

Varicocele and male infertility: Part I

Preface

Susan Benoff^{1,2,3,5} and Bruce R. Gilbert⁴

¹Center for Molecular and Cell Biology, North Shore-Long Island Jewish Research Institute, Manhasset, New York, ²Department of Obstetrics and Gynecology, North Shore University Hospital, Manhasset, New York, ³Departments of Obstetrics and Gynecology and Cell Biology, New York University School of Medicine, New York, New York, and ⁴Department of Surgery (Urology), North Shore University Hospital, Manhasset, New York, USA

⁵To whom correspondence should be addressed at: NS-LIJ Research Institute, 350 Community Drive, Room 125, Manhasset, New York 11030 USA. E-mail: sbenoff@nshs.edu

The term 'varicocele', which was coined by Curling in 1843 (Noske and Weidner, 1999), refers to an abnormal dilation of the testicular veins within the pampiniform plexus. Varicocele occur more frequently on the left side (Riba, 1947), most probably due to asymmetry of the internal spermatic veins resulting in alterations in biochemical properties (e.g. increased extensibility in comparison with the right spermatic vein) (Lund *et al.*, 1998). Varicocele was recognized and treated as early as the 1st century AD and, over the centuries, the condition was an important exclusion criterion for military service (Noske and Weidner, 1999). However, it was not until 1952 that the possible association between the presence of a varicocele and human male subfertility was formally recognized. Such recognition was derived from a single case report, of a man diagnosed with maturation arrest by testis biopsy, in which bilateral varicocele-ectomy resulted in improved sperm count and pregnancy by coitus (Tulloch, 1952). A flurry of reports followed, suggesting that the incidence of varicocele was increased in subfertile men (Russell, 1954; Scott, 1958) and that improvement in semen parameters occurred after surgical intervention (Davidson, 1954; Tulloch, 1955; Young, 1956; Scott, 1961; Charny, 1962; MacLeod, 1965; Dubin and Hotchkiss, 1969).

In current medical practice, impairment of semen parameters suggests that a varicocele may be present. In particular, a decrease in sperm number, motility and morphologically normal spermatozoa with an increase in head abnormalities is most often found (MacLeod, 1965; Brown, 1976; Belker, 1981; Naftulin *et al.*, 1991). This finding alone should provide sufficient impetus for further evaluation of the male. Of particular clinical importance is that a significant number of men presenting with subfertility have medical issues that would not have been diagnosed if an impaired semen analysis had not led to further evaluation (Honig *et al.*, 1994; Jarow, 1994). Several investigators have also presented compelling data suggesting that a varicocele causes a progressive decline in fertility with upwards of 80% of men presenting with secondary subfertility having a varicocele (Gorelick and Goldstein, 1993; Witt and Lipschultz, 1993). This decline is

thought to be due to progressive testicular damage, as testicular mass and sperm counts in patients with varicocele decline with age (Lipshultz and Corriere, 1977). The occurrence of testicular damage with varicocele has alternatively been attributed to: (i) increased scrotal (intratesticular) temperature; (ii) venous stasis; (iii) reduced oxygen tension, and/or (iv) toxic metabolites from the adrenals or kidney (Brown *et al.*, 1967). To date, heat stress remains the favoured mechanism (Comhaire, 1991; Mieusset and Bujan, 1995; Wright *et al.*, 1997).

Despite such findings, it is apparent that >85% of men with varicocele are fertile (Sylora and Pryor, 1994). The same semen abnormalities are observed in fertile and infertile men with varicocele (Nagao *et al.*, 1986). Although varicocele repair can reduce testicular temperature (Agger, 1971; Yamaguchi *et al.*, 1989; Wright *et al.*, 1997), only about one half of the studies on the effects of varicocele repair report a significant improvement in pregnancy rates after treatment, when compared with a control (no treatment) group. In the absence of molecular markers, which discriminate between fertile and infertile men with varicocele, whether or not patient selection contributes to these disparate findings is an open question. As will be seen from the contents of this mini symposium, varicocele remains a controversial topic, which is often the subject of heated debates between andrologists and urologists, both about appropriate clinical management and about the mechanisms producing infertility with varicocele. This mini symposium was organized to address these issues.

This mini symposium is divided into two parts: both temporally and with regard to content. The first part, in this issue of *Human Reproduction Update* (Cozzolino and Lipschultz, 2001; Jarow, 2001; Kamischke and Nieschlag, 2001; Silber, 2001; Turner, 2001) covers two main questions: firstly, does varicocele produce an infertile state? and, secondly, does varicocele repair increase pregnancy rates? The second part of this mini symposium (to be published in a later issue of *Human Reproduction Update*) is directed at the identification of varicocele-associated defects in sperm function and the underlying pathophysiology of the infertile state, including the role of ancillary factors.

Detection of varicocele

The examination requires an adept clinician, a warm room and a co-operative patient for proper diagnosis. Difficulties arise with the ability to palpate a varicocele through a thickened scrotal wall or contracted scrotum. In addition, the examiner must be able to evaluate for epididymal and testicular pathology as well as document asymmetric findings. Given the subjective nature of a clinical examination, non-invasive diagnostic testing (e.g. ultrasonography) has been employed to attempt to quantify varicocele size. Although there is no universally agreed-upon standard, a vein (or vein complex) of >3 mm diameter, when measured posterior to the testis during a Valsalva manoeuvre has been used to differentiate between clinically significant and non-significant varicocele. Ultrasound has also been valuable in following the regression of varicocele after repair. The increased incidence of bilateral varicocele reported recently (Lemack *et al.*, 1998; Das *et al.*, 1999; Lund *et al.*, 1999) may reflect the increased use of ultrasonography, as may data suggesting further improvement in semen quality after delayed repair of a contralateral varicocele (Scherr and Goldstein, 1999).

Indications for treatment

Subfertility is the most common indication for treatment of varicocele, while scrotal discomfort (although at times present in the subfertile male), is infrequently the sole reason for repair. A number of studies suggest that the response to varicocele repair is related to varicocele size, with greater improvement in semen parameters obtained with larger lesions (Johnsen and Agger, 1978; Steckel *et al.*, 1993). Although controversy surrounds repair of the adolescent varicocele (Atassi *et al.*, 1995; Kass and Reitelman, 1995; Paduch and Niedzielski, 1997; Sigman and Jarow, 1997; Gershbein *et al.*, 1999), most male fertility specialists would repair large varicocele in the adolescent male when accompanied by a decrease in testicular size, even in the absence of an evaluable semen analysis. In the adolescent varicocele group there also appears to be a reversal of the testicular growth failure post-operatively (Kass and Belman, 1987; Atassi *et al.*, 1995; Paduch and Niedzielski, 1997; Gershbein *et al.*, 1999; Lund *et al.*, 1999). Recent studies have also documented a significant improvement in sperm parameters in men with both azoospermia and severe oligoasthenoteratozoospermia (Matthews *et al.*, 1998; Kim *et al.*, 1999).

Varicocele repair for pain is a less common indication. However, when present the pain is usually described as a dull throbbing ache. Few studies have followed these patients to see if symptoms are better after the procedure. In a retrospective study (Petersen *et al.*, 1998) it was found that 86% had an improvement in their symptoms while only 11% did not or had worsening of their symptoms.

