[Frontiers in Bioscience 14, 3641-3672, January 1, 2009]

Molecular and other predictors for infertility in patients with varicoceles

Susan Benoff[1,2,3,5], Joel L. Marmar[4], Ian R. Hurley[1]

[1]Fertility Research Laboratories, Center for Oncology and Cell Biology, The Feinstein Institute for Medical Research, North Shore-Long Island Jewish Health System, Manhasset, New York, [2]Department of Obstetrics and Gynecology, North Shore University Hospital, Manhasset, New York, [3]Departments of Obstetrics and Gynecology and Cell Biology, New York University School of Medicine, New York, New York, [4]Department of Urology, Robert Wood Johnson School of Medicine, Camden, New Jersey, [5]350 Community Drive, Room 125, Manhasset, New York 11030

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1. ABSTRACT

Varicoceles are a treatable cause of male infertility, but very clinically diverse. Both histologic and molecular changes occur in the testes of men with varicocele. Physical measurements (scrotal temperature, testicular volume, pressure within the pampiniform plexus, basal lamina thickness) correlate with prognosis, but these correlations have not been accepted as predictors of successful repair because of variation within patient populations. Conventional semen parameters similarly correlate, but these correlations apply only to men with >5 $x10^6$ sperm/ejaculate. Levels of toxicants (e.g. norepinephrine, cadmium), reactive oxygen species byproducts, and hormones, their receptors and modulators have been evaluated as predictors in small-scale studies. Medical therapies (antoxidants, anti-inflammatories and hormones) have been applied empirically to small groups of patients with positive results that have not been verified in large-scale trials. Thus, urologists still face a challenge to determine which patients will benefit from varicocelectomies and/or medical interventions. In this review we summarize our current understanding of the pathophysiology of varicoceles, and discuss some of the new findings that may be applicable to specific clinical situations.

2. DEFINITION

Varicoceles are dilated veins within the pampiniform plexus that develop from retrograde blood flow and an increased hydrostatic pressure in the internal spermatic veins and cremasteric veins (1). These lesions develop because of absent or faulty internal valves. Varicoceles frequently occur unilaterally on the left side (2), probably due to asymmetry of the internal spermatic veins that alters its properties (hypertrophy of longitudinal smooth muscles and increased extensibility of the left compared to the right spermatic vein) (3,4). The "nutcracker effect" maybe an added influence, whereby the left renal vein is compressed by the aorta and the superior mesenteric artery leading to blood stasis in the pampiniform plexus. In the early clinical studies, the diagnosis was based on palpation while the patient stood in the up-right position and performed the Valsalva maneuver, but palpation of the "reflexive thrill" may be difficult especially in men with short thick scrotal walls (5). More recently, venography became the "gold standard" for documentation of reflux, but these studies were invasive. Currently, varicoceles have been graded by ultrasound: Grade 1 (audible by Doppler sonography, not visible, not palpable), Grade 2 (audible, palpable, not visible) or Grade 3 (audible, palpable and visible) (7-9). Some consider Grade 1 as "subclinical"; but the reflux may be significant in these cases if the retrograde flow is continues throughout the Valsalva maneuver (7). Grades 2 and 3 are considered as "clinical".

3. FREQUENCY OF OCCURRENCE AND PRESENTATION

Varicoceles often appear at puberty (review, 10,11). Clinical varicoceles occur in about 12% of the general population of men with normal semen parameters (9). The frequency of varicocele occurrence is higher in men with primary infertility, ranging between 20% to 40% (9,12-15). Increased prevalence of variococeles with increasing age has recently been demonstrated (16). The frequency of varicoceles maybe even higher in men with secondary infertility, with some estimates as high as 80% (17,18). While at least one report argues against a connection between varicocele and secondary infertility by presenting data suggesting that the incidence of varicocele among this group was similar in men with primary infertility (19), the majority of the literature associates varicocele with time-dependent testicular damage and decrease in semen quality (review, 10,11).

Two recent publications indicate that the prevalence of varicoceles is increased among first-degree relatives of men with previously diagnosed varicoceles (20,21). These data provide suggestive data for the existence of a genetic component that is not linked to either varicocele size or laterality of the varicocele.

Typically, the pathologic state for a patient with a varicocele is associated with a persistent abnormality of at least one semen parameter (count, motility or morphology) or an abnormal sperm function test. Decreased sperm counts are accompanied by elevated levels of apoptosis among testicular and ejaculated sperm (22-25). Even with unilateral lesions, there may be bilateral changes in blood flow, testicular temperature and histology (review, 24,26). Some of these changes may be initiated by excess nitric oxide released by elevated vein pressures, and this gas may diffuse bilaterally.

3.1. Association with infertility and current treatment

Although varicoceles were recognized as early as the first century AD (27), the treatment of these lesions was limited to men with pain. The possible association between varicoceles and male infertility was only first recognized in 1952 based on a case report of an azoospermic male in which bilateral varicocelectomy was followed by an improvement in sperm count and pregnancy by coitus (28). Subsequent early studies suggested that the varicocele incidence was increased in infertile men (29,30) and that semen parameters were improved following surgical varicocele repair (31-37), but these studies were not controlled.

These lesions have now been surgically repaired for the treatment of infertility for almost 50 years and approximately 20,000-40,000 varicocelectomies are performed each year (38-40). A body of literature indicates that varicocele ligation results in improved semen parameters and leads to pregnancy (in 24% to 53% of cases, varying by study) (1,7,14,15,41,42). Thus, varicoceles are thought by some to be among the most common treatable causes of male infertility based on data from case controlled studies.

Varicocelectomy has been empirically recommended based on history of infertility and two or

more semen analyses demonstrating at least one parameter below normal levels (e.g., 7,14,43,44).

4. THE VARICOCELE CONTROVERSY

Study-to-study variations have perpetuated the controversy of varicocele surgery for decades (e.g., 45-47). Some studies "flat out" have concluded that varicocele surgery has no benefit (e.g., 48). We believe that these differences of opinion may be due, in part, to clinical diversity that includes variations in scrotal temperatures, in lesion size, in bilaterality, in internal spermatic vein hydrostatic pressures, in degrees of blood flow stasis, in histological findings, and in effects on spermatogenesis (26). It is also due in part to selection bias among studies to be included in meta-analyses to determine clinical efficacy (49).

Members of our group recently developed new meta-analyses from existing studies of varicocelectomy's effects on semen parameters and pregnancy outcome (49,50). They included both randomized control trial and observational studies that were examined for bias, but these studies were limited to those of infertile men with palpable varicoceles, surgical repairs, and natural pregnancy outcomes. They determined that varicocelectomy in men with a minimum of one abnormal semen parameter increased the odds of spontaneous pregnancy by 2.87 (49). We believe that these meta-analyses refute the earlier studies and establish a statistical justification for varcocele repair. The increased pregnancy rates that our members found likely resulted from surgery-associated increases in sperm count (50), since there are data indicating that fertility status is linked to sperm density (51-53) and that the sperm density increase in response to varicocelectomy can predict pregnancy post-surgery (24,54,55).

Reproductive endocrinologists often favor IVF/ISCI as the treatment of varicocele related infertility, but the economics of varicocele repair suggest a different treatment paradigm. Combining varicocele surgery and assisted reproduction could maximize the delivered baby rate/couple at a lower cost. The cost per delivered baby by IVF/ISCI is approximately $89,091, while after varicocelectomy it is only $26,268 (42). IVF/ICSI over one cycle and varicocelectomy have similar delivery rates of 28% and 30%, respectively. By pre-selecting patients likely to benefit from a varicocele repair and utilizing post-op IUI, the couple can save up to $62,823 per live birth (55). Members of our group have performed a similar calculation using their repair statistics that suggests 52% of infertile couples should achieve pregnancy by combining surgery followed by IUI and IVF/ICSI for salvage, the cost was $26,163/baby/couple (56).

However, the Varicocele Controversy will persist because the following questions remain unanswered. "Why do some men with varicoceles father children and have normal semen analyses?", "Why do some men fail to improve following varicocelectomy?", "Why do small lesions have the same affects as large lesions?" and "Why does a left-sided lesion cause bilateral effects and infertility?" We think it unlikely that varicocele repair will be accepted as standard of care by both reproductive endocrinologists and urologists until the underlying mechanism (s) producing infertility with varicoceles is identified and validated markers that will add selectivity to varicocele surgery become available. Identification of such markers, however, requires an understanding of the underlying mechanism (s) producing infertility in the presence of varicoceles.

5. POTENTIAL MECHANISMS

Hypotheses proposed to explain the deleterious effects of varicoceles include: (1) scrotal/testicular hyperthermia (retrograde blood flow and venous stasis reducing heat exchange by the pampiniform plexus), (2) increased venous pressures (from retrograde blood flow), (3) accumulation of toxic substances (adrenal by-products, components of cigarette smoke), (4) hypoxia (from venous stasis), and (5) hormonal imbalance. Of these, increased testicular temperature remains the most widely accepted, as varicocele repair lowers testicular temperatures (57-59).

Elevated testicular temperatures, however, cannot solely be responsible for the adverse effects of varicoceles because of: (1) significant overlap between the range of scrotal temperatures from infertile men with varicoceles and fertile men (60-63) or infertile men without varicoceles (61,64), (2) considerable intra-male variability in scrotal temperature measurements (65), and (3) scrotal temperature varies with body position (66-68). Therefore, we (10,24,26) and others (69) share the opinion that it is the interaction of varicoceles with intrinsic or extrinsic "co-morbidity factors" that induces the infertile state.

6. DEFICITS ALREADY IDENTIFIED

The data presented below links observed deficits to hypothesized mechanisms and outcome of varicocele surgery. What is apparent is that the various deficits to not act *independently* but rather interact.

6.1. Scrotal/testicular hyperthermia

Scrotal/testicular hyperthermia with varicoceles is a consequence of retrograde blood flow and stasis that interferes with countercurrent cooling of arterial blood by the pampiniform plexus (70-72). Arrest of sperm maturation in infertile men with varicoceles has been linked to the degree of scrotal hyperthermia (73). This is in agreement with histological analyses of testicular biopsies that demonstrated varicoceles are associated with damage to the seminiferous epithelium resulting in hypospermatogenesis or maturation arrest (74-76). Scrotal hyperthermia has also been linked to elevations in serum gonadotropin levels (61). The connection between spermatogenic arrest and scrotal hyperthermia was examined by a recent randomized clinical trial. Scrotal hyperthermia has also lead to elevated germ cell apoptosis and suppression of spermatogenesis (77). This agrees with earlier histological studies that found germ cell apoptosis is increased with varicoceles (22-25).

Several plausible components of an underlying mechanism (s) have been identified. Testes biopsies showed that apoptosis is effected via activation of the death receptor pathway, through a decrease in soluble Fas (sFas) concentration in seminal plasma (78) and/or an increase in Fas ligand (FasL) levels (79). The effects of apoptosis are likely amplified due to a decrease in DNA synthesis in testes from infertile men with varicoceles in comparison to normal control testes, as measured by decreased expression of proliferating cell nuclear antigen (PCNA) (80,81). One trigger of apoptosis may involve a cold-inducible RNA-binding protein (Cirp) that appears required for normal spermatogenesis and is related to a candidate for human azoospermia factor (RBM1), which is expressed in normal animal testes and is reduced when testicular temperature is elevated by cryptorchidism or hot baths (82). Cirp is also down-regulated in human testis in human males with varicoceles as compared to controls without varicocele (82). Heat shock proteins (HSPs) required for normal spermatogenesis may also be involved. These are inducible in the testis by stresses such as hyperthermia, radiation and chemicals (83). However, the study of heat shock protein expression in varicocele-associated infertility has been at best limited. Where studied, mutations in heat shock proteins have not been linked to infertility with varicoceles (84). HSPA2 appears associated with thermal tolerance, in that its expression is down-regulated in adolescents and adults with varicoceles and oligospermia (85,86). Nonetheless, that sperm HSPA2 activity is increased after varicocelectomy (85) is counter-intuitive.

Consistent with this mechanism, post-operative reductions in scrotal temperature have been observed (57-59). Comparison of matched tissues from individual patients pre- and post-varicocelectomy has revealed that spermatogenesis is improved (35,75,87), potentially through increased levels of sFas (81) and PCNA (81) seen post-operatively.

6.2. Increased venous pressures

Competent venous valves are commonly absent in varicocele patients (88-92). With no valves to prevent it, retrograde blood flow leads to increased venous tension in the cord veins of infertile men with varicoceles and impaired venous drainage (91,94-98). However, the increased venous tension is unlikely to be continuous, as internal spermatic vein hydrostatic pressures are dependent on changes in body position (99,100).

Retrograde blood flow has more than one deleterious effect. In addition to leading to testicular hyperthermia (101,102), the increased venous tension damages the seminiferous endothelium (103) and increases both and interstitial fluid pressure and interstitial fluid volume (104), The damage to the epithelium potentially assists entry of toxic substances into the testis (24) while the impaired venous drainage of the intersititium induces hypoxia (97,98).

Varicocelectomy reduces venous tension and is associated with improved semen parameters and pregnancy rates (100).

6.3. Accumulation of toxic substances

Accumulation of intrinsic or extrinsic toxic substances may impair spermatogenesis in varicoceles.

6.3.1 Intrinsic toxins

Intrinsic toxins include adrenal steroids and catecholamines. A varicocele should increase their levels in the testis through retrograde blood flow. MacLeod (36) suggested that reflux of adrenal steroids was responsible for impaired spermatogenesis with varicoceles. However, direct measurements of cortisol and dehydroepiandrosterone found similar levels in spermatic venous blood and peripheral venous blood (99,105-107). In contrast, catecholamines (in particular, norepinephrine) and noradrenaline are elevated in the refluxing venous blood (107,108). Higher catecholamines should induce endothelial hyperplasia, which could impair testicular perfusion irreversibly, while noradrenaline might act via vasoconstriction. Both would lead to testicular hypoxia (109). Varicocele repair should reduce exposure to catecholamines and subsequently improve semen parameters (108).

6.3.2. Extrinsic toxins

Extrinsic toxins include components of cigarette smoke. These components concentrate in semen (110,111). Cigarette smoking is associated with an increase in oligospermia with varicoceles (112).

