to the right in order to visualize the left kidney, left adrenal vein, the left renal vein and the left spermatic vein as it inserts into the left renal vein (Figure 1a). Using careful blunt dissection, a tunnel is made in the fat and connective tissues surrounding the left renal vein, and the vein is cleared of adhering tissue in a position medial to the insertion of the left spermatic vein and left adrenal vein. Blind dissection behind the vein can sometimes result in inadvertent puncture or tear of the vein. While bleeding might seem excessive, the tear is often minor and a few minutes of topical pressure will stop the bleeding. In such an instance with the dog model, exposure of the vessel and control with an absorbable suture might be necessary.

A 4-0 silk suture is used to partially occlude the left renal vein at the point the vessel has been cleared of other tissue. This occlusion causes increased intravenous pressure lateral to the obstruction, and the pressure is transmitted to the left spermatic vein. This phenomenon mimics the ‘nutcracker’ phenomenon believed by some to be a major cause of varicocele in humans (Sayfan et al., 1984; Mali et al., 1986). This phenomenon occurs in humans when the left renal vein becomes compressed between the aorta and the superior mesenteric artery, and suffers a partial occlusion.

To ensure a consistent application of occlusive pressure in the dog, the suture is tied down around the vein and the interposed tip of a haemostat. The haemostat is then withdrawn, and the constricted vessel is allowed to expand into the available space. This results in a consistent constriction of the vessel to a diameter of ~3 mm at the point of the ligature. In the rat, the ligature is made around a metal wire of 0.85 mm diameter. The wire is removed from the ligature, and the vessel expands to an external diameter of ~1 mm.

It is our experience in rats and dogs that both a correct and consistent degree of obstruction is necessary for reliable development of the varicocele. Too much reduction in renal vein size will result in actual or virtual occlusion of the vein and eventual necrosis of the kidney. Too little constriction will not produce sufficient increases in lateral intrarenal vein pressure to force the development of the varicosity in the left spermatic vein. In our hands, using large, adult, Sprague–Dawley rats (450–550 g) we have found the 0.85 mm wire to be critical in this regard.

It is important at the time of subsequent study (the time after the varicocele-inducing surgery at which the study is to be performed) to check the fidelity of the varicocele model. That is, investigators must determine whether the inducing surgery actually caused the development of a persistent left varicocele. When there is no demonstration that a spermatic varicosity has developed, it leaves open the question whether a claimed effect of ELV or the absence of effect is really related to the presence or absence of the lesion. Variability of the development of varicoceles in different hands, or in different laboratories, might be the cause of some reports finding effects of experimental varicocele where others do not. In the case of our laboratory, a direct measurement of the diameter of the left spermatic vein is taken at the level of the crossing iliolumbar vein. Measurement is made using a metal micrometer, viewing through loupes if necessary. In our hands, when care has been taken to occlude abdominal collaterals (see below), the proportion of animals failing to form a varicocele is ~10%.

Spermatic veins in adult Sprague–Dawley rats are typically 0.15–0.20 mm in diameter, and reach ~1.0–1.5 mm diameter within 30 days after surgery (Rajfer et al., 1987; Turner and Howards, 1994; Figure 2). Also, while measurement of vessels in the pampiniform plexus is difficult, it is useful for the investigator at least to inspect the pampiniform plexus and to note whether the varicosity extends there. In measurement of the internal spermatic vein, it is important to distinguish between the spermatic vein and the nearby ureteral vein (Figure 1b).

It is not only the potential variability and effectiveness of the renal vein ligation that causes a variability in the development of ELV; the variable venous anatomy of the experimental animal can also be a causative factor. At the time of the inducing surgery, the
abdominal left spermatic vein should be inspected, and any significant abdominal collaterals should be ligated or cauterized. The occurrence of these collaterals is highly variable, often not occurring at all but sometimes occurring consistently in a series of animals. When such vessels do occur, they will expand under the varicocele and provide an effluent route of spermatic venous blood. If not eliminated, they will release the pressure needed to cause ELV development.