Treatment options for varicocele

Clearly, the overall goal of varicocele treatment is to improve pregnancy rates (by improving testicular function and semen parameters). However, the timing of varicocele repair is an area of debate. With the great success of assisted reproductive procedures, couples approaching 40 are often advised to proceed with

this modality first, prior to varicocele repair. However, for the younger male varicocele repair is considered to be the best cost-effective option (Schlegel, 1997), as it potentially allows the couple to conceive without assisted reproductive procedures. Results of controlled trials of varicocele ligation (Schlegel, 1997), have convincingly demonstrated an improvement in both semen quality and fertility. On average, after varicocele ligation there was a 33% pregnancy rate (confidence interval, 28–39%) as compared to a treatment independent rate of 16% (95% confidence interval, 13–20%). However, which patient may benefit from varicocele ligation and who should be referred for in-vitro procedures has not yet been defined.

Non-surgical approaches

Varicocele can be treated by either surgical or non-surgical approaches. In addition to the assisted reproductive procedures of intrauterine insemination (when poor post-coital testing is observed) or IVF, non-invasive approaches include the use of prednisone to treat impaired sperm motility associated with the presence of anti-sperm antibodies, external appliances to cool the scrotum, adjuvant hormonal therapy and percutaneous venous occlusion (Girardi and Goldstein, 1997).

Hormonal values are often in the normal range in men with varicocele (Swerdlow and Walsh, 1975; Bablok *et al.*, 1985; Haans *et al.*, 1991), although this is not a constant finding (Micic *et al.*, 1986). There appears to be a correlation with impairment in spermatogenesis and Leydig cell function that can be corrected by varicocele repair (Castro-Magana *et al.*, 1989, 1990). Several investigators have, in fact, suggested that hormonal concentrations prior to varicocele ligation are indicative of improvement in semen quality after repair (Girgis *et al.*, 1981; Hudson *et al.*, 1986; Bablok *et al.*, 1997). In this context, adjuvant hormonal therapy using human chorionic gonadotrophin (HCG) for 10 weeks after varicocele surgery has been found to be effective in improving semen quality, in men who also had Leydig cell dysfunction (Yamamoto *et al.*, 1995).

Percutaneous venous occlusion through the use of detachable balloons or coils have gained some acceptance as a non-surgical approach for treatment of varicocele. The use of sclerosing agents has been used. However, it requires a considerable amount of time and an increased duration of radiation exposure to the subfertile male. It is usually often used only in conjunction with detachable balloons or coils. With detachable balloons either a femoral or jugular approach is used. The advantage with their use is the ability to test the occlusion prior to completion of the procedure. The disadvantages include the cost of the balloon, the possibility of balloon migration and the inability to cannulate small collateral veins. Detachable coils have the advantage of being inexpensive. However, the adequacy of the occlusion cannot be verified prior to deployment of the coil. Although these percutaneous techniques are considered non-surgical with a minimal recuperative time they are often technically difficult and require a longer procedural time than varicocele ligation. The success rate for these procedures, when occlusion and non-recurrence are considered to be the end point, is reported to be only 69% (Nagler *et al.*, 1997). The overall complication rate (balloon migration, contrast extravasation, vascular injury) is in the range of 11% (Pryor and Howards, 1987).

Surgical approaches

Surgical approaches have involved laparoscopic, retroperitoneal and inguinal approaches (Girardi and Goldstein, 1997). The scrotal approach is primarily of historic interest. The rich anastomotic network of the pampiniform plexus of veins makes both the failure rate and complication rate of a scrotal approach unacceptably high. The microsurgical inguinal (Marmar *et al.*, 1985; Gilbert and Goldstein, 1988; Goldstein *et al.*, 1992) and more recently the subinguinal approach (Marmar and Kim, 1994) has gained widespread use among male reproductive surgeons. The benefits of this approach include the ability to individually ligate all varicocele veins while preserving the arterial and lymphatic vessels of the spermatic cord. This is done with minimal tissue disruption, which translates into minimum morbidity and a rapid recovery after the procedure. The primary complications of surgical repair have been varicocele recurrence and hydrocele formation. Varicocele recurrence has been reported to occur in as few as 0.6% (Goldstein *et al.*, 1992) to as much as 35% (Yavetz *et al.*, 1992) of patients after surgical repair. The lower rates are associated with the use of surgical magnification and operative technique (inguinal/subinguinal approach with ligation of the collateral venous channels). Hydrocele formation appears to be the most common complication after non-microscopic varicocele ligation, with an incidence of 3–33% (Goldstein, 1995). Use of magnification (operating microscope or loops) can assist in preventing the inadvertent tying of lymphatic channels and the production of a hydrocele, which would serve to blunt the beneficial effect of varicocele repair. Damage to the testicular blood supply and a subsequent impairment in spermatogenesis is not likely through many approaches due to the contribution of the testicular as well as the vasal arterial supply.

Testis biopsy as an investigational tool

Testicular biopsies have long been employed to investigate the underlying pathology of infertility with varicocele. Most studies have taken these biopsies at the time of varicocele ligation. Testis biopsies from infertile adult males with varicocele indicate that the predominant pathology is one of decreased spermatogenesis associated with premature sloughing of immature germ cells into the lumen of the seminiferous epithelium and, in some cases, maturation arrest (Etriby *et al.*, 1967; Dubin and Hotchkiss, 1969; Ibrahim *et al.*, 1977; McFadden and Mehan, 1978). Thickening of the tubular basement membranes and interstitial hyperplasia are also noted. The similar but less severe histological changes, with early focal oedema and focal damage to the peritubular basal lamina, which are observed in testis biopsies of boys aged 12–15 years are taken as additional evidence for progressive deterioration of the testis with varicocele and is used as an argument for early repair (Hienz *et al.*, 1980; Lyon *et al.*, 1982; Kass *et al.*, 1987; Santoro *et al.*, 2000). Changes in the vascular endothelium occur before damage in the seminiferous epithelium is evident (Hadziselimovic *et al.*, 1989). The latter is consistent with arguments that the underlying mechanism of infertility with varicocele involves the observed disturbances in testicular blood flow (Hienz *et al.*, 1980). Testicular damage appears progressive and generally is observed on both sides even with unilateral

varicocele (Charny, 1962; Etriby *et al.*, 1967; Dubin and Hotchkiss, 1969; Gasser, 1971). However, biopsy findings are not uniform among men with varicocele, normal hormonal profiles and primary infertility (Dubin and Hotchkiss, 1969; Ibrahim *et al.*, 1977).

Two reports suggest that a good correlation exists between the Johnsen (1970) score of the testicular biopsy from infertile men with varicocele and total sperm count in the ejaculate (Johnsen and Agger, 1978; Abdelrahim *et al.*, 1993). Comparison of testis biopsies taken pre- and post-operatively indicates that depressed spermatogenesis may be improved after varicocele repair (Charny, 1962; Johnsen and Agger, 1978; Abdelrahim *et al.*, 1993). Sperm motility may also be improved after varicocele repair (Johnsen and Agger, 1978). However, neither testicular histology nor semen quality is improved in all cases by surgical treatment (Etriby *et al.*, 1967; Dubin and Hotchkiss, 1969; Abdelrahim *et al.*, 1993). Pregnancy rates are often not improved post-operatively when multiple biopsy deficits are present at time of surgery (Etriby *et al.*, 1967; McFadden and Mehan, 1978).