Cadmium, a major toxin in cigarette smoke, is also present at significant levels in environmental exposures from food (113). Cadmium is elevated in testis tissue and seminal plasma from infertile men with varicoceles, even if they were not exposed occupationally or did not smoke cigarettes (24,114). We have observed that approximately 60% of infertile men with varicoceles present with elevated testicular cadmium (S. Benoff and J.L. Marmar, unpublished observations). Cadmium enters seminiferous epithelium through L-type calcium channels (115). Cadmium entry is associated with elevated germ cell apoptosis (24), possibly through : (1) altered calcium homeostasis (115-117), (2) cadmium-induced scrotal temperature increase (118), and/or (3) induction of nitric oxide expression and oxidative stress (119,120). Mechanism (1) suggests why elevated testicular cadmium is associated with more estensive expression of splice variants of the L-type calcium channel (115,116). These splice variants are associated with decreased testicular apoptosis (121), suggesting that the testis mounts a protective response if calcium homeostasis is altered (115,116). Mechanisms (2) and (3) should intensify injuries intiated by varicoceles. Mechanism (2) is supported by *in vitro* analysis of the combined effects of cadmium and elevated temperature on sperm function compared to their action separately (114). Elevated cadmium could induce vascular endothelial growth factor (VEGF) expression (122) and increase angiogenesis. VEGF is elevated in testes from infertile men with varicoceles (123,124).

While elevated VEGF is often deleterious (e.g., 124; also see below), we suggest that it might protect the testis from cadmium insult (123). Somatic cell studies

indicate that VEGF interferes with apoptosis. In leukemic cells for example, VEGF, among other effects, increases anti-apoptotic Bcl-2 by inducing HSP-90 and promotes Bcl-2 binding to HSP-90 and HSP-90 binding to Apaf-1 (125). In endothelial cells, VEGF inhibits apoptosis by up-regulating survivin, a member of the inhibitors of apoptosis family (IAP) 10-20 fold (126). In our pilot study (123), we found an inverse relationship between VEGF expression and germ cell apoptosis.

Since cadmium induces both nitric oxide production and VEGF expression, it could amplify the effects of hypoxia (see below). In our clinical study we found that varicocele repair was not effective when testicular cadmium levels were elevated (24,127).

6.4. Hypoxia

While the clinical literature does not clearly show that varicoceles are associated with testicular hypoxia (128,129), association with stagnation of the blood in the testicular blood vessels has been convincingly demonstrated (130), so hypoxia can be reasonably inferred. Recent clinical findings that venous reflux correlates with histopathological evidence for testicular blood stasis and with ischaemic damage to the seminiferous epithelium is in agreement with this inference (97,98).

Biochemical changes associated with hypoxia often associate with varicoceles. These changes involve hypoxia-inducing factor 1 alpha, VEGF, nitric oxide and reactive oxygen species. Hypoxia-inducible factor-1alpha is detected in the internal spermatic veins of patients with varicoceles (131). VEGF, which can be induced by hypoxia (132), is elevated in the testis of subjects with varicocele (124). Testicular VEGF levels are inversely related to sperm count, sperm motility and with testicular volume in patients with varicoceles (124). Nitric oxide production within the spermatic vein and by Leydig cells (which is normally turned on at puberty) is differentially up-regulated in varicocele testes (133-136). Nitric oxide can react with reactive oxygen species (hydrogen peroxide and free radicals such as $OH^{\cdot}$ and O_2^{-}) that are elevated in blood plasma, seminal plasma and testicular tissue from infertile men with varicoceles (e.g., 137-140). An increase in reactive oxygen species, which correlated with varicocele grade (141), is linked to the production of infertility. In contrast, a recent prospective study reported that reactive oxygen species are not increased in fertile men with varicocele irrespective of varicocele grade (142).

Elevated nitric oxide seen in varicocele patients is limited to the spermatic vein in adolescents but is widespread within adult testes (143). This is in line with the general clinical opinion that testicular damage from varicoceles is progressive. Elevated nitric oxide levels have been linked to increased lipid peroxidation (144) and protein tyrosine nitration (143) as well as elevations in apoptotic cells within the seminiferous epithelium (120). Increases in nitric oxide concentrations are correlated with increased expression of both endothelial nitric oxide synthase (eNOS) (120) and inducible NOS (iNOS) (120,136). The negative effects of elevated nitric oxide may be enhanced by a decrease in melatonin, a nitric oxide antagonist (145-147).

There are several lines of clinical evidence connecting oxidative damage downstream of hypoxia with varicoceles. Elevated concentrations of reactive oxygen species are associated with detrimental effects on sperm (e.g., 148) even though low levels of reactive oxygen species may be involved in capacitation and induction of sperm motility. Among men with varicoceles, there are (1) elevated levels of protein carbonyls in blood plasma from the spermatic vein (149); elevated 8-hydroxy-2'-deoxyguanosine in leukocyte DNA of the spermatic vein (150); and increased levels of 8-hydroxy-2'-deoxyguanosine in testicular and ejaculated sperm (151), (2) greater sperm DNA fragmentation (152), (3) greater testicular lipid peroxidation (153,154), (4) appearance of 4-hydroxy-2-nonenal modified proteins in testes (155) that increase with increasing age (156), (5) increased fraction of immature sperm with cytoplasmic droplets in the ejaculate (157), (6) higher levels of pro-inflammatory cytokines in semen (158), and (7) irrespective fo semen parameters, increased percentages of apoptotic (TUNEL-positive) sperm in the ejaculate (152). The variety and uniformity of these observations argues strongly that hypoxia induces oxidative damage in varicocele testes.

The 4-hydroxy-2-nonenal modified proteins may be more than an indicator of reactive oxygen species; they may also damage seminiferous tubules. These species are both products and significant mediators of oxidative stress *in vivo*. As a mediator they act in part, by inducing apoptosis (159). Recent evidence from human testis biopsies from infertile men with varicoceles, suggests a relationship between 4-hydroxy-2-nonenal modified proteins and (1) loss of proliferating germ cells (as measured by a decrease in proliferating cell nuclear antigen) and (2) up-regulation of p53 protein expression (160). Since p53 acts via the extrinsic death receptor pathway (161), hypoxia can exacerbate the effects of elevated temperatures as described above. p53 can also trigger apoptosis via the intrinsic mitochondrial pathway (161).

Effects of hypoxia can be amplified by co-existing factors. For example, differential expression of alleles of an antioxidant enzyme, glutathione S-transferase M1, may make a man with varicoceles more sensitive to reactive oxygen species. Individuals with the homozygous glutathione S-transferase M1 null genotype display elevated levels of 8-hydroxy-2'-deoxyguanosine in their sperm DNA (162). Such patients also present with a large deletion in their mitochondrial DNA (162) that is associated with reduced sperm motility (163). A reduction in the sperm membrane polyunsaturated fatty acid composition associated with varicoceles may also be contributory (164). Such lipid membrane disturbances may be systemic (164,165) and might be detected by testing peripheral blood. As another example, normal antioxidant activities may be inefficiently utilized when FSH levels are elevated (166), as has been observed in many infertile men with varicoceles (167). Antioxidant enzyme activities may

be concomitantly reduced in varicoceles, magnifying the effects of increased reactive oxygen species (139,168,169), but not all agree (166,170). Cigarette smoking could further exacerbate this damage as it increases levels of oxidative stress (171).

The testis appears to mount a protective response to reactive oxygen species by increasing expression of inducible heme oxygenase-1 in Leydig cells (156). The products of heme oxygenase-1 have anti-oxidant activities. We view this response as analogous to the one described above for cadmium-induced alterations in calcium homeostasis.

Varicocelectomy reduces generation of reactive oxygen species and nitric oxide, increases the antioxidant activities of seminal plasma, reduces lipid peroxidation, and reduces retention of the cytoplasmic droplet during spermiation (157,172,173). However, the role of oxidative stress in the etiology of infertility with varicoceles remains controversial because two reports suggest that the levels of oxidative stress in infertile men with varicoceles and fertile men with varicoceles do not differ (137,139) and one report indicates that total seminal antioxidant capacity is unaffected by surgical varicocele repair (166).

6.5. Hormonal imbalance

As mentioned in sections 6.3 and 6.4, germ cell apoptosis is increased with varicoceles. Such apoptosis is modulated, at least in part, by hormone levels (review, 174). This finding suggested that androgen deprivation is a major contributory factor to decreased spermatogenesis with varicoceles (26). However, clinical hormone levels in infertile men with varicoceles vary over a wide range (175), explaining why this suggestion has not been been greeted with enthusiasm.

Testosterone (both total and free) has been reported to be decreased in blood plasma, seminal plasma and testis tissue in varicocele patients (176-181). A number of studies indicate the cause lies in a late stage(s) of Leydig cell testosterone biosynthesis (182-185). Testosterone levels were correlated with Leydig cell number (186) and were inversely related to age (187,188). Nevertheless, controversies exist. The number of Leydig cells detected in testis biopsies from infertile men with varicoceles have variously been reported to increase (74,189) or to decrease (190). In some cases, decreased testosterone levels were linked to Leydig cell hyperplasia (thought to be a compensatory response to reduced testosterone; 191) or to sexual dysfunction (192). One study suggests that increased ghrelin expression by Leydig cells is responsible for the decrease in serum testosterone (193). It may be significant that the normal increase in testosterone in response to hCG injection is blunted with varicoceles (194,195) while estradiol is increased (184). The estradiol increase has been linked to development of gynecomastia in adolescents with varicoceles (184).

Our own observations lead us to believe that varicoceles affect Leydig cells by several interacting pathways. We have recently reported that Leydig cell number is decreased and is accompanied by morphological changes (an increase in cell size and a change in nuclear shape) in the remaining Leydig cells in testis biopsies from infertile men with varicoceles as compared to controls with normal spermatogenesis (196). Neuronal NOS (nNOS) and Fas expression as well as germ cell apoptosis increased as Leydig cell structural abnormalities increased.

Nevertheless, not all studies support a role for androgen deprivation with varicoceles. Normal testosterone levels have been reported (197-201). Further, other studies indicated that testosterone levels do not differ between infertile men with varicoceles and fertile men with varicoceles or fertile men without varicoceles (202). Not all varicocele patients respond abnormally to the hCG stimulus (199).

One way to reconcile these findings is to recognize there may be other signals from varicoceles that affect the pituitary-hypothalamic-gonadal axis. These are evidenced by elevations in baseline FSH and LH levels (179,180,203), which in some cases was correlated with histological findings of depressed spermatogenesis (198,204), and marked increases in serum FSH and LH levels to stimulation by GnRH (203,205,206) or LH-RH (207,208). Elevated baseline FSH may predict an exaggerated GnRH test response (167) and/or an association with impotence (180). Excessive response to GnRH appears associated with a decreased free testosterone and increased levels of free estradiol and free sex binding hormone globulin (178). Exaggerated response to GnRH stimulus may increase with age (209,210), supporting the hypothesis of progressive testicular damage.

However these pituitary-hypothalamic-gonadal axis signals may not be significant in all varicoceles cases. Normal baseline levels of FSH and LH have been observed (201). Elevations in serum FSH levels and a marked increase in serum FSH following GNRH stimulation have been observed in fertile men with varicoceles (211). Similarly, increased gonadotropin responses were observed after LHRH stimulation were detected in fertile men with varicoceles (212).

There is also evidence for Sertoli cell dysfunction in varicoceles at the molecular level. In contrast to findings in fertile men, inhibin B levels in varicocele subjects were not positively correlated with sperm concentration or negatively related to FSH levels (211,213). In other studies inhibin B levels have been reported to decrease (214) and increase (215) with severity of varicoceles. The age of the subjects may play a role since Trigo *et al.* (215) studied adolescents rather than adults. These inhibin B changes affecting Sertoli cells must occur early in the pathway leading to damage, since a study that compared serum levels of inhibin B with testicular histology (216) failed to find a correlation. At later stages of testes damage, there is reduced expression of E-cadherin and alpha-catenin at intercellular junctions between adjacent Sertoli cells (217).

Varicocelectomy is associated, at least in some cases, with an increase in serum testosterone, serum free

testosterone and inhibin levels (176,179,181,192,214,218-220) and a decrease in FSH and LH (179). Testosterone response to hCG is also improved (221). Abrogation of the exaggerated FSH and LH response to bolus GnRH (206,209,222-224) and elevated LH to LH-RH (208) has also been reported. However, normalization of hormone levels that suggested an improvement in testis function and were linked to an increase in testicular volume may or may not be associated with an increase in semen parameters and/or pregnancy rates.

6.6. Additional molecular changes

It is hypothesized that reduced expression of E-cadherin and alpha-catenin at intercellular junctions between adjacent Sertoli cells contributes to a disruption of the blood-testis barrier with varicocele. This abnormality has the potential to synergize with the effects of cadmium. Results from animal studies indicate that, while cadmium-sensitive murine strains accumulate more cadmium than do the resistant strains (225-227), the permeabilities of isolated seminiferous tubules from cadmium-sensitive and resistant murine strains to exogenous cadmium are similar. This means that cadmium is able to gain entry into the seminferous tubule fluid by crossing both the myoid and Sertoli cell barriers (228).

Changes is testicular and ejaculated sperm protein composition with loss of cytoskeletal elements has been reported (10,114,204,229,230). A reduced ability to under go the acrosome reaction (231-233) may be linked to the loss of sperm head actin (114,229). Varicocelectomy is associated with an increase in the ability of sperm to acrosome react (232,233).

A defect in acrosin activity may be contributory to a reduction in sperm-egg interactions with varicoceles (234). Smoking similarly reduces acrosin activity (234). The latter may help to explain, at least in part, why smoking exacerbates the deleterious effects of varicoceles.

Antisperm antibodies have been documented to affect the acrosome reaction (e.g., 235). However, although antisperm antibodies are postulated to result from disruption of the blood-testis barrier, the evidence as to whether antisperm antibodies contribute to the etiology of varicocele-associated infertility is equivocal. In the studies examining this relationship, the percentages of varicocele patients presenting with antisperm antibodies has ranged from 3% to 91% as compared with control group (non-varicocele) values of 0% to 41% (236-243). These findings lead to the conclusion that antisperm immunity is higher in infertile men with varicoceles than in the general population. Despite this, there is no correlation between antibody titres and varicocele grade (237,243). The sperm antigens recognized by antisperm antibodies from infertile men with varicoceles are not necessarily those that are recognized by antibodies from infertile men without varicoceles (244). Varicocelectomy has been reported to reduce (associated with an increase in semen quality), increase (having no effect on semen quality) or have no effect upon antibody titres (242,243). At least one study reports that the presence of sperm bound antibodies has no effect on the response to surgical treatment (242).