Venous anatomy of the ELV

The pertinent venous anatomy of the rat has been described (Turner and Howards, 1994; Figure 3). The conventional, or ‘textbook’ anatomy is illustrated in Figure 3A, but close inspection of a series of animals reveals a number of vascular variations which can relate to the success of the model (see Figure 3B; also previous comments on variability of collaterals). When pertinent collaterals are found and occluded the vascular response to the partial left renal obstruction is relatively consistent (Fig. 3C). In routine documentation of the varicocele only the spermatic vein diameter at the crossing iliolumbar vein is measured. This is done for the sake of consistency; nevertheless, Figure 3C shows more comprehensively the vessels that are expanded due to the varicocele-inducing surgery. Notably, the entire spermatic vein (including the pampiniform plexus) is dilated. Importantly, several collaterals arising from or near the pampiniform plexus are also dilated, and all of these lead to the iliac vein (Figure 3C).

Comparison of the rat anatomy (see Figure 3) with maps of pertinent venous anatomy in the human (Figure 4A) illustrates the similarities and differences between the two species. Importantly, a map of vessels consistently reported to be expanded in radiographical and anatomical studies of the human with varicocele (Figure 4B), when compared with the homologous map from the ELV rat (see Figure 3C), shows that in both cases the varicocele causes dilation of the pampiniform plexus, the
spermatic vein, and the collaterals leading to the iliac (Turner and Howards, 1994). The importance of the iliac communication in varicocele has been addressed (Mali, 1984), but this has not received follow-up study.

The collaterals to the iliac might be the same as the infrainguinal perforating veins reported in humans by some surgeons. The ligation of those vessels is asserted to be important to the elimination of the varicocele (Coolstaet, 1980). In short, studies of the ELV rat vasculature show it to be consistent with the human varicocele vasculature, and provide evidence for the appropriateness of the rat ELV model in the study of varicocele. Such architectural studies, as tedious as they might be, must be performed in other animal models in order to ensure that the vascular response to the ELV is as expected in those species.

Finally, the basic venous anatomy of the rat compares favourably with that of humans, and may provide a partial explanation of how varicocele increases testicular blood flow and varicocele repair returns blood flow to normal. The means by which the spermatic vein and collateral vessels increase in diameter under the influence of experimental varicocele are illustrated in Figure 3C; a similar situation exists in the human (Figure 4B). Thus, collateral vessels expand under varicocele and develop the capacity to carry more blood from the testis after induction of varicocele and increased resistance of blood flow via the spermatic vein. It is also possible that local mitogenic factors or vasoactive substances might increase in-flow on the arterial side, though as yet these substances have not been investigated. If there is an increased capacity to carry blood away from the testis under varicocele, then total ligation of the spermatic vein could reduce that capacity and return blood flow to within normal ranges. This might explain why varicocele repair in the ELV model returns testicular blood flow and spermatogenesis to normal. Even this speculation, however does not address a bilateral response to varicocele repair.

Use of the ELV model to understand varicocele pathophysiology

Early reports of testicular hypoxia and stagnation of blood flow in the human testis (for review see Turner, 1983; Chakraborty et al., 1985) seemed intuitively consistent with the idea of increased resistance to venous return up the spermatic vein. Unfortunately, data supporting this idea were inconsistent, and the concept developed despite existing evidence that human testicular
temperature was decreased bilaterally (for review see Turner, 1983). At the temperature. As described previously, the first uses of animal was increased bilaterally by varicocele, and that spermatogenesis MacLeod, 1973). Reduced testicular blood flow would be expected to have the effect of reducing, not increasing, testicular temperature was not mediated by the ipsilateral testis (Green et al., 1987). Importantly, it was shown that the increased blood flow and temperature and decreased spermatogenesis were returned to control values by varicocele ligation (Green et al., 1984; Hurt et al., 1986). Later, it was demonstrated that the effect of ELV on contralateral flow and temperature was not mediated by the ipsilateral testis (Green et al., 1985; Hurt et al., 1987). This latter point is in contrast to other reports that reduction of spermatogenesis in the ipsilateral rat testis after ELV results in an immunological consequence to the contralateral testis (Shook et al., 1988).

In additional studies, intravitral microscopy was used to demonstrate microvascular blood flow changes in the rat testis after varicocele (Nagler et al., 1987), while others (Choi et al., 1990) showed that ELV imposed on adolescent rats caused testicular dysfunction. All of these investigations probed pathophysiological mechanisms in a scientific manner not possible in the human patient population. Interestingly, in the animal studies increases in testicular temperature were documented directly in the testis, whereas clinical data had been limited to determining surface scrotal temperatures. Also, in several animal studies blood flow was measured directly and quantitatively, rather than indirectly and qualitatively. Obtaining such data in a controlled way is a primary advantage of using animal models for human lesions, in general. Nevertheless, later studies did obtain direct measurements of human intratesticular temperatures and confirmed that testicular temperature was increased in human varicocele patients (Goldstein and Eid, 1989). Moreover, colour Doppler ultrasonography, which provides a qualitative estimation of blood flow, has demonstrated tendencies towards increased blood flow in the human testis with varicocele (Mellinger, 1981; Ross et al., 1994).