The immature forms found in the lumen of the seminiferous epithelium and in the ejaculate of men with varicocele are similar (Dubin and Hotchkiss, 1969). This observation, when considered in the light of the findings described above, suggests that the main damage of varicocele to human spermatozoa occurs in the testis, not after ejaculation. However, in reviewing the literature and in preparing this mini symposium, we found it striking that there was a paucity of studies using testicular biopsies to assess varicocele-associated defects at the molecular level. Perhaps this is because of past failures in identifying a relationship between pre-operative testicular histopathology and sperm count with pregnancy outcome post-surgery (Dubin and Hotchkiss, 1969; McFadden and Mehan, 1978). Nevertheless, what limited analysis of testis biopsies is currently available provides evidence for biochemical defects associated with varicocele and should serve as impetus for additional studies.

Varicocele is associated with progressive sclerosis of the lamina propria. A recent electron microscopic and immunohistochemical study of testis biopsies revealed loss of α -laminin and α -collagen type IV of the basal lamina (Santoro *et al.*, 2000). Peritubular myoid cells are also affected. A loss of actin- and desmin-immunoreactive cells is reported, with observations suggesting that the missing myoid cells have acquired fibroblastic characteristics (Santamaria *et al.*, 1992). Given evidence for cross-talk between myoid cells and Sertoli cells (Santamaria *et al.*, 1992) and for a role of Sertoli cell actin in spermiogenesis and movement of spermatocytes across the blood-testis barrier (Russell *et al.*, 1989; Vogl, 1989) and our own preliminary studies indicating that spermatozoa from infertile men are actin-depleted (Benoff, 1997; Benoff *et al.*, 1997), we believe that premature sloughing which characterizes testis biopsies from many varicocele subjects might be indicative of a cytoskeletal defect.

To test this hypothesis, we compared actin immunoreactivity in biopsy sections from men with obstructive azoospermia and normal spermatogenesis with biopsy sections from infertile men with varicocele, having either normal or reduced spermatogenesis. Typical results are shown in Figure 1 (left series). A bright uniform fluorescence is observed in control biopsies from men with obstructive azoospermia. In contrast, the overall fluorescence

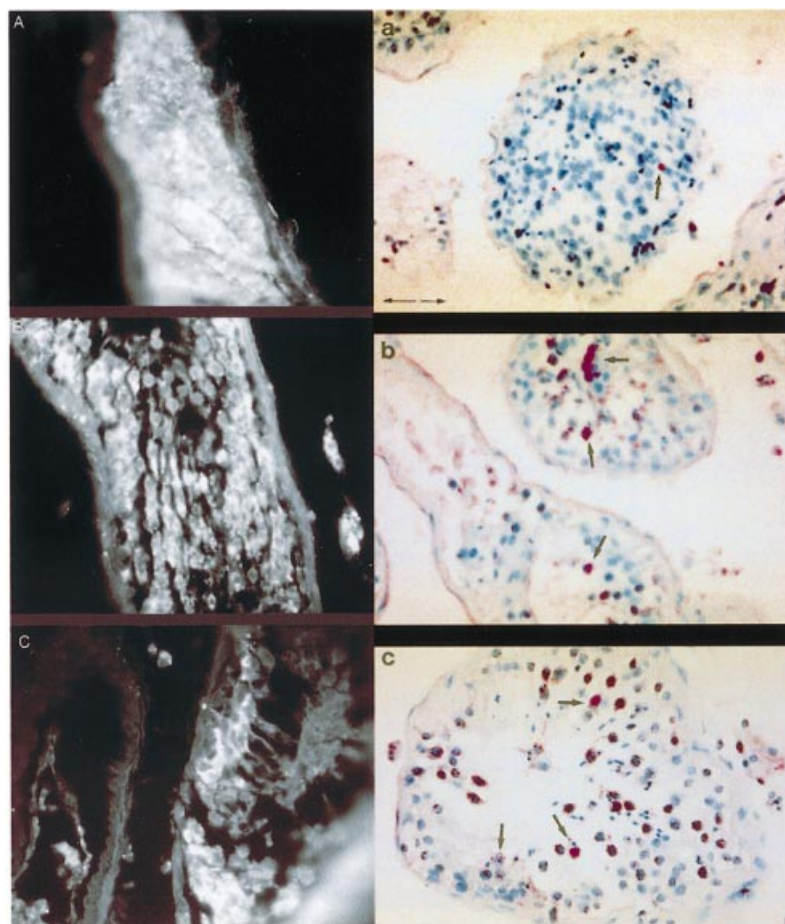


Figure 1. Loss of cytoplasmic actin filaments is correlated with an increase in germ cell apoptosis. A 10 mHz pencil Doppler was used to detect audible sounds of retrograde blood flow. Testicular tissue was obtained by percutaneous needle aspiration biopsy (Marmar, 1998) and immediately fixed in formalin. Typical results are shown. **Left:** indirect immunofluorescence (anti-actin) images of tubules from formalin-fixed testicular biopsy sections (9 μ m) were prepared by standard laboratory protocols (Benoff, 1997; Benoff *et al.*, 1997). (A) Patient with obstructive azoospermia (Johnsen score ≥ 8), scale bar = 40 μ m. (B) Patient with varicocele-associated infertility and normal spermatogenesis (Johnsen score ≥ 8). (C) Patient with varicocele-associated infertility and hypospermatogenesis (Johnsen score ≤ 8). **Right:** Duplicate testis sections were assessed for apoptosis by TUNEL assay (TACS 2 TdT *in situ* Apoptosis Detection Kit) according to manufacturer's instructions. The arrows in the figure point to the red-brown diamminobenzidine staining of apoptotic nuclei.

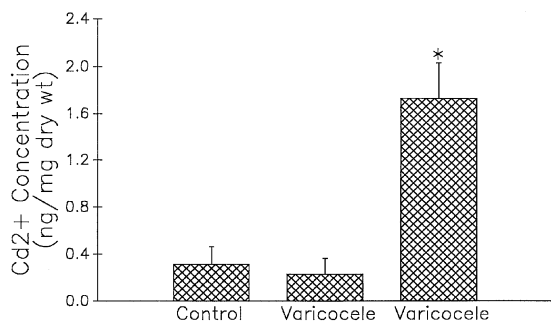


Figure 2. Initial analyses of testicular Cd²⁺ content. To determine the concentration of Cd²⁺ in testis biopsies, nine biopsy fragments (wet weight >40 mg) were dried to a constant weight *in vacuo*, digested with acid and the Cd²⁺ concentrations determined by graphite furnace atomic absorption spectroscopy (Benoff, 1997; Benoff *et al.*, 1997) using the method of standard additions to correct for matrix effects. These assays show that testicular Cd²⁺ concentrations in men with varicocele-associated infertility divide into two groups, one similar to measured values in control 'normal' testes (from men with obstructive azoospermia) and one significantly elevated (**P* < 0.02).

intensity of sections from subjects with varicocele is decreased and the pattern of actin immunostaining also differs from the controls, in that the immunofluorescence decreases with increasing differentiation within the seminiferous tubules and with severity of the spermatogenic lesion.