Damage to the seminiferous epithelium is correlated with increased levels of lactate dehydrogenase-X (LDH-X) in seminal plasma (245). Post- surgery, improvement in semen parameters is associated with a decrease in the concentration of this enzyme in the ejaculate. LDH-X activity is also increased in ejaculated sperm from infertile men with varicoceles, but varicocelectomy has no effect on its elevated activity (85).

A point is here added as food for thought, Morgentaler *et al.* (246) suggested an “intrinsic” defect in scrotal cooling may exist in some infertile men and that this defect predisposes the men to infertility. This suggestion was based on observations that elevated scrotal temperatures (1) were detected in more than 40% of infertile men without varicoceles (61,64), (2) were associated with poor semen quality (61,64), and (3) scrotal cooling improved semen parameters and increased pregnancy rates (66,248-250). If this defect co-exists in some men with varicoceles and is absent in others, it could help to explain why only a small fraction of men with varicoceles are infertile.

7. USE OF ANIMAL MODELS

Although many groups including our own have easy access to human tissue, it is not feasible to study the time course of varicocele development as testis biopsies represent a “snapshot in time”. We cannot ethically study a human varicocele as it develops by repeated testes biopsies of individuals for research purposes alone. Thus, studies in the human are often either incomplete or suffer from varied interpretation.

7.1. Experimental left varicocele

Varicoceles occur naturally only in human males and in the ram (251-254). However, varicoceles can be surgically created in animal models. Partial constriction of the left testicular vein of animals produces more uniform varicocele effects than found in man (26,255). Varicocele models have been created in rat (256,257); dog (38); rabbit (258,259) and monkey (260-262). In the models that achieved visible evidence of venous distension, there was a reproducible increase in bilateral blood flow, intratesticular temperature and interstitial pressures in all species. In addition, although animals were fertile prior to varicocele creation, over time there was a uniform progression to hypospermatogenesis with basement membrane thickening and premature bilateral sloughing of spermatocytes and spermatids. Where examined, unilateral varicoceles produced bilateral effects. However, in contrast to man, it is thought that spermatic vein reflux does not occur. The latter conclusion, however, has been challenged (263).

Important results from animal models include the following. Experimental varicoceles are most damaging when created at puberty (264,265), when the testis is growing most rapidly and when these lesions are first detected in man. Neither the left testis nor the adrenals nor venous reflux are required for a right testicular response (266,267), suggesting that left venous distention is involved in the contralateral effects. Most importantly, release of the

partial renal vein ligation reverses these effects (268,269), providing direct evidence of the benefit of varicocele repair.

7.1.1. Recapitulation of effects observed in human males with varicoceles

The rat model is the extensively studied. Although it is not identical to naturally occurring human varicoceles, the redistribution of blood flow from the spermatic vein to the iliac vein is similar in rats and man (270). In addition, the rat model recapitulates many of the other human events.

Intrinsic toxins may be contributory but findings are equivocal. Comparison of the effects of experimental left varicocele in the presence or absence of the left adrenal gland revealed that, although in both cases testicular volume was reduced, FSH was elevated and testosterone reduced, structural abnormalities of the seminiferous epithelium were more severe when the left adrenal was present (263). In contrast, another group reported that left adrenalectomy does not inhibit the development of varicocele-associated physiologic changes (271).

Germ cell apoptosis is elevated (272-276), and increases with time after varicocele creation (277). Although one study showed that elevated temperature was responsible for increased apoptosis (278), hypoxia-inducible factor-1alpha is detected (279) and hypoxia appears to be the major contributory factor. Levels of p53 (280), VEGF and neoangiogenesis (279), pro-inflammatory cytokines (154), reactive oxygen species (275,281), nitric oxide levels (145,154,281-284) and lipid peroxidation (276) are elevated. (Note that elevated nitric oxide levels are the result of an increase in iNOS, in contrast to human patients where nNOS is increased; 285.) Antioxidant defenses are decreased (284) and antioxidants preserve testicular function (275,286). However, not all studies report support a role for germ cell apoptosis (280,287) or oxidative stress (288). An open question is whether problems associated with the creation of the varicocele (255) are responsible for these contradictory findings.

There is an increase in local venous pressure in experimental varicocele (289). An increase in testicular vascular permeability and histologic alterations of the structure of the blood-testis barrier has also been reported (290,291). These phenomena are consistent with disruption of the blood-testis barrier as described in human subjects (103,217) and may also be correlated with increased VEGF expression. However, not all studies indicate that the blood-testis barrier is breached with varicoceles (292,293).

Finally, two studies report changes consistent with involvement of immunological mechanisms contributing to testicular pathology with varicoceles (264,290), leading to the suggestion that autoimmune orchitis could serve as an experimental model for varicoceles (290). However, this approach has not yet been utilized.

Overall, these data suggest that animal models can be employed to study the effects of potential medical therapies.

7.1.2. Additional changes not yet reported in human subjects

A number of additional changes have not yet been identified in human subjects. These include the following. Transient induction of a variety of HSPs has been observed in the rat model (294). Increased expression of pro-apoptotic Bax protein and decreased expression of anti-apoptotic Bcl-2 protein have been reported (276). Down-regulation of Notch-1 and Notch-2 has been reported in experimental left varicocele (295). Looking at the last of these changes, we hypothesize that Notch-signaling might interact with varicocele-induced hypoxia to control angiogenesis. This is based on in part on studies in somatic tissues (296). Notch signaling contributes to developmental cell fate decisions, including those involved in normal spermatogenesis (297) and decreased expression is associated with maturation arrest and development of germ cell tumors (297,298). Notch signaling is effected via a family of genes. Notch-1 signaling may be involved in acrosome formation while Notch-2 appears to participate in spermatogenic cell proliferation and differentiation.

7.2. Elevated temperature

Apoptosis can be induced in testes more easily than in other tissues (299) by a short-term exposure to temperatures in the range 106 – 108^{o} Fahrenheit. This experimental system has been extensively studied in rats, mice and cynomolgus monkeys, largely by Swerdloff and coworkers (300,301). Overall, an intrinsic (mitochondrial) pathway is induced. Within 6 hrs of heat exposure, endoplasmic reticulum and mitochondria congregate paranuclear, co localizing with Bax, mitochondrial cytochrome C is released, caspases -9, -3, -6 and -7 are activated and poly-ADP polymerase (PARP) is cleaved, indicative of DNA damage. Fas and FasL (extrinsic pathway for apoptosis) are not involved, as apoptosis proceeds in hybrid mice where the intrinsic pathway was ablated, so receptor-mediated pathways are not obligatory (302). Ectopic expression of a natural calpain inhibitor in mutant mice suppressed apoptosis (303), in agreement with an intrinsic pathway. A study in iNOS knock-out mice showed iNOS facilitates, not inhibits, apoptosis in late stages of germ cell development (304). Rockett *et al.* (83) studied testicular mRNA expression changes induced in mice by short 109^{o}F temperature shock. These changes tracked with increased HSP-70 protein levels and were followed after some delay by testicular apoptosis. Among genes in apoptosis pathways, HSPs -25, -40 and -60, heme oxygenase, oxidative stress-induced protein and VEGF were up-regulated, while procaspase 2, Bax, Bag, Dad, HST-1 and two superoxide dismutases were down-regulated. Most of these changes are understandable as a protective response against apoptosis. Interestingly, temperature shock at 102^{o}F did not induce apoptosis.

This experimental protocol does not match well with testicular heating in varicocele, where temperatures are raised ~ 2^{o}C from normal and maintained over a period

of months or years. It is possible that adaptive responses develop in varicocele, which are absent from the acute testicular heating model. Nonetheless, as the effects of acute heating (301) and of testicular heating with experimental left varicoceles (268,269) are fully reversible, acute heating studies can be employed to suggest molecular candidates for a varicocele study (e.g., 174).

Cryptorchidism, natural or experimentally induced, may provide a better model for study of effects of chronic testicular heating as observed with varicoceles. Histological changes, including elevations in germ cell apoptosis and vacuolization in the seminiferous epithelium, parallel the changes observed in testis biopsies from infertile men with varicoceles (246,305). Elevations in oxidative stress from reactive oxygen species (306-308) and nitric oxide (309-312), increasing expression of pro-apoptotic Bax1 and decreasing expression of anti-apoptotic Bcl-2 (313-315), appear responsible for induction of apoptosis. eNOS synthase appears to be the mediator of elevations in nitric oxide (157,311). Biphasic elevations in oxidative stress (316) are consistent with biphasic elevations in apoptosis, the initial wave being p53-dependent and the second being p53-independent/Fas-dependent (317,318).

An early study combining experimental cryptochidism with external cooling resulted in preserved spermatogenesis (319). When taken with observations that an increase in apoptosis occurs prior to a reduction in intratesticular testosterone (320), these data suggest that heat, and not endocrine deprivation, is the major effector of apoptosis with cryptorchidism. Studies of experimental cryptorchidism also provide evidence for a genetic component regulating response to elevated testicular temperatures, as induction of testicular apoptosis varies among mouse strains (321). Similarly, ethnic differences in susceptibility of germ cells to apoptosis have been identified in man (322).

This model can be utilized to identify potential targets for medical therapies for varicocele-induced infertility (e.g., 306,307,310,312,316,323,324).

7.3. Effect of toxins

Focusing on our own work, we recently questioned whether cadmium alone could be responsible for impaired spermatogenesis in infertile men with varicoceles. In this pilot study, we employed the cadmium-sensitive Wistar rat strain and oral cadmium exposures at environmentally relevant dosages. We observed that cadmium exposures reduced epididymal sperm count and motility in adult animals (117). In the testis, cadmium levels approached those seen in infertile men with varicoceles (117). Cadmium-induced alterations in gene expression (as detected with DNA microarrays and confirmed by immunocytochemistry) were associated with elevations in eNOS and iNOS, FasL and germ cell apoptosis (325) as well as Leydig cell dysfunction resulting in a decrease in intratesticular testosterone (326). These data support our hypothesis that cadmium is a significant co-morbidity factor with varicoceles.

7.3.1. A potential role for genetics in sensitivity or resistance to cadmium-induced testicular damage

Surveys of inbred murine strains as F1 hybrids as well as segregation and linkage studies utilizing backcross and F2 generations all indicate that resistance to cadmium-induced testicular damage is the result of a single, recessive, fully penetrant gene ("cdm"; 327-329). Cadmium apparently co-opts a transport system for zinc (ZIP8) to cross the blood-testis (and blood-brain) barrier (228,330-332). Zinc and cadmium transport rates are similar in a given murine strain but differ between sensitive and resistant strains. Specificity of transport is demonstrated by the fact that calcium does not compete with cadmium transport. These data are consistent with *in vitro* findings that zinc offers protection against cadmium cytotoxicity in cultured vascular endothelial cells by decreasing intracellular cadmium accumulation (333). However, there are still gaps in our knowledge. Although ZIP8 expression differs between sensitive and resistant mouse strains, no difference is detected in the DNA sequence (331). Other as yet undefined factors must be controlling ZIP8 expression in the testis.

Recently, additional analyses of the mouse chromosome 3 in the region encoding cdm revealed the presence of 6 more genes. One of these genes, calcineurin (Ppp3ca; a serine threonine phosphatase) is now considered another candidate for the cdm gene, as an inhibitor of its activity (FK506), prevents cadmium-induced testicular damage (334).

An extrapolation of these findings is that genetic variations in the human population may determine individual sensitivity to cadmium and that multiple genes may be involved in this process. This argument is consistent with observations that there are striking inter-individual differences in effects of cadmium among people from the same area, the same age group, and presumably the same amounts of cadmium exposure (335). The potential existence of such genes adds another layer of complexity to the etiology of infertility with varicoceles.

7.4. Androgen deprivation

GnRH antagonists have been employed to study the effects of hormone deprivation on spermatogenesis in adult rodent models. GnRH antagonists induce a time and spermatogenic-stage dependent increase in germ cell apoptosis (336-339) via the intrinsic pathway (340). As with the induction of apoptosis by testicular heating (302), the death receptor pathway was not activated (340). Apoptosis is initiated by activation of p38 MAPK, which is concomitant with a decrease in intratesticular testosterone (340). p38 MAPK-signaling increases iNOS levels and Bax translocation to the mitochondria, thereby stimulating the intrinsic pathway (340). Selective iNOS inhibition blocks the effects of the GnRH anatagonists (340). It has been suggested that stage-specificity of GnRH antagonist effects may be related to stage-specificity of androgen receptor expression (338).

That androgen deprivation could have similar effects in the human testis was recently demonstrated using

human seminiferous tubules cultured in serum-free (therefore hormone-free) conditions which induce apoptosis (340). Both p38 MAPK or iNOS inhibitors blocked apoptosis of human testicular germ cells.

8. MEDICAL THERAPIES

The role of nutritional supplements/medicines for treatment of infertility with varicoceles has been studied in both animal models and humans. Such empirical therapies have focused primarily on reducing oxidative stress but other approaches are possible. The outcomes of attempted therapies are also helpful in guiding the search for predictor variables.

8.1. Oxidative stress

8.1.1. Antioxidants

Several antioxidants have been used clinically or in animal studies**.** Glutathione is the substrate for glutathione peroxidase, an antioxidant enzyme whose levels in semen are increased by varicocelectomy (172). Several studies have demonstrated that administration of glutathione to infertile men with varicoceles results in an increase in sperm motility (165,341,342), potentially through effects to reduce lipid peroxidation (165). Similar effects have been reported in *in vivo* and *in vitro* animals (342). Vitamin C is a water soluble reactive oxygen species scavenger that protects against DNA damage in human sperm (343). Vitamin C levels in seminal plasma and in internal spermatic vein blood are reduced in infertile men with varicoceles (149,162,344), being linked to the glutathione S-transferase M1 genotype (162). Vitamin C in seminal plasma is increased after varicocelectomy (172). Although potentially efficacious, vitamin C therapy has not been attempted in animal models or human subjects with varicoceles.

Vitamin E is lipid soluble and acts on the sperm membranes to protect against lipid peroxidation (345). Vitamin E may reduce apoptosis in rats with experimental left varicoceles (275). Although this therapeutic approach has not yet been applied to infertile men with varicoceles, the results of the animal study are contrary to what would be expected, as vitamin E levels in seminal plasma appear reduced following varicocele surgery (172).