**Specific pathophysiological mechanisms**

Insufficiency along the hypothalamo-hypophyseal axis has been proposed as an important causative factor in varicocele-associated infertility (Hudson, 1985; Nagao et al., 1986), though results from human studies have been variable. Studies in the ELV rat have shown that bilateral intratesticular testosterone concentrations do decline significantly within weeks of the varicocele-inducing surgery (Rajfer et al., 1987), but follow-up experiments examining LH secretion by anterior pituitary cells (Pryor et al., 1989), gonadotroph responsiveness to gonadotrophin-releasing hormone, and Leydig cell responsiveness to LH (Turner et al., 1990) found no differences between control and ELV animals or tissues. As a result, it has been postulated that the decrease in intratesticular testosterone concentrations found in the ELV rat are due to a washout phenomenon subsequent to the increased testicular blood flow. This could also be a factor in the human testis, and might mean that intratesticular testosterone concentrations in humans with varicocele are lower than normal, despite there being a normal testosterone concentration in peripheral plasma.

It is often presumed by some that retrograde blood flow down the spermatic vein is, a priori, a part of the condition of varicocele. Venographic evidence has reinforced this impression, as has the filling of the varicocele during the Valsalva manoeuvre, or when the patient stands upright from a horizontal position. Unfortunately, several factors are often not taken into account when assessing retrograde blood flow: (i) retrograde flow is not the only explanation for scrotal filling after Valsalva or after rising; (ii) common venographic techniques block normal venous outflow, potentially forcing blood or venography dyes to go in directions they would not otherwise go; (iii) the perfusion pressure (which is not reported or commented upon in retrograde venography studies) can artificially cause retrograde filling of vasculature with contrast dye; and (iv) radiocontrast dyes have a much higher specific gravity than blood, and can gravitate downward against the direction of a positive left spermatic vein blood flow. Nevertheless, the idea of retrograde blood flow has been used to explain how renal or adrenal metabolites supposedly arrive at the testis (or both testes for that matter) and cause changes in testicular function (for review see Turner, 1983). These ideas have persisted even though studies in the human have...
found natural reflux down the spermatic vein (Netto et al., 1980) or Valsalva-induced reflux (Hirsch et al., 1980) to occur equivalently in normal men and men with varicocele. These reports, as well as the concerns listed above, raise questions about the phenomenon of reflux and whether it is a significant factor in varicocele-associated infertility. This issue has been addressed with the ELV model.

In one study using the ELV rat, $^{85}$Sr-labelled microspheres were injected into the left renal vein lateral to the insertion of the left spermatic vein (Turner and Lopez, 1989). In ELV animals, the injection site was also lateral to the ELV ligature around the renal vein. Microsphere infusion was carried out in a volume and at a rate that was trivial relative to total blood effluent in the renal vein. This was an important consideration in order to avoid unintentionally forcing fluids or microspheres in a direction they would not otherwise go. Should reflux occur, radiolabelled microspheres would pass down the spermatic vein toward the testis, and if refluxing blood reached either the vein or the testis then $^{85}$Sr radioactivity would be detected there. In fact, radioactivity was not detected in either testis, and only in very few animals were small numbers of microspheres detected in the spermatic vein from the ipsilateral testis or the contralateral testis. In these same studies, some animals showed a significant increase in testicular blood flow (Turner and Lopez, 1989). Also in a separate study, no venous collaterals could be found communicating from the ipsilateral to the contralateral testis of the ELV testis (Turner et al., 1989). While such collaterals have been called upon in the human as the route of delivery of spermatic vein reflux (Hsu et al., 1996a), other studies using the ELV model have shown that reflux from the ipsilateral adrenal gland; and (iii) collateral circulation to the testis does not occur in the ELV rat; (ii) anomalous blood flow does not occur in the ELV rat; (iii) collateral circulation to the testis does not occur in the ELV rat.