Actin loss by somatic cells is associated with an increase in apoptosis (Russo *et al.*, 1982; Tsudikate *et al.*, 1993) and apoptosis is reported to be increased in the testis of infertile men with varicocele (Simsek *et al.*, 1998). Therefore, we assessed the level of apoptosis in duplicate biopsy sections (Figure 1, right series). We observed that the percentage of apoptotic nuclei increases as the actin immunoreactivity decreases.

Elevated concentrations of a metal ion, cadmium (Cd²⁺), can result in actin loss (Wang *et al.*, 1996) and can increase apoptosis in the testis (Jones *et al.*, 1997; Yan *et al.*, 1997). Therefore, as we had previously observed Cd²⁺ concentrations to be elevated in the seminal plasma of infertile men with varicocele (Benoff, 1997; Benoff *et al.*, 1997), we assessed intratesticular Cd²⁺ concentrations in portions of the same biopsies used for anti-actin staining and for terminal deoxynucleotidyl transferase-mediated dUDP

nick-end labelling (TUNEL) analyses (Figure 2, typical results). We observed that the testicular Cd^{2+} concentrations of the testis biopsies from obstructive azoospermia were low (e.g. Figure 1A, 0.31 ng/mg dry weight), consistent with published reports on testicular Cd^{2+} content (Oldereid *et al.*, 1993). Cd^{2+} concentrations in testis biopsies from men with varicocele and normal spermatogenesis are similar (e.g. Figure 1B, 0.23 ng/mg dry weight) but are significantly increased in some testis biopsies from men with varicocele and hypospermatogenesis (e.g. Figure 1C, 1.1 ng/mg dry weight). These observations are interpreted to indicate that anti-actin staining decreases as testicular temperature and/or Cd^{2+} concentrations increase and that both loss of actin and increased intratesticular Cd^{2+} concentrations contribute to increased germ cell apoptosis.

The clinical diversity of human varicocele (e.g. inter-male differences in scrotal temperatures, semen analysis and histology of testis biopsies) as compared to the relative uniformity of findings in experimental animal models becomes clear from the current minisynposium. With this in mind, we suggest that other mechanisms to produce apoptosis and an irreversible infertile state may be operational, in addition to heat stress and increased intratesticular Cd^{2+} concentrations/decreased actin, e.g. androgen receptor defects may be a contributing factor (J.L.Marmar, personal communication). We can take Marmar's suggestion one step further: in Figure 3, we propose a model for the interaction of three pathways in the production of varicocele-associated apoptosis and oligozoospermia: (i) elevated scrotal temperature (heat stress); (ii) increased intratesticular Cd^{2+} concentrations and (iii) androgen deprivation. Pathway I invokes heat stress (elevated scrotal temperature), which has been documented to occur with varicocele (Agger, 1971; Yamaguchi *et al.*, 1989; Mieusset and Bujan, 1995; Wright *et al.*, 1997) and which may be reduced by varicocele repair (Agger, 1971; Yamaguchi *et al.*, 1989; Wright *et al.*, 1997). In proposing this pathway, we consider observations indicating that: (i) cryptorch-

idism in man is associated with high scrotal temperature, impaired spermatogenesis and infertility (Mieusset *et al.*, 1995); (ii) heat stress induces germ cell apoptosis (Hsueh *et al.*, 1996; Yin *et al.*, 1997); (iii) actin loss increases apoptosis by somatic cells (Russo *et al.*, 1982; Tsukidate *et al.*, 1993); and (iv) intracellular actin in the testis of animal models is disrupted by a brief local heating of the scrotum (McLaren *et al.*, 1994). We have observed that actin loss from cells within the seminiferous epithelium in testis biopsies from infertile men with varicocele is correlated with increased germ cell apoptosis (Figure 1), with severity of spermatogenic defects (see Figure 1), and with decreased sperm count in the ejaculate (S.Benoff and J.L.Marmar, unpublished observations). Pathway II invokes a mechanism based on increased intratesticular Cd^{2+} concentrations (see Figure 2), which can also cause actin disassembly and degradation (Wang *et al.*, 1996), and is the pathway we are currently attempting to dissect (Benoff, 1999; Benoff *et al.*, 1999; Benoff *et al.*, 2000a,b; Hurley *et al.*, 2000). In this pathway, the interstitial fluid in the testis is a filtrate from blood plasma, with capillary hydrostatic pressure and interstitial fluid pressure determining the volume of interstitial fluid (Nagler, 1996). Varicocele causes an increase in testicular venous pressure (Shafik and Bedeir, 1980; Sweeney *et al.*, 1995), increasing fluid transport. Thus, the elevation in testicular Cd^{2+} concentration that we have observed in varicocele-associated infertility (Figure 1) is likely to be derived from the increased transvascular fluid exchange, which occurs with varicocele (Sweeney *et al.*, 1991, 1995), potentially allowing more of the Cd^{2+} in serum to enter the testis. Such entry is likely to occur as animal studies have provided clear evidence that Cd^{2+} exposure alters the permeability of the testicular vascular endothelium (Setchell and Waites, 1970). In the absence of an active pump to remove this Cd^{2+} (Gunn *et al.*, 1961), we predict that testicular Cd^{2+} concentrations would be elevated over time. This prediction is supported by studies in man and in animal models (Oldereid *et al.*, 1993; Yan *et al.*, 1997). In addition,

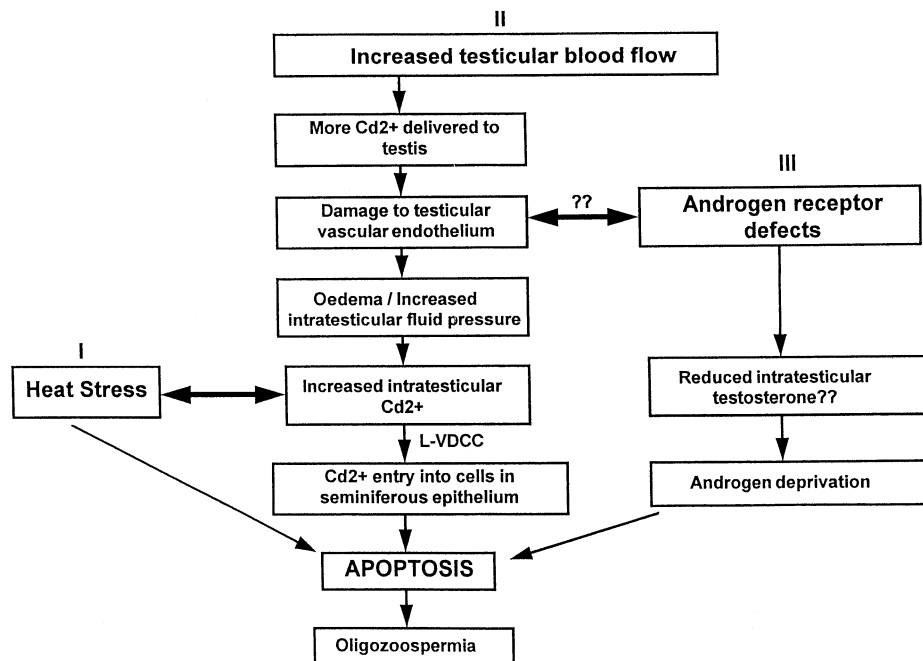


Figure 3. Proposed model for multifactor aetiology of apoptosis leading to oligozoospermia in varicocele-associated infertility.