Although not yet tested in human subjects, melatonin administration appears protective to the testis. When given to rats with experimental left varicocele, it reduces nitric oxide levels and increases antioxidant enzyme activity (145,276). It also decreased expression of pro-apoptotic proteins (276).

Another more drastic therapy not yet tested in humans is chemical sympathectomy via intraperitoneal 6-OH dopamine. Chemical sympathectomy in rats with experimental left varicocele results in a decrease in lipid peroxidation and an increase in antioxidant enzyme activities (346).

8.1.2. Supplementary zinc

Zinc is a powerful antioxidant (347). Abnormally low seminal plasma zinc levels occur in many cases with varicoceles (114,348-350). Testicular zinc is also lowered in varicocele patients (S. Benoff, J.L. Marmar and I.R. Hurley, unpublished observations). Some men who remain infertile post-varicocelectomy have low seminal plasma zinc levels after varicocele correction (348-350). Prior reports (e.g., 348), including one from our group (351), suggest that oral zinc supplementation in these cases improves semen quality and pregnancy rates.

8.1.3. Anti-inflammatory drugs

Several anti-inflammatories have been tried clinically.The non-steroidal anti-inflammatory drug, cinnoxicam, administered as a suppository to elevate bioavialability in the pelvic region has been shown to increase sperm counts (352) and, in a placebo-controlled trial, to also increase pregnancy rates (353) among infertile men with varicoceles. In our opinion, these findings highlight the importance of pro-inflammatory cytokines in the etiology of the disease process. Patients taking a combination therapy employing cinnoxicam and oral L-carnitine/acetyl-L-carnitine displayed significantly increased semen parameters and pregnancy rates as compared to those taking cinnoxicam alone (353) although free-L-carnitine levels, a marker of epididymal function, are similar in semen from infertile men with varicoceles and normal controls (349). The increased efficacy of this combination therapy is attributed to antioxidant properties of carnitine (353), which significantly increased sperm motility in infertile men with varicoceles (354) and in some (355,356), but not all, infertile men without varicoceles (357). However, the utility of anti-inflammatories is probably limited. The efficacy of cinnoxicam alone or in combination with L-carnitine/acetyl-L-carnitine decreased with increasing grade of varicoceles (352,353).

8.2. Hormones

Since spermatogensis is under hormonal control and since there is a body of evidence indicating hormonal imbalance with varicoceles, some clinicians have used hormonal supplements as empirical therapy for men who do not respond to varicocelectomy. Clomiphene citrate (Clomid) improved semen parameters and increased pregnancy rates, especially in men with sperm counts less than 10 million/ml or with testosterone deficiency (358-360). However, when administered prior to varicocelectomy, clomophene citrate was not as effective as surgery in terms of improvement in semen parameters and pregnancy rates (361,362). Tamoxifen citrate has successfully been employed in the treatment of idiopathic oligospermia (363). When tested in oligospermic subjects with varicoceles, those with normal hormonal parameters displayed a significant increase in spermatogenesis while only a marginal response was obtained in hypogonadotropic subjects (364). Human chorionic gonadotropin (hCG) has been administered post-surgery to patients with pre-operative sperm count of less than 10 million/ml and to patients with sustained Leydig cell dysfunction demonstrated using LHRH stimulation. A significant fraction of those treated displayed improved semen parameters and increased pregnancy rates (43,365)

8.3. Toxic substance antagonists

Focusing on cadmium, studies in animal models provide evidence that testicular toxicity can be abrogated by administration of anti-oxidant vitamins C and E (366), by the anti-oxidant effects of zinc (367,368), and by the nitric oxide antagonist melatonin (369). Vitiman C or zinc restores testosterone production which is inhibited by cadmium (368,370). Additional effects ascribed to zinc include immunomodulation (371) and competition with cadmium for effects on calcium-dependent functions (372-376**).** Finaly, both selenium and vitamin C suppress cadmium-induced germ cell apoptosis (370,377).

9. PREDICTOR VARIABLES

The variables which have been suggested as predictive of the outcome of varicocelectomy are discussed below. Not all are associated with clinically useful thresholds. Also, studies vary in their definition of outcome, e.g., whether a marker is linked to semen parameters, hormone levels or pregnancy rates.

Note that both simple (histologic), hormonal and complex molecular markers are considered, as not all clinicians have ready access to advanced technologies and assays that can be performed in their own offices are more likely to become widely accepted.

9.1. Semen parameters

Several groups have reported that the pre-operative semen parameters are predictive of spontaneous pregnancy rates after surgical varicocele repair. However threshold values differ between practices and over time. Early studies suggested that men with a sperm concentration greater than 10 million sperm/ml displayed a greater increase in post-operative semen parameters (378,379) and pregnancy rates (379) than did men with pre-operative sperm concentrations less than 10 million /ml. In another study, sperm density greater than 50 million per ml and sperm motility greater than or equal to 60%, but not sperm morphology or sperm forward progression, were predictors of pregnancy success (380). More recently, total motile sperm count was reported as predictor of pregnancy outcome and a threshold value of greater than or equal to 5 million motile sperm was associated with elevated spontaneous pregnancy rates (381).

9.2. Testis volume and histology

Varicoceles are often associated with decreased testicular volume. Lack of testicular atrophy, but not varicocele size or laterality, has been associated with postoperative pregnancy success (380). Similarly, a combined testicular volume of at least 30 ml has been correlated with fertility post-surgery (382).

Analysis of matched testis biopsies taken pre- and post-operatively from individual men demonstrates that varicocelectomy improves spermatogenesis and Leydig cell defects in some men (75), but not all (383). This is of particular importance as some infertile men with varicoceles who are azoospermic will have sperm in their ejaculate after surgery (384), which can shift them from ICSI candidates to intrauterine insemination or spontaneous pregnancy candidates (55). Testis biopsies indicate that such men present with hypospermatogenesis or late maturation arrest (383,385-390). This indicates that, at a minimum, the presence of spermatids is prognostic of a positive response to varicocelectomy. However, one report suggests that the fraction of azoospermic men post-surgery with sufficient sperm in their ejaculate to obviate the need for testicular sperm extraction to achieve pregnancies is less than previously estimated (391). These contrary findings may be attributable to the failure of the latter study to consider testicular histology when assessing success of varicocelectomy.

The histology of Leydig cells in testis biopsies has been tested as a predictor of varicocelectomy outcomes. Sperm counts were not increased and pregnancies were not obtained post-surgery in subjects where there was severe atrophy of Leydig cells (392). This pathology was associated with elevated LH and FSH levels but markedly decreased testosterone levels.

Proliferating cell nuclear antigen labeling of basal cells, has been proposed a prognostic factor for surgical outcome of varicocele repair (81). Proliferating cell nuclear antigen is a marker of cell proliferation detectable in testis biopsies by immunocytochemistry, A threshold value for number of basal cells labeled of greater than or equal to 31%, in conjunction of pre-operative sperm count of greater than 1 million/ml, predicts a greater than 50% in sperm count post-operatively.

Thickening of the seminiferous tubule basement membrane is an early event and is progressive over time, being focal in adolescents and widespread in adults (review, 10). One report suggests that increased seminiferous tubule basement membrane thickness is predictive of poor surgical outcome (74). This suggestion is in contrast to our own findings that basement membrane thickness was not a prognostic variable (393). Our findings are supported by the analysis of matched testis biopsies taken pre- and post-operatively from individual men (75). This study demonstrated that basement membrane thickness was not reduced post-surgery.

9.3. Scrotal/testicular hyperthermia

Clearly, the considerable variability in scrotal temperature measurements and overlap in temperatures between subjects with and without varicoceles (as described above) serves as indications that scrotal temperatures could not be employed as a predictor of the success of varicocelectomy. However, preliminary studies suggest that another measure of heat stress, the presence or absence of HSP 70-1 (a protein expressed in mammalian testis only after heat stress; 83) might be more informative (394,395).

In a pilot study of testis biopsies from 45 infertile men with varicoceles, we found that HSP 70-1 expression varied between subjects. When HSP 70-1 mRNA was detected, the percentages of germ cells undergoing apoptosis via the death receptor pathway were lower and

pre-operative sperm counts were higher than when HSP 70-1 mRNA was absent. In the HSP 70-1 mRNA-positive group, pre-operative sperm counts exceeded >10 million sperm/ml, a value that in previous studies was a positive predictor of pregnancy outcome post-surgery (379).

9.4. Toxic substances

With regard to toxic substances, one marker might be elevations in spermatic vein norepinephrine concentrations as compared to the right femoral vein. Cohen *et al.* (108) reported that subjects with elevated norepinephrine levels displayed better increases in semen parameters and pregnancy rates post varicocele correction than did subjects with normal spermatic vein norepinephrine levels. However, no cut-off values have been published.

Cadmium is elevated in seminal plasma and in testis biopsies from a significant fraction of infertile men with varicoceles. Preliminary studies suggest that seminal plasma cadmium levels less than 0.4 micrograms/l are predictive of an improvement in sperm density and a positive pregnancy outcome post-surgery (396). More extensive studies have established that testicular cadmium levels greater than or equal 0.453 ng/mg dry weight of tissue predicts a poor response (less than or equal to a 50% increase in post-operative sperm count) (24). When only left testis biopsies are analyzed, this test has a positive predictive value of 75%, a negative predictive value of 70.6%, an overall accuracy of 73.2%, a specificity of 66.7% and a sensitivity of 78.3% (127). When bilateral testis biopsies were analyzed, these values increased to, respectively, 86.4%, 78.9%, 82.9%, 83.3% and 82.6% (127). We attribute the increased efficacy of bilateral analyses to occasional discordance in marker expression observed between matched biopsies (24,127).

We have published evidence that cadmium enters the cells of the seminiferous epithelium via L-type calcium channels and that elevated testicular cadmium levels are associated with production of deletion variants of the channel (10,115,117). Using an RT-PCR-based assay, absence of an undeleted L-type calcium channel mRNA predicts elevated testicular cadmium levels with a positive predictive value of 84%, a negative predictive value of 84%, an overall accuracy of 83.3%, a specificity of 69.2% and a sensitivity of 91.3%. As would be predicted on the basis of the relationship to testicular cadmium levels, absence of an undeleted L-type calcium channel mRNA predicts a poor response (less than or equal to a 50% increase in post-operative sperm count) with a positive predictive value of 78.6%, a negative predictive value of 85.7%, an overall accuracy of 80.9%, a specificity of 66.7% and a sensitivity of 91.6% (127). As with cadmium determinations, bilateral analyses increased these values to, respectively, 92.8%, 85.7%, 90.5%, 85.7% and 92.8%. Given the accessibility of thermal cyclers, this assay may be preferred over the graphite furnace atomic absorption spectroscopic determination of tissue cadmium levels.

9.5. Factors related to hypoxia

It has been reported that the expression of 4-hydroxy-2-nonenal modified proteins in the testis of varicocele patients predicts improvement in spermatogenesis after varicocelectomy (154). This suggestion is based on comparison of semen parameters and pregnancy rates post-surgery of “responder” patients versus “non-responder”. However, the definitions of “responders” and “non-responders” is unclear and no threshold value is reported for 4-hydroxy-2-nonenal modified protein levels. More importantly, the reported positive relationship between 4-hydroxy-2-nonenal modified protein levels and proliferating cell nuclear antigen expression or testicular volume is not intuitively obvious and is inconsistent with more recent findings from the same group that 4-hydroxy-2-nonenal modified protein levels and proliferating cell nuclear antigen expression are negatively correlated (160).

Polymorphisms in glutathione S-transferase enzymes T1 and M1 have been evaluated with regard to predicting an increase in semen parameters after varicocele repair (397). In this study, a positive response to varicocele surgery was defined as a 100% increase in motile sperm concentration. Individuals with the glutathione S-transferase T-positive and the M1-positive genotypes were found to have a higher positive response rate to varicocelectomy than individuals with the null genotypes for either enzyme. The response rate was even higher when an individual was double positive for these enzymes.

9.6. Hormones

Baseline serum FSH levels less than 300 ng/ml or 11.7 mIU/ml have been suggested as a marker predicting successful conception after varicocele repair (380,382).

As mentioned above, some men, but not all, with varicoceles display a 2-3-fold increase in FSH and 3-5-fold increase in LH in response to gonadotropin-relasing hormone (GnRH) as compared to normal controls (e.g., 205). Testosterone levels did not increase. Seminal plasma DHT levels lower than normal were detected in these subjects (206,222). Surgery seems to resolve the exaggerated response to GnRH and improves seminal DHT levels (206,222-224). Studies in man and animal models suggest that these exaggerated responses may be related to an abnormality in steroid biosynthesis as a result of a deficiencies in 17,20 demolase and/or 17alpha-hydroxylase (194,398). Therefore, in addition to Leydig cell histology (392; see above), the GnRH stimulation has been employed to predict the outcome of varicocelectomy in adults. However, inconsistent findings have been reported. Some groups claim that a positive (exaggerated) response to the GNRH stimulation test predicts a statistically significant increase in semen parameters and pregnancy rates post-surgery (399), especially in men who are severely oligospermic (200,205,224). Others report that no relationship was detected (223,381). Further, the efficacy of this test in determining the outcome of adolescent varicocele repair is also debatable (221,400). These contrary findings may be the result of mode of GnRH administration (intranasal spray, continuous infusion or

bolus injection) and/or the lack of consistent cut-off values. Only one study (224) actually gives values which define a "positive" response to GnRH stimulation, e.g., >2-fold increase in FSH and >5-fold increase in LH.

LH levels in response to LHRH stimulation has been linked to pregnancy outcome. Responders (men who exhibit a return to fertility) have statistically lower maximal LH levels as compared to non-responders (208).

Inhibin B, a product of Sertoli cells, regulates serum FSH levels via a negative feedback loop. Serum inhibin B levels are reduced in adolescents and adults with varicoceles and impaired spermatogenesis (401,402), suggesting a use of inhibin B measurements to predict the outcome of varicocele surgery. However, preoperative measurements of inhibin B have produced equivocal results (403,404). Nevertheless, it has been postulated that an increase in inhibin B levels post-surgery indicate an improvement in testicular function (404).