Conclusions

Animal models of varicocele have now been used for 20 years, and have contributed significantly to our current understanding of the lesion. Such studies have shown that testicular blood flow increases bilaterally in response to unilateral varicocele, and that there is a concomitant increase in testicular temperature. Such a temperature increase is what would be expected with increased blood flow, since increased arterial flow through the pampiniform plexus reduces the efficiency of the countercurrent heat exchange mechanism there. It is well accepted that increased temperature of the kind seen in the ELV testis is sufficient to disrupt spermatogenesis, primarily through the alteration of intracellular enzymes. An example of this is the decreased 17 $\alpha$-hydroxylase activity (Rajfer et al., 1987) which was associated with the reduction of intratesticular testosterone found in that same study. Other studies using the ELV model have shown that the hypothalamic-pituitary-gonadal axis functions normally in ELV rats with testicular blood flow measurements. Others (Li et al., 1999) also reported the appearance of varicocele within minutes of ELV-inducing surgery, but in our hands the spermatic varicosity develops over a period of 2-4 weeks (see Figure 2); thus, there may be something fundamentally different in the ELV technique used and the anatomical result obtained by these other groups (Hsu et al., 1994; Li et al., 1999). Some years previously, another group (Laven and Wensing, 1989) had not been able to produce varicoceles in the dog using the left renal vein ligation technique that has successfully produced varicoceles in dogs in other laboratories (Al-Juburi et al., 1979; Saypol et al., 1981); thus using an animal model to study a human lesion does not guarantee that different laboratories will also achieve consistent results. However, it does mean that procedures may be repeated and techniques refined in a manner not possible in the patient population.

Some recent studies have gone on to use the ELV model to study intracellular phenomena. For example, defective mitochondrial oxidative-phosphorylation was found in rat testes with varicocele (Hsu et al., 1995), while others (Sofikitis et al., 1996) used the rabbit to show that round spermatids from the varicocele-bearing testis have less fertility potential than similar gametes from control testes. Both of these studies relate most strongly to germ cells, and point to the cellular consequences of disturbed testicular blood flow and temperature. In contrast, studies of rat Sertoli cell protein synthesis and luminal secretion in vivo have not demonstrated an ELV-induced change in this aspect of Sertoli cell function (Turner and Miller, 1996).

Study of varicocele using animal models

Animal models of varicocele have now been used for 20 years, and have contributed significantly to our current understanding of the lesion. Such studies have shown that testicular blood flow increases bilaterally in response to unilateral varicocele, and that there is a concomitant increase in testicular temperature. Such a temperature increase is what would be expected with increased blood flow, since increased arterial flow through the pampiniform plexus reduces the efficiency of the countercurrent heat exchange mechanism there. It is well accepted that increased temperature of the kind seen in the ELV testis is sufficient to disrupt spermatogenesis, primarily through the alteration of intracellular enzymes. An example of this is the decreased 17 $\alpha$-hydroxylase activity (Rajfer et al., 1987) which was associated with the reduction of intratesticular testosterone found in that same study. Other studies using the ELV model have shown that the hypothalamic-pituitary-gonadal axis functions normally in ELV rats with testicular blood flow measurements. Others (Li et al., 1999) also reported the appearance of varicocele within minutes of ELV-inducing surgery, but in our hands the spermatic varicosity develops over a period of 2-4 weeks (see Figure 2); thus, there may be something fundamentally different in the ELV technique used and the anatomical result obtained by these other groups (Hsu et al., 1994; Li et al., 1999). Some years previously, another group (Laven and Wensing, 1989) had not been able to produce varicoceles in the dog using the left renal vein ligation technique that has successfully produced varicoceles in dogs in other laboratories (Al-Juburi et al., 1979; Saypol et al., 1981); thus using an animal model to study a human lesion does not guarantee that different laboratories will also achieve consistent results. However, it does mean that procedures may be repeated and techniques refined in a manner not possible in the patient population.

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and such studies should continue to produce interesting results in the future. Finally, a warning seems appropriate. A model is like an analogy; it can always be stretched too far. As investigators move to studies of the cellular and molecular aspects of varicocele pathology, the investigators must have the expertise to perform correctly the basic ELV surgery, as well as to develop an understanding of the basic physiology and anatomy pertinent to varicocele. In the absence of this expertise, investigators run the risk of studying the cell or molecular biology of tissues which do not relate to the human lesion that they wish to understand. Many questions concerning varicocele remain to be answered, significant among which, for example, is how unilateral varicocele causes a bilateral testicular response. It is possible that this and other questions will be addressed in the future by investigators using animal models to study this still enigmatic lesion.

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References


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