animal modelling studies indicate that Cd^{2+} exposures damage vascular endothelial cells, resulting in oedema, allowing greater access to testicular parenchyma (Mason *et al.*, 1964; Gunn *et al.*, 1968; Clegg *et al.*, 1969; Aoki and Hoffer, 1978; Gazid and Kaminski, 1984). Our data suggest that entry of Cd^{2+} into the cells of the seminiferous epithelium can occur via L-type voltage-dependent calcium channels (L-VDCC) (Benoff *et al.*, 2000a), which are expressed in all cells within the seminiferous epithelium (Goodwin *et al.*, 1998). Cd^{2+} action in the testis is likely to be cell-specific and stage-specific (Clegg *et al.*, 1969; Wong and Klaassen, 1980; Hew *et al.*, 1993b). Under these conditions, Sertoli cells are differentially sensitive to Cd^{2+} (Clough *et al.*, 1990). Intracellular actin of Sertoli cells apparently regulates spermiogenesis and movement of spermatocytes through the blood–testis barrier (Russell *et al.*, 1989; Vogl, 1989). A single low dose of Cd^{2+} causes both disruption of Sertoli cell microfilaments (Hew *et al.*, 1993b) and failure of spermiogenesis (Hew *et al.*, 1993a), potentially contributing to oligozoospermia. By analogy with findings in somatic cells (e.g. Russo *et al.*, 1982; Tsukidate *et al.*, 1993), Cd^{2+} -induced loss of cytoplasmic actin filaments should increase apoptosis and contribute to the production of oligozoospermia. Intracellular Cd^{2+} should also directly stimulate the Ca^{2+} -dependent endonuclease, which produces the DNA fragmentation leading to apoptosis (Lohmann and Beyersmann, 1993). It is likely that the effects of heat stress and increased testicular Cd^{2+} concentrations are synergistic. In the cryptorchid rat, exposure of the testis to the higher abdominal temperature potentiates the toxic effects of Cd^{2+} exposure (Chatterjee and Ray, 1972; Fende and Niewenhuys, 1977). Pathway III invokes androgen deprivation (Sinha-Hakim and Swerdloff, 1995; Hseuh *et al.*, 1996), which may result from endocrine dysfunction (Weiss *et al.*, 1978; Ando *et al.*, 1983; Sirvent *et al.*, 1990) or from an androgen receptor defect (Yoshida *et al.*, 1999).

We now propose an interaction between Cd^{2+} and androgen deprivation based on two sets of observations. First, varicocele often appear at puberty (Steen *et al.*, 1976; Hienz *et al.*, 1980). Second, the susceptibility of the testis of animal models to Cd^{2+} -induced damage is thought to be androgen dependent (Clegg *et al.*, 1969), as newborn animal testes are resistant and the severity of Cd^{2+} -induced damage increases with age (Wong and Klaassen, 1980; Phelps and Laskey, 1989). This interaction is eminently testable through the examination of human testis biopsies, such as used in this study.

Although some authors (McFadden and Mehan, 1978) suggest that routine biopsies of varicocele patients are of limited benefit in clinical management, another early study recommends the prognostic value of testis biopsy when taken at the time of varicocelectomy (Ertib *et al.*, 1967). We concur with the latter and suggest that testis biopsy could become part of the standard clinical evaluation of varicocele patients in future.

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References

- Abdelrahim, F., Mostafa, A., Hamdy, A. *et al.* (1993) Testicular morphology and function in varicocele patients: pre-operative and post-operative histopathology. *Br. J. Urol.*, **72**, 643–647.
- Agger, P. (1971) Scrotal and testicular temperature: its relation to sperm count before and after operation for varicocele. *Fertil. Steril.*, **22**, 286–296.
- Ando, S., Giacchetto, C., Colpi, G. *et al.* (1983) Plasma levels of 17-OH-progesterone and testosterone in patients with varicocele. *Acta Endocrinol.*, **102**, 463–469.
- Aoki, A. and Hoffer, A.P. (1978) Reexamination of the lesions in rat testis caused by cadmium. *Biol. Reprod.*, **18**, 579–591.
- Atassi, O., Kass, E.J. and Steinert, B.W. (1995) Testicular growth after successful varicocele correction in adolescents: comparison of artery sparing techniques with the Palomo procedure [See comments.] *J. Urol.*, **153**, 482–483.
- Bablok, L., Janczewski, Z. and Czaplicki, M. (1985) Testosterone, FSH and LH in human spermatic and cubital venous plasma in varicocele patients. *Andrologia*, **17**, 346–351.
- Bablok, L., Czaplicki, M., Fracki, S. *et al.* (1997) Relationship between semen quality improvement after varicocelectomy and preoperative levels of hypophyseal and gonadal hormones. *Int. Urol. Nephrol.*, **29**, 345–349.
- Belker, A.M. (1981) The varicocele and male infertility. *Urol. Clin. North Am.*, **8**, 41–51.
- Benoff, S. (1997) Environmental toxins and varicocele. *Assist. Reprod. Technol. Androl.*, **9**, 261–284.
- Benoff, S. (1999) Receptors and channels regulating acrosome reactions. *Hum. Fertil.*, **2**, 42–55.
- Benoff, S., Hurley, I.R., Barcia, M. *et al.* (1997) A potential role for cadmium in the etiology of varicocele-associated infertility. *Fertil. Steril.*, **67**, 336–347.
- Benoff, S., Hurley, I.R., Jacob, A. *et al.* (1999) Increased testicular cadmium (Cd^{2+}) levels contribute to infertility with varicocele. [Abstr. No. O-064.] *American Society for Reproductive Medicine, 55th Annual Meeting. Abstracts of the Scientific Oral and Poster Session*. p. S25.
- Benoff, S., Hurley, I.R., Pergolizzi, R.G. and Goodwin, L.O. (2000a) Variation in region IS6 of the L-type voltage-dependent calcium channel $\alpha 1$ subunit in testis and sperm: implications for role of cadmium in varicocele-associated infertility. [Abstr. No. O-147.] *American Society for Reproductive Medicine, 56th Annual Meeting. Abstracts of the Scientific Oral and Poster Session*. p. S55–S56.
- Benoff, S., Jacob, A. and Hurley, I.R. (2000b) Male infertility and environmental exposure to lead and cadmium. *Hum. Reprod. Update*, **6**, 107–121.
- Brown, J.S. (1976) Varicocelectomy in the subfertile male: a ten-year experience with 295 cases. *Fertil. Steril.*, **27**, 1046–1053.
- Brown, J.S., Dubin, L. and Hotchkiss, R.S. (1967) The varicocele as related to fertility. *Fertil. Steril.*, **18**, 46–56.
- Castro-Magana, M., Angulo, M.A., Canas, J.A. and Uy, J.S. (1989) Improvement of Leydig cell function in male adolescents after varicocelectomy. *J. Pediatr.*, **115**, 809–812.
- Castro-Magana, M., Angulo, M., Canas, A. and Uy, J. (1990) Leydig cell function in adolescent boys with varicoceles. *Arch. Androl.*, **24**, 73–79.
- Charny, C.W. (1962) Effects of varicocele on fertility. *Fertil. Steril.*, **13**, 47–56.
- Chatterjee, A. and Ray, P. (1972) An early differential effect of cadmium on the scrotal and contralateral cryptorchid testes of the rat. *J. Reprod. Fertil.*, **30**, 297–300.
- Clegg, E.J., Niemi, M. and Carr, I. (1969) The age at which the blood vessels of the rat testis become sensitive to cadmium salts. *J. Endocrinol.*, **43**, 445–449.
- Clough, S.R., Welsh, M.J., Payne, A.H. *et al.* (1990) Primary rat Sertoli cells and interstitial cells exhibit a differential response to cadmium. *Cell Biol. Toxicol.*, **6**, 63–79.
- Comhaire, F. (1991) The pathogenesis of epididymo-testicular dysfunction in varicocele: factors other than temperature. *Adv. Exp. Biol. Med.*, **286**, 281–285.
- Cozzolino, D.J. and Lipshultz, L.I. (2001) Varicocele and male infertility Part I: Varicocele as a progressive lesion: positive effect of varicocele repair. [Minisymposium.] *Hum. Reprod. Update*, **7**, 55–58.
- Das, K.M., Prasad, K., Szmigielski, W. and Noorani, N. (1999) Intratesticular