Androgen deprivation may contribute to infertility with varicoceles (review: 10,26). Mutations which increase the polyglutamine repeat number in the androgen receptor are associated with androgen insensitivity and male infertility (405,406) and may co-exist with varicoceles (26). The effects of androgen receptor mutations may be modified depending on genetic background (406). Patients with such androgen receptor mutations should be counseled that they may not be good candidates for varicocele surgery.

9.7. Other prognostic factors

As mentioned above, varicoceles may co-exist with genetic abnormalities such as alleles of antioxidant enzymes (397) or androgen receptor defects (26).

Additional genetic problems include abnormal karyotypes/Y-chromosome deletions (54) and congential absence of the vas deferens (CAVD; 407). While men with CAVD generally have normal spermatogenesis and co-existence with varicocele should not impact on the outcome of varicocele repair, preliminary findings indicate that semen parameters do not improve post-surgery in men with co-existing abnormal karyotypes or Y-chromosome deletions. Genetic counseling should be offered to patients with varicoceles and co-existing defined genetic infertility.

An inverse relationship between the concentration of fibronectin in seminal plasma and sperm motility has been reported (408). Surprisingly, fibronectin concentrations in varicocele subjects were lower than the norm and were unrelated to sperm motility. Thus, fibronectin is not a diagnostic tool for varicoceles, but may be applicable to patients with unexplained infertility.

10. FUTURE DIRECTIONS

This review began as a short introduction to a research paper on markers to predict the outcome of varicocelectomy, but as it expanded as we realized how much is not known. We hope, by assembling what is known, to clarify our thinking as to what should be done next.

We believe that the preponderance of evidence shows that two or more factors are necessary for varicocele-related infertility. This concept serves as the basis for the "second hit" hypothesis, and the problem maybe separated into two parts, the lesion itself and its local manifestations, and the active testes, with its Leydig cells and seminiferous tubules. The lesion communicates with the testes by sending signals. If the testis is receptive to these signals, there maybe a reduction of sperm production and sperm quality. If the testis is resistant or not receptive, then the man remains fertile. If varicocele repair restores fertility, then the testis has repaired itself following removal of the signals from the lesion. If surgery is not successful, (assuming that the repair was not poorly executed), then the testes has been transformed so that it is no longer capable of repair despite removal of the signals from the lesion.

There are several pathways towards identifying the critical transformation. One is brute force: gene expression profiling of enough testes from infertile men with varicoceles to characterize a profile representing those patients that will not respond to correction. This approach is not likely to be successful for several reasons, not least because of random genetic variation in the patient population. A second would involve gene expression profiling of the lesion and of the active testes separately, to characterize potential signals from the lesion consequent changes in RNAs in the active testes. There are several possible variants of this approach which place increasingly greater demands on the pathologist, including microdissection of different cell types from each tissue, and amplification of gene products. All are open to the criticism that this approach ignores protein and small molecular messengers, whose levels may change significantly yet with absent noticeable change in RNA levels. Also, the DNA microarrays presently available are not sensitive to mRNA splicing changes that might change intracellular targeting, or abrogate or alter function. The third principle pathway is to pick a gene and follow its transcription, posttranscriptional processing, translation, glycolsylation (and other post-translational modifications) and then assay for its activity. This third approach is likely to be the one most followed, if only because of preferences of grant review panels. But which gene?

Histological characterization of the testis biopsies from varicocele patients is replete in the old literature, with most publication dating from before laser capture microdissection, RT-PCR and the advent of gene expression profiling of primary cell cultures. However, we believe that the new technology may be applied to testis tissue biopsies, and the percutaneous acquisition of the seminiferous tubules from these men can be done safely (127). The molecular/genetic studies on testis issue should yield new and possibly predictive information in men with varicoceles.

The deleterious effects of varicoceles are first evidenced by progressive thickening of the vascular endothelium in both the varicocele vein itself and in the

associated testis, with focal edema in the testis and increased interstitial pressures (26,103,409). The change in endothelial thickness is attributed to proliferation and hyperplasia of the endothelial cells rather than hypertrophy (103). These findings are consistent with arguments that the underlying mechanism of infertility with varicoceles involves disturbances in testicular blood flow (410,411). Thus, there may be a backdoor to address signaling from the varicocele lesion to the active testes.

There is a body of literature on varicose veins in the extremities (for a review, see: 412-415). The normal microanatomy of the testicular veins and veins of the extremities (e.g., greater saphenous veins) are similar. Outside of the largely endothelial intima, there are two medial layers of smooth muscle, an inner circumferential layer surrounded by a second layer of longitudinally oriented smooth muscle. The whole is embedded in an extracellular matrix. Other commonalities include reflux of blood through incompetent venous values, leading to hypoxia, venous hypertension and inflammatory cascades. Both are surgically treated to reduce reflux.

To a first approximation, the changes in cell types, compartmentalization and gene products in greater saphenous veins as varicosity increases should be the same as in the pampiniform plexus. What has been found here?

10.1. Etiology of varicose veins

As the varicose vein in the leg develops, blood flow drops and hypoxia occurs, the endothelium becomes activated and porous to white blood cell invasion, and leads to releases of fibroblast growth factor (bFGF) and cytokines. Transcriptional activation of VEGF and its receptors are observed (review, 414). The level of noradrenaline secreted by the vascular endothelium falls, while TGF-beta-1 levels rise and cGMP regulated nitric oxide synthesis by iNOS is increased, especially in highly tortuous regions of the media (416). The increased nitric oxide antagonizes the effects of angiotensin, leading to a relaxation of the vein wall. Smooth muscle cells migrate to the intima and dedifferentiate to a collagen-synthesizing phenotype, possibly under the influence of nitric oxide, or changes in steroid hormones. Initially, the medial layers of smooth muscle decrease in thickness, which is attributed to apoptosis of the smooth muscle cells. This is followed by down-regulation of pro-apoptotic factors and up-regulation of anti-apoptotic proteins (417-420) with smooth muscle cell hypertrophy (418,419). Alterations in gene expression were consistent with changes in smooth muscle actin functionality and contractility (421). The ratio of collagen I to collagen II in the extracellular matrix falls as does the elastin:collagen ratio. Levels of some matrix metalloproteases (MMP), e.g., MMP-1 and MMP-9, are upregulated, as is the matrix metalloprotease inhibitor TIMP-1. In some regions, the extracellular matrix of the medial layer thickens, in others the matrix is degraded. All of these changes affect the extensibililty, contractility and tortuosity of varicose veins.

Agents which can block unwanted growth of blood vessels and also reduce oxidative stress have been proposed as therapy to limit formation of varicose veins (e.g., 422).

10.2. Points for investigation in infertile men with varicoceles

The next few paragraphs discuss factors altered as varicose veins develop that also appear in the infertility literature. These look to be early candidates for investigation.

10.2.1. Smooth muscle cell proliferation

The histological changes in testicular veins as a varicocele develops are presently controversial. One report shows that smooth muscle atrophies as the varicoceles develop (423). In contrast, Tanji *et al.* (4) shows that smooth muscle cell layers thicken with increasing stage. This disagreement may be due to differences in the tissue samples evaluated. The varicose vein literature reports local atrophy and distensions at various levels of the same tissue as responsible for the characteristic tortuosity of the affected vein (413). While a larger survey of more tissue samples is needed to clarify this point, recent evidence supports a role for smooth muscle cell hypertrophy as a result of over-expression of the anti-apoptotic protein, Bcl-2 (424).

Aberrations in smooth muscle are not limited to the internal spermatic vein and can be seen in relation to smooth muscle of testicular blood vessels and the myoid cells in the seminiferous tubule basal lamina. These aberrations are associated with changes in cell shape, collagen IV biosynthesis, and basement membrane thickening as well as reductions in the size of the lumen of the seminiferous epithelium and in spermatogenesis (76,425-428).

10.2.2. VEGF

VEGF controls both vascular permeability and angiogenesis. Altered transcription of VEGF and its receptors are observed in varicose veins (414,429). To the best of our knowledge, VEGF expression in varicocele veins has not yet been studied. However, its overexpression may be inferred from the increase in vascular permeability observed in animal models (81). A bigger question is why ectopic expression of VEGF within the seminiferous epithelium has been reported both in human subjects (123) and in the rat model for experimental varicocele (279).

10.2.3. TGF-beta-1

The cytokine TGF-beta-1 and the intercellular messenger nitric oxide released by varicose veins seem to have role in varicocele infertility. In normal testes, highest concentrations of TGF-beta-1 and its receptor are found in Leydig cells. This receptor is also present on elongating spermatids but not in other cells of the seminiferous tubule (430). Intracellular levels of TGF-beta-1 are significantly raised in varicocele testes compared to normal controls, especially in Sertoli and germ cells (431). This raises two questions. First, does nitric oxide from smooth muscle layer of the vein affect the TGF-beta-1 activity in Leydig cells? Second, are TGF-beta-1 and iNOS levels in the varicocele vein predictors of varicocelectomy success?

10.2.4. Angiotensin

Angiotensin level modulation occurs as a varicose vein relaxes, which may help explain why varicocele sperm are less motile. The mice knockout of testicular angiotensin converting enzyme (ACE) is almost infertile because its sperm are largely immotile (432). This finding translates to the human case. The progressive motility of ejaculated human sperm correlates inversely with their ACE activity (433,434). But this situation is complicated by the existence of two isoforms, a somatic isozyme present in blood and a shorter testes-specific form that has a greater affinity for angiotensin converting enzyme. It is clear that the levels of the somatic form are the same in peripheral and spermatic veins of both varicocele and normal testes (435), but this does not address intracellular concentrations in testes. It is possible that local modulation of tissue levels of somatic angiotensin in the active testes may alter synthesis or transport of the testes isoform.

10.2.5. Fibroblast growth factors

Fibroblast growth factors (FGF) have roles in sperm synthesis and maturation (for a review, see 436). As examples, FGF1 and FGF2 regulate of steroid hormone by Leydig cells. Several FGF receptors (FGFR) are present in developing germs cells, and seem expressed in a stage dependent manner, possibly regulating progression, while FGFR1 and FGFR4 are present on the tails of elongating spermatids and may be involved in development of sperm motility. FGFs excretion by the endothelium of varicose veins is upregulated in response to hypoxia and consequent modulation of ion pump activity (412). This is a general phenomenon seen in other tissues (437) so is likely to occur in the pampiniform plexus.

10.2.6. Matrix metalloproteases

Matrix metalloproteases (including MMP9) and their inhibitors modulate Sertoli–germ cell interactions (438), so it is possible that enzymes released during vein extracellular matrix remodeling could disrupt spermatogenesis. Direct action seems improbable since these enzymes are usually anchored, but indirect mechanisms are at least not impossible.

10.2.7. Other possibilities

If, on the other hand, more than the varicocele itself is responsible for the infertility of varicocele patients, it is unlikely that a trigger for the critical transformation will be identified by the lines of investigation outlined above. However, the knowledge gained will not be wasted, since they will clarify type and extent of communications between the varicocele lesion and the active testes.

11. CONCLUDING COMMENTS

It may be that none of the factors common to both varicose veins and varicoceles are the signal(s) connecting a vascular injury to irreversible damage to the active testes. This signal or signals may lie further upstream and may act separately at sensitive sites in both tissues, and perhaps other sites (such as the hypothalamus). However, if we can idenfity a fingerprint of factors (in vascular tissue, and the principal cell lineages of the active testes) that distinguishes human subjects with varicocele that regain fertility after repair from those that do not, we will be well on our way to narrowing down the pathway(s) in which the signals could act, as an intermediate step towards identifying the critical signal(s) themselves. The animal varicocele models should become exquisitely valuable at that time as systems to experimentally manipulate putative signals. One believes that a structured approach of the sort outlined above may unravel the science underling the confused picture that has arisen from the small scale and, sometimes, haphazard studies we have here reviewed.

12. ACKNOWLEGEMENTS

Original studies by the authors cited in this manuscript were supported by Grant Nos. ES06100 and ES10496 to S.B. from the National Institute of Environmental Health Sciences, National Institutes of Health, Bethesda, Maryland.

13. REFERENCES

1. Pryor J.L. & S.S. Howards: Varicocele *Urol Clin NA* 14, 499-513 (1987)

2. Riba L.W: Excision of the internal spermatic vein for varicocele. *J Urol* 57, 889-893 (1947)

3. Lund E, E. Ernst, H.T. Sorensen & H. Oxlund: Biochemical properties of normal and varicocele internal spermatic veins. *Scand J Urol* 32, 47-50 (1998)

4. Tanji N, T. Fujiwara, H. Kaji, S. Nishio & M. Yokokama: Histologic evaluation of spermatic veins in patients with varicocele. *Int J Urol* 7, 365-360 (1999)

5. Bushci A.J, R.B. Harrison, A. Norman, A.G. Brenbridge, B.R. Williamson, R.R. Gentry and R. Cole: Distended left renal vein: CT/sonographic normal variant. *Am J Roentgenol* 135, 339-342 (1980)

6. Zorgniotti A.W: Hypothesis to explain subfertile semen. *Adv Exp Biol Med* 286, 221-223 (1991)

7. Marmar J.L. & Y. Kim: Subinginual microsurgical varicocelectomy: a technical critique and statistical analysis of semen and pregnancy data. *J Urol* 152, 1127-1132 (1994)

8. Jarow J.P.: Effects of varicocele on male fertility. *Hum Reprod Update* 7, 59-64 (2001)

9. W.H.O. (World Health Organization). The influence of varicocele on parameters of fertility in a large group of men presenting to infertility clinics. *Fertil Steril* 57:1289-1293 (1992)

10. Benoff S.B. & B.R. Gilbert: Varicocele and male infertility: Part I. Preface. *Hum Reprod Update* 7, 47-54 (2001)

11. Santoro G. & C. Romeo: Normal and varicocele testis in adolescents. *Asian J Androl* 3, 259-262 (2001)

12. Dubin L. & R.D. Amelar: Etiologic factors in 1294 consecutive cases of male infertility. *Fertil Steril* 22, 469-474 (1971)

13. Greenberg S.H, L.I. Lipshultz & A.J. Wein: Experience with 425 subfertile male patients. *J Urol* 119, 507-510 (1978)

14. Goldstein M, B. Gilbert, A. Dicker, J. Dwosh & C. Gnecco: Microsurgical inguinal varicocelectomy with delivery of the testis: an artery and lymphatic sparing technique. *J Urol* 148, 1808-1811 (1992)

15. Schlesinger M.H, I.F. Wilets & H.M. Nagler: Treatment outcome after varicocelectomy. *Urol Clin N Am* 21, 517-529 (1994)