- varicocele: evaluation using conventional and Doppler sonography. *Am. J. Roentgenol.*, **173**, 1079–1083.
- Davidson, H.P. (1954) Treatment of male subfertility, testicular temperature and varicocele. *Practitioner*, **173**, 703.
- Dubin, L. and Hotchkiss, R.S. (1969) Testis biopsy in subfertile men with varicocele. *Fertil. Steril.*, **20**, 50–57.
- Etriby, A., Girgis, S.M., Hefnawy, H. and Ibrahim, A.A. (1967) Testicular changes in subfertile males with varicocele. *Fertil. Steril.*, **18**, 666–671.
- Fende, P.L. and Niewenhuis, R. (1977) An electron microscopic study of the effects of cadmium chloride on cryptorchid testes of the rat. *Biol. Reprod.*, **16**, 298–305.
- Gazid, T. and Kaminski, M. (1984) Ultrastructural study of development of the rat testis. II. After injection of CdCl₂. *Folia Morphol.*, **32**, 218–222.
- Gasser, G. (1971) Varicocele. *Wien. klin. Wschr.*, **83**, 484–487.
- Gershbein, A.B., Horowitz, M. and Glassberg, K.I. (1999) The adolescent varicocele I: left testicular hypertrophy following varicocelectomy. *J. Urol.*, **162**, 1447–1449.
- Gilbert, B.R. and Goldstein, M. (1988) New directions in male reproductive microsurgery. *Microsurgery*, **9**, 281–285.
- Girardi, S.K. and Goldstein, M. (1997) Varicocele. *Curr. Therapy Endocrinol. Metabol.*, **6**, 355–358.
- Girgis, S.M., Abdalla, M.I., Ibrahim, I.I. *et al.* (1981) Clinical and hormonal studies of subfertile males with varicocele. *Arch. Androl.*, **6**, 267–271.
- Goodwin, L.O., Leeds, N.B., Hurley, I. *et al.* (1998) Alternative splicing of exons in the alpha-1 subunit of the rat testis voltage-dependent calcium channel generates germ-line specific dihydropyridine binding sites. *Mol. Hum. Reprod.*, **4**, 215–226.
- Goldstein, M. (1995) Complications and results of varicocelectomy. In Goldstein, M. (ed.) *Surgery of Male Infertility*. W.B.Saunders Co, Philadelphia, USA, pp. 194–196.
- Goldstein, M., Gilbert, B.R., Dicker, A.P. *et al.* (1992) Microsurgical inguinal varicocelectomy with delivery of the testis: an artery and lymphatic sparing technique. *J. Urol.*, **148**, 1808–1811.
- Gorelick, J.I. and Goldstein, M. (1993) Loss of fertility in men with varicocele. *Fertil. Steril.*, **59**, 613–616.
- Gunn, S.A., Gould, T.C. and Anderson, W.A.D. (1961) Competition for zinc in rat testis and dorsolateral prostate. *Acta Endocrinol.*, **37**, 24–30.
- Gunn, S.A., Gould, T.C. and Anderson, W.A.D. (1968) Mechanisms of zinc, cysteine and selenium protection against cadmium-induced vascular injury to the mouse testis. *J. Reprod. Fertil.*, **15**, 65–70.
- Haans, L.C., Laven, J.S., Mali, W.P. *et al.* (1991) Testis volumes, semen quality, and hormonal patterns in adolescents with and without a varicocele. *Fertil. Steril.*, **56**, 731–736.
- Hadziselimovic, F., Herzog, B., Liebungut, P. *et al.* (1989) Testicular and vascular changes in children and adults with varicocele. *J. Urol.*, **142**, 583–585.
- Hew, K.-H., Ericson, W.A. and Welsh, M.J. (1993a) A single low cadmium dose causes failure of spermiogenesis in the rat. *Toxicol. Appl. Pharmacol.*, **121**, 15–21.
- Hew, K.-H., Heath, G.L., Jiwa, A.H. and Welsh, M.J. (1993b) Cadmium *in vivo* causes disruption of tight junction-associated microfilaments in rat Sertoli cells. *Biol. Reprod.*, **49**, 840–849.
- Hienz, H.A., Voggenthaler, J. and Weissbach, L. (1980) Histological findings in testes with varicocele during childhood and therapeutic consequences. *Eur. J. Pediatr.*, **133**, 139–146.
- Honig, S.C., Lipshultz, L.I. and Jarow, J. (1994) Significant medical pathology uncovered by a comprehensive male infertility evaluation. *Fertil. Steril.*, **62**, 1028–1034.
- Hsueh, A.J.W., Eisenhauer, K., Chun, S.-Y. *et al.* (1996) Gonadal cell apoptosis. *Recent Prog. Hormone Res.*, **51**, 433–456.
- Hudson, R.W., Perez-Marrero, R.A., Crawford, V.A. and McKay, D.E. (1986) Hormonal parameters in incidental varicoceles and those causing infertility. *Fertil. Steril.*, **45**, 692–700.
- Hurley, I.R., Cooper, G.W., Napolitano, B. *et al.* (2000) High testicular cadmium levels in varicocele-associated infertility. *Andrologia*, **32**, 191.
- Ibrahim, A.A., Awad, H.A., El-Haggag, S. and Mitawi, B.A. (1977) Bilateral testicular biopsy in men with varicocele. *Fertil. Steril.*, **28**, 663–667.
- Jarow, J.P. (1994) Life-threatening conditions associated with male infertility. *Urol. Clin. North. Am.*, **21**, 409–415.
- Jarow, J.P. (2001) Varicocele and male infertility Part I: Varicocele and male infertility. [Minisymposium.] *Hum. Reprod. Update*, **7**, this issue.
- Johnsen, S.G. (1970) Testicular biopsy score count – a method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males. *Hormones*, **1**, 2–25.
- Johnsen, S.G. and Agger, P. (1978) Quantitative evaluation of testicular biopsies before and after operation for varicocele. *Fertil. Steril.*, **29**, 58–63.
- Jones, M.M., Xu, C. and Ladd, P.A. (1997) Selenite suppression of cadmium-induced testicular apoptosis. *Toxicology*, **116**, 169–175.
- Kamischke, A. and Nieschlag, E. (2001) Varicocele and male infertility Part I: Varicocele treatment in the light of evidence-based andrology. [Minisymposium.] *Hum. Reprod. Update*, **7**, this issue.
- Kass, E.J. and Belman, A.B. (1987) Reversal of testicular growth failure by varicocele ligation. *J. Urol.*, **137**, 475–476.
- Kass, E.J. and Reitelman, C. (1995) Adolescent varicocele. *Urol. Clin. North. Am.*, **22**, 151–159.
- Kass, E.J., Chandra, R.S. and Belman, A.B. (1987) Testicular histology in the adolescent with a varicocele. *Pediatrics*, **79**, 996–998.
- Kim, E.D., Leibman, B.B., Grinblat, D.M. and Lipshultz, L.I. (1999) Varicocele repair improves semen parameters in azoospermic men with spermatogenic failure. *J. Urol.*, **162**, 737–740.
- Lemack, G.E., Uzzo, R.G., Schlegel, P.N. and Goldstein, M. (1998) Microsurgical repair of the adolescent varicocele. *J. Urol.*, **160**, 179–181.
- Lipshultz, L.I. and Corriere, J.N. Jr. (1977) Progressive testicular atrophy in the varicocele patient. *J. Urol.*, **117**, 175–176.
- Lohmann, R.D. and Beyersmann, D. (1993) Cadmium and zinc mediated changes of the Ca²⁺-dependent endonuclease in apoptosis. *Biochem. Biophys. Res. Commun.*, **190**, 1097–1103.
- Lund, L., Ernst, E., Sorensen, H.T. and Oxlund, H. (1998) Biochemical properties of normal and varicose internal spermatic veins. *Scand. J. Urol. Nephrol.*, **32**, 47–50.
- Lund, L., Tang, Y.C., Roebuck, D. *et al.* (1999) Testicular catch-up growth after varicocele correction in adolescents. *Pediatr. Surg. Int.*, **15**, 234–237.
- Lyon, R.P., Marshall, S. and Scott, M.P. (1982) Varicocele in childhood and adolescence implication in adulthood infertility? *Urology*, **19**, 641–644.
- MacLeod, J. (1965) Seminal cytology in presence of varicocele. *Fertil. Steril.*, **16**, 735–757.
- Marmar, J.L. (1998) The emergence of specialized procedures for acquisition, processing and cryopreservation of epididymal and testicular sperm in connection with intracytoplasmic sperm injection. *J. Androl.*, **19**, 517–526.
- Marmar, J.L. and Kim, Y. (1994) Subinguinal microsurgical varicocelectomy: a technical critique and statistical analysis of semen and pregnancy data. *J. Urol.*, **152**, 1127–1132.
- Marmar, J.L., DeBenedictis, T.J. and Praiss, D. (1985) The management of varicoceles by microdissection of the spermatic cord at the external inguinal ring. *Fertil. Steril.*, **43**, 583–588.
- Mason, K.E., Brown, J.A., Young, J.O. and Nesbit, R.R. (1964) Cadmium-induced injury of the rat testis. *Anat. Rec.*, **149**, 135–148.
- Matthews, G.J., Matthews, E.D. and Goldstein, M. (1998) Induction of spermatogenesis and achievement of pregnancy after microsurgical varicocelectomy in men with azoospermia and severe oligoasthenozoospermia. *Fertil. Steril.*, **70**, 71–75.
- McFadden, M.R. and Mehan, D.J. (1978) Testicular biopsies in 101 cases of varicocele. *J. Urol.*, **119**, 372–374.
- McLaren, T.T., Foster, P.M.D. and Sharpe R.M. (1994) Identification of stage-specific changes in protein secretion by isolated seminiferous tubules from rats following exposure to short-term local testicular heating. *J. Reprod. Fertil.*, **102**, 293–300.
- Mićić, S., Dotlić, R., Ilić, V. and Genbačev, O. (1986) Seminal plasma hormone profile in infertile men with and without varicocele. *Arch. Androl.*, **17**, 173–178.
- Mieusset, R. and Bujan, L. (1995) Testicular heating and its possible contributions to male infertility: a review. *Int. J. Androl.*, **18**, 169–184.
- Mieusset, R., Bujan, L., Mansat, A. and Pontonnier, F. (1995) Clinical and biological characteristics of infertile men with a history of cryptorchidism. *Hum. Reprod.*, **10**, 613–619.
- Naftulin, B.N., Samuels, S.J., Hellstrom, W.J. *et al.* (1991) Semen quality in varicocele patients is characterized by tapered sperm cells. *Fertil. Steril.*, **56**, 149–151.
- Nagao, R.R., Plymate, S.R., Berger, R.E. *et al.* (1986) Comparison of gonadal function between fertile and infertile men with varicocele. *Fertil. Steril.*, **46**, 930–933.
- Nagler, H.M. (1996) Commentary on the testicular microenvironment. *J. Urol.*, **155**, 800–801.
- Nagler, H., Luntz, R. and Martinis, F. (1997) Varicocele. In: Lipshultz, L. and Howards, S., editors. *Infertility in the Male*. St. Louis: Mosby, p. 336.
- Noske, H.-D. and Weidner, W. (1999) Varicocele – a historical perspective. *World J. Urol.*, **17**, 151–157.
- Oldereid, N.B., Thomassen, Y., Attramadal, A. *et al.* (1993) Concentrations of