16. Levinger U., M. Gornish, Y. Gat & G.N. Bachar: Is varicocele prevalence increasing with age? *Andrologia* 39, 77-80 (2007)

17. Gorelick J.I. & M. Goldstein: Loss of fertility in men with varicocele. *Fertil Steril* 59, 613-616 (1993)

18. Witt M.A. & L.I. Lipshultz: Varicocele: a progressive or static lesion? *Urology* 42, 541-543 (1993)

19. Jarow J.P., M. Coburn & M. Sigman: Incidence of varicoceles in men with primary and secondary infertility. *Urology* 47, 73-76 (1996)

20. Raman J.D, K. Walmsley & M. Goldstein: Inheritance of varicoceles. *Urology* 65, 1186-1189 (2005)

21. Mokhtari G.H, A. Noshad & M. Jamali: Comparison of prevalence of varicocele in first-degree relatives of patients with varicocele and male kidney donors. *Urology* 70 Suppl 3A, 208-209 (2007)

22. Baccetti B, G. Collodel & P. Piomboni: Apoptosis in human ejaculated cells. *J Submicrosc Cytol Pathol* 28, 587-596 (1996)

23. F. Simsek F, L. Turkeri, I. Cevik, K. Bircan & R. Akdas: Role of apoptosis in testicular tissue damage caused by varicocele. *Arch Esp Urol* 51, 947-950 (1998)

24. Benoff S, C. Millan, I.R. Hurley, B. Napolitano & J.L. Marmar: Bilateral increased apoptosis and bilateral accumulation of cadmium in infertile men with left varicocele. *Hum Reprod* 19, 616-627 (2004)

25. Chen C.H, S.S. Lee, D.C. Chen, H.H. Chien, I.C. Chen, Y.N. Chu, J.Y. Liu, W.H. Chen & G.J. Wu: Apoptosis and kinematics of ejaculated spermatozoa in patients with varicocele. *J Androl* 25, 348-353 (2004)

26. Marmar J.L: Varicocele and male infertility: Part II. The pathophysiology of varicoceles in light of current molecular and genetic information. *Hum Reprod Update* 7, 461-472 (2001)

27. Noske H.-D & W. Weidner: Varicocele – a historical perspective. *World J Urol* 17, 151-157 (1999)

28. Tulloch W.S: A consideration of sterility factors in the light of subsequent pregnancies. *Endinburgh Med J* 59, 29-34 (1952)

29. Russell J.K: Varicocele in groups of fertile and subfertile males. *Brit Med J* 1, 1231 (1954)

30. Scott L.S: The effect of varicocele on spermatogenesis. *Proc Soc Study Fertil* 10, 33 (1958)

31. Davidson H.P: Treatment of male subfertility, testicular temperature and varicocele. *Fertil Steril* 20, 50-57 (1954)

32. Tulloch W.S: Varicocele in subfertility, results of treatment. *Brit Med J* 2, 356 (1955)

33. Young D: The influence of varicocele on human spermatogenesis. *Brit J Urol* 28, 426 (1956)

34. Scott L.S: Varicocele as a treatable cause of subfertility. *Brit Med J* 1, 788 (1961)

35. Charny C.W: Effects of varicocele on fertility. *Fertil Steril* 13, 47-56 (1962)

36. MacLeod J: Seminal cytology in presence of varicocele. *Fertil Steril* 16, 735-757 (1965)

37. Dubin L. & R.S. Hotchkiss: Testis biopsy in subfertile men with varicocele. *Fertil Steril* 20, 50-57 (1969)

38. Saypol D.C, S.S. Howards, T.T. Turner & E.D. Miller Jr.: Influence of surgically induced varicocele on testicular blood flow, temperature, and histology in adult rats and dogs. *J Clin Invest* 68, 39-45 (1981)

39. Mali W.P: The varicocele. A hemodynamic and spermatologic study. Dordrecht, Netherlands: ICG Printing. (1984)

40. Center for Fertility & Reproductive Endocrinology at Virginia Mason. Varicocele repair or varicocelectomy. www.virginiamason.org/dbfertility/sec1596.htm 2004.

41. Marmar J.L, S.L. Corson, F.R. Batzer & B. Gocial: Insemination data on men with varicoceles. *Fertil Steril* 57, 1084-1090 (1992)

42. Schlegel P.N: Is assisted reproduction the optimal treatment for varicocele-associated infertility? A cost-effectiveness analysis. *Urology* 49, 83-90 (1997)

43. Dubin L. & R.D. Amelar: Varicocelectomy: 986 cases in a twelve-year study. *Urology* 10, 446-449

(1977)

44. Ross L.S. & N. Ruppman: Varicocele vein ligation in 565 patients under local anesthesia: a long-term review of technique, results and complications in light of proposed management by laparowcopy. *J Urol* 149, 1361-1363 (1993)

45. Madgar I, R. Weissenberg, B. Lumenfeld, A. Karasik & B. Goldwasser. Controlled trial of high spermatic vein ligation for varicocele in infertile men. *Fertil Steril* 63, 120-124 (1995)

46. Nieschlag E, L. Hertle, A. Fischedick, K. Abshagen & H.M. Behre: Update on treatment of varicocele: counseling as effective as occlusion of the vena spermatica. *Hum Reprod* 13, 2147-50 (1998)

47. Sandlow J.I: Do varicocele really affect male infertility? *Sexuality, Reprod Menapause* 2, 219-221 (2004)

48. Evers J.L.H. & J.A. Collins: Assessment of the efficacy of varicocele repair for mal subfertility: a systematic review. *Lancet* 361, 1849-1852 (2003)

49. Marmar J.L, A. Agarwal, S. Prabakaran, R. Agarwal, R.A. Short, S. Benoff & A.J. Thomas: Resassessing the value of varicocelectomy as a treatment for male subfertility with a new meta-analysis. *Fertil Steril* 88, 639-648 (2007)

50. Agarwal A, F. Deepinder, M. Cocuzza, R. Agarwal, R.A. Short, E. Sabanegh & J.L. Marmar: Efficacy of varicocelectomy in improving semen paraters: new meta-analytical approach. *Urology* 70, 532-538 (2007)

51. Ombelet W, E. Bosmans, M. Janssen, A. Cox, J. Viasselaer, W. Gyselaers, H. Vandeput, J. Gielen, H. Pollet, M. Maes, O. Steeno & T. Kruger: Semen parameters in fertile versus subfertile population: a need for change in the interpretation of semen testing. *Hum Reprod* 12, 987-993 (1997)

52. Bonde J.P, E. Ernst, T.K. Jensen, N.H. Hjollund, H. Kolstad, T.B. Henriksen, T. Scheike, A. Giwercman, J. Olsen & N.E. Skakkebaek: Relations between semen quality and fertility: a population-based study of 430 first-pregnancy planners. *Lancet* 352, 1172-1177 (1998)

53. Guzick D.S, J.W. Overstreet, P. Factor-Litvak, C.K. Brazil, S.T. Nakajima, C. Coutifaris, S.A. Carson, P. Cisneros, M.P. Steinkamph, J.A. Hill, D. Xu & D.L. Vogel: Sperm morphology, motility, and concentration in fertile and infertile men. *N Eng J Med* 345, 1388-1393 (2001)

54. Cayan S, D. Lee, L.D. Black, R.A. Reijo Pera & P.J. Turek: Response to varicocelectomy in oligospermic men with and without defined genetic infertility. *Urology* 57, 530-535 (2001)

55. Cayan S, F. Erdemir, I.Ozbey, P.J. Turek, A. Kadioglu & S. Tellaloglu: Can varicocelectomy significantly change the way couples use assisted reproductive technologies? *J Urol* 167, 1749-1752 (2002)

56. Edwards T. & J.L. Marmar: Assisted reproductive technologies (ART's) combined with varicocele surgery: pregnancy data and cost analysis. Program and Abstracts of the 29th Annual Meeting of the American Society of Andrology, Abstr. no. 145, p.86 (2004)

57. Agger P: Scrotal and testicular temperature: its relation to sperm count before and after operation for varicocele. *Fertil Steril* 22, 286-296 (1971)

58. Yamaguchi M, J. Sakotu & H. Takihara: The application of intrascrotal deep body temperature measurement for the noninvasive diagnosis of varicocele. *Fertil Steril* 52, 295-301 (1989)

59. Wright J.E, G.P.H. Young & M.Goldstein: Reduction in testicular temperature after varicocelectomy in infertile men. *Urology* 50, 257-259 (1997)

60. Zorgniotti A.W. & J. MacLeod: Studies in the temperature, human semen quality and variococele. *Fertil Steril* 24, 295-301 (1973)

61. R. Mieusset R, L. Bujan, M. Plantavid, A. Mansat, H. Grandjean & F. Pontonnier: Scrotal hyperthermia: frequency in an infertile population and associated alterations in testicular function. *Adv Exp Biol Med* 286, 203- 209 (1991)

62. Mieusset R. & L. Bujan: Testicular heating and its possible contributions to male infertility: a review. *Int J Androl* 18, 169-184 (1995)

63. Lund L. & K.T. Nielsen: Varicocele testis and testicular temperature. *Br J Urol* 78, 113-115 (1996)

64. Zorgniotti A.W: Intrinsic testicular temperature elevation and subfertile semen. *Adv Exp Biol Med* 286, 199-202 (1991)

65. Marmar J.L, E. Chenvin, S. Benoff & C. Millan: Localization of transition protein-1 (TP-1) within complete seminiferous tubules from men with Sertoli cell only syndrome (SCO). Ninety-Seventh Annual Meeting of the American Urological Association, ID # 1400 (2002a)

66. Jung A, M. Eberl & W.B. Schill: Improvement of sperm semen quality by nocturnal scrotal cooling and moderate behavioural change to reduce genital heat stress in men with oligoasthenoteratozoospermia. *Reproduction* 121, 595-603 (2001)

67. Hjollund N.H, J.P. Bonde, T.K. Jensen & J. Olsen: Diurnal scrotal skin temperature and semen quality. The Danish First Pregnancy Planner Study Team. *Int J Androl* 23, 309-318 (2000)

68. Hjollund N.H, L. Storgaard, E. Ernst, J.P. Bonde & J. Olsen: Impact of diurnal scrotal temperature on semen

quality. *Reprod Toxicol* 16, 215-221 (2002)

69. Peng B.C, P. Tomashefsky & H.M. Nagler: The cofactor effect: varicocele and infertility. *Fertil Steril* 54, 143-148 (1990)

70. Zorgniotti A.W: Elevated scrotal temperature: hypothesis for poor semen in infertile men. *Bull NY Acad Med* 58, 535-40 (1982)

71. Mirone V. & F. Iacono. Testicular hyperthermia: pahtophysiology, diagnostic and therapeutic concepts. *Adv Exp Biol Med* 286, 217-220 (1991)

72. Sealfon A.I. & A.W. Zorgniotti: A theoretical model for testis thermoregulation. *Adv Exp Biol Med* 286, 123-135 (1991)

73. Dada R, N.P. Gupta & K. Kucheria: Spermatogenic arrest in men with testicular hyperthermia. *Teratog Carcinog Mutagen* Suppl 1, 235-243 (2003)

74. McFadden M.R. & D.J. Mehan: Testicular biopsies in 101 cases of varicocele. *J Urol* 119, 372-374 (1978)

75. Abdelrahim F, A. Mostafa, A. Hamdy, M. Mabrouk, M. el-Kholy & O. Hassan: Testicular morphology and function in varicocele paitents: pre-operative and post-operative histopathology. *Br J Urol* 72, 643-647 (1993)

76. Rosenthal J, J.L. Marmar, C. Millan & S. Benoff: Measurement of the basal lamina (BL) thickness and cell types within the seminiferous tubules of varicocele patients. Twenty-Ninth Annual Meeting of the American Society of Andrology, Abstract No. 144, p.86 (2004)

77. Wang C, Y.G. Cui, X.H. Wang, Y. Jia, A. Sinha Hikim, Y.H. Lue, J.S. Tong, L.X. Qian, J.H. Sha, Z.M. Zhou, L. Hull, A. Leung & R.S. Swerdloff: Transient scrotal hyperthermia and levonorgestrel enhance testosterone-induced spermatogenesis suppression in men through increased germ cell apoptosis. *J Clin Endocrinol Metab* 92, 3292-3304 (2007)

78. Fujisawa M. & T. Ishikawa: Soluble forms of Fas and Fas ligand concentrations in the seminal plasma of infertile men with varicocele. *J Urol* 170, 2363-2365 (2003)

79. Benoff S, L. Yuan, C. Millan, I.R. Hurley & J.L. Marmar: Induction of Fas ligand (FasL) in varicocele by elevated testicular cadmium. Twenty-Ninth Annual Meeting of the American Society of Andrology, Abstract No. 35, p.49 (2004)

80. Tanaka H, M. Fujisawa, H. Okada, S. Arakawa & S. Kamidono: Assessment of germ-cell kinetics in the testes of patients with varicocele using image analysis of immunostained proliferating cell nuclear antigen. *Br J Urol* 78, 769-771 (1996)

81. Salama N, M. Tsuji, M. Tamura & S. Kagawa: Proliferating cell nuclear antigen in testes of infertile men with varicocele--preliminary results of interrelationship with sperm count before and after varicocelectomy. *Scand J Urol Nephrol* 37, 48-52 (2003)

82. Nuishiyama H, S. Danno, Y. Kaneko, K. Itoh, H. Yokoi, M. Fukumoto, H. Okuno, J.L. Millan, T. Matsuda, O. Yoshida & J. Fujita: Decreased expression of cold-inducible RNA- binding protein (CIRP) in male germ cells at elevated temperature. *Am J Pathol* 152, 289-296 (1998)

83. Rockett J.C, F.L. Mapp, J.B. Garges, J.C. Luft, C. Mori & D.J. Dix: Effects of hyperthermia on spermatogenesis, apoptosis, gene expression, and fertility in adult male mice. *Biol Reprod* 65, 229-239 (2001)

84. Filho P.A.H, A.P. Cedenho, S.B. Lima, V. Ortiz & M. Srougi: Single nucleotide polymorphisms of th heat shock protein 90 gene in varicocele-associated infertility. *Int Braz J Urol* 31, 236-244 (2005)