- lead, cadmium and zinc in the tissues of reproductive organs of men. *J. Reprod. Fertil.*, **99**, 421–425.
- Paduch, D.A. and Niedzielski, J. (1997) Repair versus observation in adolescent varicocele: a prospective study. *J. Urol.*, **158**, 1128–1132.
- Peterson, A.C., Lance, R.S. and Ruiz, H.E. (1998) Outcomes of varicocele ligation done for pain. *J. Urol.*, **159**, 1565–1567.
- Phelps, P.V. and Laskey, J.W. (1989) Comparison of age-related changes in *in vivo* and *in vitro* measures of testicular steroidogenesis after acute cadmium exposure in the Sprague-Dawley rat. *J. Toxicol. Environ. Health.*, **27**, 95–105.
- Pryor, J.L. and Howards, S.S. (1987) Varicocele. *Urol. Clin. North Am.*, **14**, 499–512.
- Riba, L.W. (1947) Excision of the internal spermatic vein for varicocele. *J. Urol.*, **57**, 889–893.
- Russell, J.K. (1954) Varicocele in groups of fertile and subfertile males. *Brit. Med. J.*, **1**, 1231.
- Russell, L.D., Saxena, N.K. and Turner, T.T. (1989) Cytoskeletal involvement in spermiation and sperm transport. *Tissue Cell*, **21**, 261–279.
- Russo, M.A., Kane, A.B. and Farber, J.L. (1982) Ultrastructural pathology of phalloidin-intoxicated hepatocytes in the presence and absence of extracellular calcium. *Am. J. Pathol.*, **109**, 133–144.
- Santamaria, L., Martin, R., Nistal, M. and Paniagua, R. (1992) The peritubular myoid cells in the testes from men with varicocele: an ultrastructural, immunohistochemical and quantitative study. *Histopathology*, **21**, 423–433.
- Santoro, G., Romeo, C., Imperlizzeri, P. *et al.* (2000) Ultrastructural and immunohistochemical study of basal lamina of the testis in adolescent varicocele. *Fertil. Steril.*, **73**, 699–705.
- Schlegel, P.N. (1997) Is assisted reproduction the optimal treatment for varicocele-associated male infertility? A cost-effectiveness analysis. *Urology*, **49**, 83–90.
- Scherr, D. and Goldstein, M. (1999) Comparison of bilateral versus unilateral varicoectomy in men with palpable bilateral varicoceles. *J. Urol.*, **162**, 85–88.
- Scott, L.S. (1958) The effect of varicocele on spermatogenesis. *Proc. Soc. Study Fertil.*, **10**, 33.
- Scott, L.S. (1961) Varicocele as a treatable cause of subfertility. *Brit. J. Med.*, **1**, 788.
- Setchell, B.P. and Waites, G.M.H. (1970) Changes in the permeability of the testicular capillaries and of the ‘blood-testis barrier’ after injection of cadmium chloride in the rat. *J. Endocrinol.*, **47**, 81–86.
- Shafik, A. and Bedeir, G.A.M. (1980) Venous tension patterns in cord veins. I. In normal and varicocele individuals. *J. Urol.*, **123**, 383–385.
- Sigman, M. and Jarow, J.P. (1997) Ipsilateral testicular hypotrophy is associated with decreased sperm counts in infertile men with varicoceles. *J. Urol.*, **158**, 605–607.
- Silber, S.J. (2001) Varicocele and male infertility Part I: The varicocele dilemma. [Minisymposium.] *Hum. Reprod. Update*, **7**, 70–77.
- Simsek, F., Turkeri, L., Cevik, I. *et al.* (1998) Role of apoptosis in testicular damage caused by varicocele. *Arch. Esp. Urol.*, **9**, 947–950.
- Sinha-Hakim, A.P. and Swerdloff, R.S. (1995) Temporal and stage-specific effects of recombinant human follicular stimulating hormone on the maintenance of spermatogenesis in gonadotropin releasing hormone antagonist treated rat. *Endocrinology*, **136**, 253–261.
- Sirvent, J.J., Bernat, R., Navarro, M.A. *et al.* (1990) Leydig cell in idiopathic varicocele. *Eur. Urol.*, **17**, 257–261.
- Steen, O., Knops, J., Declerk, L. *et al.* (1976) Prevention of fertility disorders by detection and treatment of varicocele at school and college age. *Andrologia*, **8**, 47–53.
- Steckel, J., Dicker, A.P. and Goldstein, M. (1993) Relationship between varicocele size and response to varicoectomy. *J. Urol.*, **149**, 769–771.
- Sweeney, T.E., Rozum, J.S., Desjardins, C. and Gore R.W. (1991) Microvascular pressure distribution in the hamster testis. *Am. J. Physiol.*, **260**, H1581–H1589.
- Sweeney, T.E., Rozum, J.S. and Gore R.W. (1995) Alteration of testicular microvascular pressures during venous pressure elevation. *Am. J. Physiol.*, **269**, H37–H45.
- Swerdloff, R.S. and Walsh, P.C. (1975) Pituitary and gonadal hormones in patients with varicocele. *Fertil. Steril.*, **26**, 1006–1012.
- Sylora, J.A. and Pryor, J.L. (1994) Varicocele. *Curr. Therapy Endocrinol. Metabol.*, **5**, 309–314.
- Tsukidate, K., Yamamoto, K., Snyder, J.W. and Farber, J.L. (1993) Microtubule antagonists activate programmed cell death (apoptosis) in cultured rat hepatocytes. *Am. J. Pathol.*, **143**, 918–925.
- Tulloch, W.S. (1952) A consideration of sterility factors in the light of subsequent pregnancies. *Edinburgh Med. J.*, **59**, 29–34.
- Tulloch, W.S. (1955) Varicocele in subfertility, results of treatment. *Brit. Med. J.*, **2**, 356.
- Turner, T.T. (2001) Varicocele and male infertility Part I: The study of varicocele through the use of animal models. [Minisymposium.] *Hum. Reprod. Update*, **7**, 78–84.
- Vogl, A.W. (1989) Distribution and function of organized concentrations of actin filaments in mammalian spermatogenic cells and Sertoli cells. *Int. Rev. Cytol.*, **119**, 1–56.
- Wang, Z., Chin, T.A. and Templeton, D.M. (1996) Calcium-independent effects of cadmium on actin assembly in mesangial and vascular smooth muscle cells. *Cell Motil. Cytoskel.*, **33**, 208–222.
- Weiss, D.B., Rodriguez-Riguau, L.J., Smith, K.D. and Steinberger, E. (1978) Leydig cell function in oligospermic men with varicocele. *J. Urol.*, **120**, 427–430.
- Witt, M.A. and Lipshultz, L.I. (1993) Varicocele: a progressive or static lesion? *Urology*, **42**, 541–543.
- Wong, K. and Klaassen, C.D. (1980) Age differences in the susceptibility to cadmium induced testicular damage in rats. *Toxicol. Appl. Pharmacol.*, **55**, 456–466.
- Wright, E.J., Young, G.P.H. and Goldstein, M. (1997) Reduction in testicular temperature after varicoectomy in infertile men. *Urology*, **50**, 257–259.
- Yamaguchi, M., Sakotu, J. and Takihara, H. (1989) The application of intrascrotal deep body temperature measurement for the noninvasive diagnosis of varicocele. *Fertil. Steril.*, **52**, 295–301.
- Yamamoto, M., Hibi, H., Katsuno, S. and Miyake, K. (1995) Human chorionic gonadotropin adjuvant therapy for patients with Leydig cell dysfunction after varicoectomy. *Arch. Androl.*, **35**, 49–55.
- Yan, H., Carter, C.E., Xu, C. *et al.* (1997) Cadmium-induced apoptosis in the urogenital organs of the male rat and its suppression by chelation. *J. Toxicol. Environ. Health*, **52**, 149–168.
- Yavetz, H., Levy, R., Papo, J. *et al.* (1992) Efficacy of varicocele embolization versus ligation of the left internal spermatic vein for improvement of sperm quality. *Int. J. Androl.*, **15**, 338–344.
- Yin, Y., Hawkins, K.L., Dewolf, W.C. and Morgentaler, A. (1997) Heat stress causes testicular germ cell apoptosis in adult mice. *J. Androl.*, **18**, 159–165.
- Yoshida, K.I., Yano, M., Chiba, K. *et al.* (1999) CAG repeat length in the androgen receptor gene is enhanced in patients with idiopathic azoospermia. *Urology*, **54**, 1078–1081.
- Young, D. (1956) The influence of varicocele on human spermatogenesis. *Brit. J. Urol.*, **28**, 426.