85. Yesilli C, G. Mungan, I. Seckiner, B. Akduman, S. Acikgoz, K. Altan & A. Mungan: Effect of varicocelectomy on sperm creatine kinase, HspA2 chpaerone proteins (creatine kinase-M type), LDH, LDH-X, and lipid peroxidation product levels in infertile men with varicocele. *Urology* 66, 610-615 (2005)

86. Lima S.B, M.A. Cenedeze, R.P. Bertolla, P.A. H. Filho, S. Oehninger & A.P. Cedenho: Expression of the HSPA2 gene in ejaculated spermatozoa from adolescents with and without varicocele. *Fertil Steril* 86, 1659-1663 (2006)

87. Johnsen S.G. & P. Agger: Quantitative evaluation of testicular biopsies before and after operation for varicocele. *Fertil Steril* 29, 58-63 (1978)

88. Ahlberg N.E, O. Bartley, N. Chidekel and A. Fritjofsson: Phlebography in varicocele scroti. *Acta Radiol Diagn* (Stockh) 4, 517-528 (1966)

89. Kohler F.P: On the etiology of varicocele. *J Urol* 97, 741-742 (1967)

90. Coolsaet B.L.R.A: The varicocele syndrome: venography determining the optimal level for surgical management. *J Urol* 124, 833-839 (1980)

91. Comhaire F M. Simons, M. Kunnen & L. Vermeulen: Testicular arterial perfusion in varicocele: the role of rapid sequence scintigraphy with technetium in varicocele evaluation. *J Urol* 130, 923-926 (1983)

92. Braedel H.U, J. Steffens, M. Ziegler, M.S. Polsky & M.L. Platt: A possible ontogenic etiology for idiopathic left varicocele. *J Urol* 151, 62-66 (1994)

93. Comhaire F. & M. Kunnen: Selective retrograde venography of the internal spermatic vein: a conclusive approach to the diagnosis of varicocele. *Andrologia* 8, 11-27 (1976)

94. Shafik A. & G.A.M. Bedeir: Venous tension patterns in cord veins. I. In normal and varicocele individuals. *J Urol* 123, 383-385 (1980)

95. Comhaire F, R. Monteyne & M. Kunnun: Radiologic anatomy of the internal spermatic vein(s) in 200 retrograde venograms. *Int J Androl* 4, 379-387 (1981)

96. Mali W.P, J.W. Ardnt, B.ZL. Coolsaet, J. Kremer & H.Y. Oei: Haemodynamic aspects of left-sided varicocele and its association with so-called right-sided varicocele. *Int J Androl* 7, 297-308 (1984)

97. Y. Gat Y, Z. Zukerman, J. Chakraborty & M. Gornish: Varicocele, hypoxia and male infertility. Fluid mechanics analysis of the impaired testicular venous drainage system. *Hum Reprod* 20, 2614-261 (2005)

98. Gat Y, M. Gornish, U. Navon, J. Chakraborty, G.N. Bachar & I. Ben-Schlomo: Right varicocele and hypoxia, crucial factors in male infertility: fluid mechanics analysis of the impaired testicular drainage system. *Reprod Biomed Online* 13, 510-515 (2006)

99. Sayfan J. & Y. Adam: Varicocele subfertility and venous pressure in the left internal spermatic vein. *Fertil Steril* 29, 366-367 (1978)

100. Shafik A: Venous tension patterns in cord veins. II. After varicocele correction. *J Urol* 129, 749-751 (1983)

101. Goldstein M. & J.-F. Eid: Elevation of intratesticular and scrotal skin surface temperature in men with varicocele. *J Urol* 142, 743-745 (1989)

102. Zorgnotti A.W. (ed.): Temperature and Environmental Effects on the Testis. Plenum Press, New York (1991)

103. Hadziselimovic F, B, Herzog, P. Liebungut, P. Jenny & M. Buser: Testicular and vascular changes in children and adults with varicocele. *J Urol* 142, 583-585 (1989)

104. Nagler H.M: Commentary on the testicular microenvironment. *J Urol* 155, 800-801 (1996)

105. Koumans J, O. Steeno, W. Heyns & J.P. Michielsen: Dehydroepiandrosterone sulfate, androsterone sulfate and corticoids in spermatic vein blood of patients with left varicocele. *Andrologia* 1, 87 (1969)

106. Agger P: Plasma cortisol in the left spermatic vein in patients with varicocele. *Fertil Steril* 22, 270-274 (1971)

107. F. Comhaire F. & A. Vermeulen: Varicocele sterility: cortisol and catecholamines. *Fertil Steril* 25, 88-95 (1974)

108. Cohen M.S., L. Plaine & J.S. Brown: The role of internal spermatic vein plasma catecholamine determinations in subfertile men with varicoceles. *Fertil Steril* 26, 1243-1249 (1975)

109. Comhaire F: The pathogenesis of epididymo-testicular dysfunction in varicocele: factors other than temperature. Adv *Exp Biol Med* 286, 281-287 (1991)

110. Chia S.E, B. Xu, C.N. Ong, F.H.M. Tsakok & S.T. Lee: Effect of cadmium and cigarette smoking on human semen quality. *Int J Fertil* 39, 292-298 (1994)

111. Vine M.F, C.-K.J. Tse, P.-C. Hu & K.Y. Truong. Cigarette smoking and semen quality. *Fertil Steril* 65, 835-842, 1996.

112. Klaiber E.L, D.M. Broverman, T.P. Pokoly, A.J. Albert, P.J. Howard Jr. and J.F. Sherer Jr.: Interrelationships of cigarette smoking, testicular varicoceles, and seminal fluid indexes. *Fertil Steril* 47, 481-486 (1987)

113. Benoff S, A. Jacob & I.R. Hurley: Male infertility and environmental exposure to lead and cadmium. *Hum Reprod Update* 6, 107-121 (2000)

114. Benoff S, I.R. Hurley, M. Barcia, F.S. Mandel, G.W. Cooper & A. Hershlag: A potential role for cadmium in the etiology of varicocele-associated infertility. *Fertil Steril* 67, 336-347 (1997)

115. Benoff S, L.O. Goodwin, C. Millan, I.R. Hurley, R.G. Pergolizzi & J.L. Marmar: Deletions in L-type calcium channel alpha1 subunit testicular transcripts correlate with testicular cadmium and apoptosis in infertile men with varicoceles. *Fertil Steril* 83, 622-634 (2005)

116. Benoff S, C.C. Chu, J.L. Marmar, R.Z. Sokol, L.O. Goodwin & I.R. Hurley: Voltage-dependent calcium channels in mammalian spermatozoa revisited. *Front Biosci* 12, 1420-1449 (2007)

117. Benoff S, K. Auborn, J.L. Marmar & I.R. Hurley: Link between low-dose environmentally relevant cadmium exposures and asthenozoospermia in a rat model. *Fertil Steril* 89 (2 Suppl):e73-79 (2008)

118. Johnson A.D, W.R. Gomes & N.L. VanDemark: Early actions of cadmium in the rat and domestic flow testis. *J Reprod Fert* 21, 383-393 (1970)

119. Chen L, J. Zhou, W. Gao & Y.Z. Jiang: Action of NO and TNF-alpha release of rats with cadmium loading in malfunction of multiple system organ. *Sheng Li Xue Bao* 25, 535-540 (2003)

120. Benoff S, I. Hurley, L. Yuan & J. Marmar: Endothelial nitric oxide synthase (eNOS) and inducibile nitric oxide synthase (iNOS) are separately induced and have separable effects in varicocele infertility. Thirty-First Annual Meeting of the American Society of Andrology, Abstract no. 53, p.57 (2006)

121. Millan C, I.R. Hurley, J.L. Marmar & S. Benoff: Can varicocelectomy outcome be altered by selective defects in testis L-type voltage-dependent calcium channels (L-

VDCC)? Twenty-Eighth Annual Meeting of the American Society of Andrology, P120 (2003)

122. Shin H.J, K.K. Park, B.H. Lee, C.K. Moon & M.O. Lee: Identification of genes that are induced after cadmium exposure by suppression subtractive hybridization. *Toxicology* 191, 121-131 (2003)

123. Benoff S.H, L. Yuan, C. Millan, I.R. Hurley & J.L. Marmar: Induction of CD34 and VEGF in varicocele by elevated testicular cadmium (Cd) and the outcome of varicocelectomy. Sixtieth Annual Meeting of the American Society for Reproductive Medicine, Abstract No. O-52, p.S20-S21 (2004)

124. Shiraishi K. & K. Naito: Involvement of vascular endothelial growth factor on spermatogenesis in testis with varicocele. *Fertil Steril* (2007) Dec 3. (Epub ahead of print)

125. Dias S, S.V. Shmelkov, G. Lam & S. Rafii: VEGF (165) promotes survival of leukemic cells by Hsp90-mediated induction of Bcl-2 expression and apoptosis inhibition. *Blood* 99, 2532-2540 (2002)

126. Tran J, Z. Master, J.L. Yu, J. Rak, D.J. Dumont & R.S. Kerbel: A role for survivin in chemoresistance of endothelial cells mediated by VEGF. *Proc Nat Acad Sci* USA 99, 4349-4354 (2002)

127. Marmar J.L. & S. Benoff: The safety of ultrasonically guided testis aspiration biopsies and efficacy of use to predict varicocelectomy outcome. *Hum Reprod* 20, 2279-2288 (2005)

128. Netto N.R. Jr, G.C. Lemos & G.M. Goes: Varicocele: relation between anoxia and hypospermatogenesis. *Int J Fertil.* 22:174-178 (1977)

129. Girgis S.M, Y. Abd el-Rahman, H. Awad, I. Eisa, N. Younan, B. Mittawy & Q.A. el-Saleh: Lactate and pyruvate levels in the testicular vein of subfertile males with varicocele as a test for the theory of underlying hypoxia. *Andrologia* 13, 16-19 (1981)

130. Chakraborty J, A.P. Hikim & J.S. Jhunjhunwala: Stagnation of blood in the microcirculatory vessels in the testes of men with varicoceles. *J Androl* 6, 117-126 (1985)

131. Lee J.D, S.Y. Jeng & T.H. Lee: Increased expression of hypoxia-inducible factor-1alpha in the internal spermatic vein of patients with varicocele. *J Urol* 175, 1045-1048 (2006)

132. Sullivan R. & C.H. Graham: Hypoxia-driven selection of the metastatic phenotype. *Cancer Metastasis Rev* 26, 319-331 (2007)

133. Mitropoulos D, G. Deliconstantinos, A. Zervas, V. Villiotou, C. Dimopoulos & J. Stavrides: Nitric oxide synthase and xanthine oxidase activities in the spermatic vein of patients with varicocele: a potential role for nitric oxide and peroxynitrite in sperm dysfunction. *J Urol* 156, 1952-1958 (1996)

134. Middendorf R, D. Mueller, S. Wichers, A.F. Holstein & M.S. Davidoff: Evidence for production and functional activity of Nitric Oxide in seminiferous tubules and blood vessels of the human testes. *J Clin Endocrin Metabol* 82, 4154-4161 (1997)

135. Romeo C, R. Gentile, G Santoro, P. Impellizzeri, N. Turiaco, P. Impala, S. Cifala, G. Cutroneo, F. Trimarchi & C. Gentile: Nitric oxide is increased in the spermatic veins of adolescents with left idiopathic varicocele. *J Pediatr Surg* 36, 389-393 (2001)

136. Santoro G, C. Romeo, P. Impellizeri, R. Ientile, G. Cutroneo, F. Trimarci, S. Pedale, N. Turiaco & C. Gentile: Nitric oxide synthase patterns in normal and varicocele testes in adolescents. *BJU International* 88, 967-973 (2001)

137. Weese D.L, M.L. Peaster, K.K. Himsl, G.E. Leach, P.M. Lad & P.E. Zimmern: Stimulated reactive oxygen species generation in the spermatozoa of infertile men. *J Urol* 149, 64-67 (1993)

138. Sharma R.K. & A. Agarwal: Role of reactive oxygen species in male infertility. *Urology* 48, 835-850 (1996)

139. Hendin B.N, P.N. Kolettis, R.K. Sharma, A.J. Thomas Jr. & A. Agarwal: Stimulated reactive oxygen species generation in spermatozoa of infertile men. *J Urol* 161, 1831-1834 (1999)

140. Koksal I.T, M. Usta, I. Orhan, S. Abbasoglu & A. Kadioglu: Potential role of reactive oxygen species on testicular pathology associated with infertility. *Asian J Androl* 5, 95-99 (2003)

141. Allamaneni S.S, C.K. Naughton, R.K. Sharma, A.J. Thomas Jr. & A. Agarwal: Increased seminal reactive oxygen species levels in paitents with varicoceles correlate with varicocele grade but not with testis size. *Fertil Steril* 82, 1684-1686 (2005)

142. Cocuzza M, K.S. Athayde, A. Agarwal, R. Pagani, S.C. Sikka, A.M. Lucon, M. Srougi & J. Hallak: Impact of clinical varicocele and testis size on seminal reactive oxygen species levels in a fertile population: a prospective controlled study. *Fertil Steril* (2007) Nov 15. (Epub ahead of print)

143. Romeo C, R. Ientile, P. Imellizzeri, N. Turiaco, M. Teletta, P. Antonuccio, M. Basile & C. Gentile: Preliminary report on nitric oxide-mediated oxidative damage in adolescent varicocele. *Hum Reprod* 18, 26-29 (2003)

144. Turkyilmaz Z, S. Gulen, K., Sonmz, R. Karabulut, S. Dincer, A. Can Basaklar & N. Kale: Increased nitric oxide is accompanied by lipid oxidation in adolescent varicocele. *Int J Androl* 27, 183-187 (2004)

145. Semercioz A, R. Onur, S. Ogras & I Orhan: Effects of melatonin on testicular tissue nitric oxide level and antioxidant enzyme activities in experimentally induced left varicocele. *Neuro Endocrinol Lett* 24, 86-90 (2003)

146. Esrefoglu M, M. Gul, H. Parlakpinar & A. Acet: Effects of melatonin and caffeic acide phenethyl ester on testicular injury induced by myocardial ischemia/reperfusion in rats. *Fundam Clin Pharmacol* 19, 365-372 (2005)

147. Awad H, F. Halawa, T. Mostafa & H. Atta: Melatonin hormonal profile in infertile males. *Int J Androl* 30, 409-413 (2006)

148. de Lamirande E. & C. Gagnon: Impact of reactive oxygen species on spermatozoa: a balancing act between beneficial and detrimental effects. *Hum Reprod* 10, 15-21 (1995)

149. Chen S.-S, L.S. Chang & Y.-H. Wei: Oxidative damage to proteins and decrease of antioxidant capacity in patients with varicocele. *Free Radic Biol Med* 30, 1328-1334 (2001)

150. Chen, S.-S, W.J. Huang, L.S. Chang & Y.H. Wei: 8-hydroxy-2'-deoxyguanosine in leukocyte DNA of spermatic vein as a biomarker of oxidative stress in patients with varicocele. *J Urol* 172, 1239-1240 (2004)

151. Ishikawa T, H. Fujioka, T. Ishimura, A. Takenaka & M. Fujisawa: Increased testicular 8-hydroxy-2'-deoxyguanosine in patients with varicoceles. *BJU Int* 100, 868-866 (2007)

152. Smith R, H. Kaune, D. Parodi, M. Madariaga, R. Rios, I. Morales & A. Castro: Increased sperm DNA damage in patients with varicocele: relation with seminal oxidative stress. *Hum Reprod* 21, 986-993 (2006)

153. Koksal I.T, A. Tefekli, M. Usta, H. Erol, S. Abbasoglu & A. Kadioglu: The role of reactive oxygen species in testicular dysfunction associated with varicocele. *BJU Int* 86, 549-552 (2000)

154. Chen L.P, S.Q. Wang, Y.Z. Zhao, L. Liu, S.X. Wang & Y.Z. Lu: The changes in IL-1 and NO levels in the testes of rats with experimental varicocele. *Zhonghua Nan Ke Xue* 8, 125-126 (2002)

155 Shiraishi K. & K. Naito: Generation of 4-hyroxy-2-nonenal modified proteins in testes predicts improvement in spermatogenesis after varicocelectomy. *Fertil Steril* 86, 233-235 (2006)

156. Shiraishi K. & K. Naito: Increased expression of Leydig cell haem oxygenase-1 preserves spermatogenesis in varicocele. *Hum Reprod* 20, 2608-2613 (2005)

157. Zini A, M. Buckspan, M. Jamal & K. Jarvi: Effect of varicocelectomy on the abnormal retention of residual cytoplasm by human spermatozoa. *Hum Reprod* 14, 1791-1793 (1999)

158. Nallella K.P, S.S. Allamameni, F.F. Pasqualotto, R.K. Sharma, A.J. Thomas Jr. & A. Agarwal: Relationship of interleukin-6 with semen characteristics and oxidative stress in patients with varicocele. *Urology* 64, 1010-1013 (2004)

159. Uchida K: 4-Hydroxy-2-nonenal: a product and mediator of oxidative stress. *Prog Lipid Res* 42, 318-343 (2003)

160. Shiraishi K & K. Naito: Effects of 4-hydroxy-2-nonenal, a marker of oxidative stress, on spermatogenesis and expression of p53 protein in male infertility. *J Urol* 178, 1012-1017 (2007)

161. Haupt S, M. Berger, Z. Goldberg & Y. Haupt: Apoptosis – the p53 network. *J Cell Sci* 116, 4077-4085 (2003)

162. Chen S.-S, L.S. Chang, H.-W. Chen & Y.-H. Wei: Polymorphisms of glutathione S-transferase M1 and male infertility in Taiwanese patients with varicocele. *Hum Reprod* 17, 718-725 (2002)

163. Kao S.H, H.T. Chao & Y.H. Wei: Mitochondrial deoxyribonucleic acid 4977-bp deletion is associated with diminished fertility and motility of human sperm. *Biol Reprod* 52, 729-736 (1995)

164. Lenzi A, M. Picardo, L. Gandini & F. Dondero: Lipids of the sperm plasma membrane: from polyunsaturated fatty acids considered as markers of sperm function to possible scavenger therapy. *Hum Reprod Update* 2, 246-256 (1996)

165. Lenzi A, M. Picardo, L. Gandini, F. Lombardo, O. Terminali, S. Passi & F. Dondero: Glutathione treatment of dyspermia: effect on the lipoperoxidation process. *Hum Reprod* 9, 2044-2050 (1994)

166. Mecucci E, D. Lilardi, A. Mordente, G.E Martorana, E. Giacchi, L. De Marinis & A. Mancini: Total antioxidant capacity in patients with varicoceles. *Fertil Steril* 79 Suppl 3, 1577-1583 (2003)

167. Bach T, D. Pfeiffer & R. Tauber: Baseline follicle-stimulating hormone is a strong predictor for the outcome of the gonadotropin-releasing hormone test in young men with unilateral medium- or high-grade varicocele. *BJU Int* 98, 619-622 (2006)

168. Mancini A, G. Conte, D. Milardi, L.de Marinis & G.P. Littarru: Relationship between sperm cell ubiquinone and seminal parameters in subjects with and without varicocele. *Andrologia* 30, 1-4 (1998)

169. Barbieri E.R, M.E. Hidalgo, A. Venegas, R. Smith & E.A. Lissi: Varicocele-associated decrease in antioxidant defenses. *J Androl* 20, 713-717 (1999)

170. Mancini A, E. Meucci, D. Milardi, E. Giacchi, A. Bianchi, A.L. Pantano, A. Mordente, G.E. Martorana & L. de Marinis: Seminal antioxidant capacity in pre- and postoperative varicocele. *J Androl* 25, 44-49 (2004)

171. Saleh R.A, A. Agarwal, R.K. Sharma, D.R. Nelson & A.J. Thomas Jr.: Effect of cigarette smoking on levels of seminal oxidative stress in infertile men: a prospective study. *Fertil Steril* 78, 491-499 (2002)

172. Mostafa T, T.H. Anis, A. El-Nashar, H. Imam & I.A. Othman: Varicocelectomy reduces reactive oxygen species and increases antioxidant activity of seminal plasma from infertile men with varicoceles. *Int J Androl* 24, 261-265 (2001)

173. Mancini A, D. Milardi, G. Conte, R. Festa, L. De Marinis & G.P. Littarru: Seminal antixidants in humans: preoperative and postoperative evaluation of coezyme Q10 in varicocele patients. *Horm Metab Res* 37, 428-432 (2005)

174. Sinha Hikim, A.P & R.S. Swerdloff: Hormonal and genetic control of germ cell apoptosis in the testis. *Rev Reprod* 4, 38-47 (1999)

175. Schinfeld J.S, I. Schiff & R. Newton: Testicular hormone concentraqtion in men with varicoceles: immediate effects of varicocelectomy. *Int J Fertil* 28, 177-180 (1983)

176. Comhaire F. & A. Vermeulen: Plasma testosterone on patients with varicocele and sexual inadequacy. *J Clin Endocrinol Metab* 40, 824-829 (1975)

177. Micic S, R. Dtlic, V. Ilic & O. Genbacev: Seminal plasma hormone profile in infertile men with and without varicocele. Arch Androl 17, 173-178 (1986)

178. Hudson R.W: Free sex steroid and sex hormone-binding globulin levels in oligozoospermic men with varicoceles. *Fertil Steril* 66, 299-304 (1996)

179. Cayan S, A. Kadioglu, I. Orhan, E. Kandirali, A Tefekli & S. Tellaloglu: The effect of microsugical varicocelectomy on serum follicle stimulating hormone, testosterone and free testosterone levels in infertile men with varicocele. *BJU Int* 84, 1046-1049 (1999)

180. Younes A.K: Low plasma testosterone in varicocele patients with impotence and male infertility. *Arch Androl* 45, 187-195 (2000)

181. Y. Gat Y, M. Gornish, A. Belenky & G.N. Bachar: Elevation of serum testosterone and free testosterone after embolization of the internal spermatic vein for the treatment of varicocele in infertile men. *Hum Reprod* 19, 2303-2306 (2004)

182. Ando S, C. Giachetto, G. Colpi, M.L. Panno, E. Beraldi, A. Lombardi & G. Sposato: Plasma levels ofs 17-OH-progesterone and testosterone in patients with varicoceles. *Acta Endocrinol* 102, 463-469 (1983)

183. Ando S, C. Giacchetto, E. Beraldi, M.L. Panno, A. Carpino & C. Brancati: Progesterone, 17-OH-progesterone, and androstenedione and testosterone levels in spermatic venous blood of normal men and varicocele patients. *Horm Metab Res* 17, 99-103 (1985)

184. Castro-Magana M, M. Angulo & J Uy: Elevated serm estradiol associated wit increased adrostenedione-testosterone ratio in adolescent males with varicoceles and gynecomastia. *Fertil Steril* 56, 515-518 (1991)

185. Hampl R, M. Lachman, Z. Novak, J. Sulcova & L. Starka: Serum levels of steroid hormones in men with varicocele and oligospermia as compared to normozoospermic men. *Exp Clin Endocrinol* 100, 117-119 (1992)

186. Weiss D.B, L.J. Rodriguez-Rigau, K.D. Smith & E. Steinberger: Leydig cell function in oligospermic men with varicocele. *J Urol* 120, 427-430 (1978)

187. Ando A, C. Giacchetto, E. Beraldi, M.L. Panno, A. Lombardi, G. Sposato & G. Golpi: The influence of age on Leydig cell function in patients with varicocele. *Int J Androl* 7, 104-118 (1984)

188. Ando S, C. Giacchetto, G. Colpi, E. Beraldi, M.L. Panno, A. Lombardi & G. Sposato: Physiopathologic aspects of Leydig cell function in varicocele patients. *J Androl* 5, 163-170 (1984)

189. Weiss D.B, L. Rodriguez-Rigau, K.D. Smith, A. Chowdhury & E. Steinberger: Quantitation of Leydig cells in testicular biopsies of oligospermic men with varicocele. *Fertil Steril* 30, 305-312 (1978)

190. Rodriguez-Rigau L.J, D.B. Weiss, Z. Zukerman, H.E. Grotjan, K.D. Smith & E. Steinberger: A possible mechanism for the detrimental effect of varicocele on testicular function in man. *Fertil Steril* 30, 577-585 (1978)

191. Sirvent J.J, R. Bernat, M.A. Navarro, J. Rodriguez Tolra, R. Guspi & R. Bosch: Leydig cell in idiopathic varicocele. *Eur Urol* 17, 257-261 (1990)

192. Younes A.K: Improvement in sexual activity, pregnancy rate, and low plasma testosterone after bilateral varicocelectomy in impotence and male infertility patients. *Arch Androl* 49, 219-228 (2003)

193. Ishikawa T, H. Fujioka, T. Ishimura, A. Takenaka & M. Fujisawa: Ghrelin expression in human testis and serum testosterone level. *J Androl* 28, 320-324 (2007)

194. Scholler R, K. Nahoul, M. Castanier, J. Rotman & J. Salat-Baroux: Testicular secretion of conjugated and unconjugated steroids in normal adults and in patients with varicocele. Baseline levels and time-course response to hCG administration. *J Steroid Biochem* 20, 203-215 (1984)

195. Takeyama M, M. Honjoh, M. Kodama, H. Sakaguchi, E. Koh, N. Kondoh, H. Fujioka, M. Nakamura, M. Okuyama, K. Matsumoto & T. Sonoda: Testicular steroids in spermatic and peripheral veins after a single injection of hCG in patients with varicocele. *Arch Androl* 24, 207-213 (1990)

196. Benoff S, I.R. Hurley, H. Xu & J.L. Marmar: Alteration in Leydig cell number and morphology among infertile men with varicoceles. Sixty-Second Annual Meeting of the American Society for

Reproductive Mdeicine, Abstract no. O-114, pS372-S373 (2006)

197. Swerdloff R.S. & P.C. Walsh: Pituitary and gonadal hormones in patients with varicoceles. *Fertil Steril* 26, 1006 (1975)

198. Micic S, V. Illic & M. Isvaneski: Correlation of hormone and histologic parameters in infertile men with varicocele. *Urol Int* 38, 187-190 (1983)

199. Pirke K.M, H.J. Vogt, R. Sintermann & B. Spyra: Testosterone in peripheral plasma, spermatic vein and in testicular tissue under basal conditions and after HCG-administration in patients with varicocele. *Andrologia* 15, 637-641 (1983)

200. Hudson R.W: The endocrinology of varicoceles. *Fertil Steril* 49, 199-208 (1988)

201. Haans L.C, J.S. Laven, W.P. Mali, E.R. te Velde & C.J. Wensing: Testis volumes, semen quality, and hormonal patterns in adolescents with and without varicoceles. *Fertil Steril* 56, 731-736 (1991)

202. Pasqualotto F.F, A.M. Lucon, P.M. de Goes, B.P. Sobreiro, J. Hallak, E.B. Pasqualotto & S. Arap: Semen profile, testicular volume, and hormonal levels in infertile patients with varicoceles compared with fertile men with and without varicoceles. *Fertil Steril* 83, 74-77 (2005)

203. N. Guarino N, B. Tadini & M. Bianchi: The adolescent varicocele: the crucial role of hormonal tests in selecting patients with testicular dysfunction. *J Pediatr Surg* 38, 120-123 (2003)

204. Pujo A, J.R. Tolra, M.A. Navarro, R. Bonnin, J.J. Sirvent, M. Pladellorens & R. Bernat: The hormonal pattern in varicocele and its relationship with the findings of testicular biopsy: preliminary results. *Br J Urol* 54, 300-304 (1982)

205. Hudson R.W. & D.E. McKay: The gonadotropin response of men with varicoceles to gonadotropin-releasing hormone. *Fertil Steril* 33, 427-432 (1980)

206. Hudson R.W, R.A. Perez-Marrerp, V.A. Crawford & D.E. McKay: Hormonal parameters of men with varicoceles before and after varicocelectomy. *Fertil Steril* 43, 905-910 (1985)

207. Freire F.R. & C.R. Nahoum: Endocrine evaluation in infertile men with varicocele. *Andrologia* 13, 395-404 (1981)

208. Bickel A. & G. Dickstein: Factors predicting the outcome of varicocele repair for subfertility: the value of the luteinizing hormone-releasing hormone test. *J Urol* 142, 1230-1234 (1989)

209. Podesta M.L, S. Gottlieb, R. Medel Jr., G. Ropelato, C. Bergada & E.M. Quesada: Hromonal parameters and testicular volume in children and adolescents with unilateral varicocele: preoperative and postoperative findings. *J Urol* 152, 794-797 (1994)

210. J.A. Osuna J.A, J.R. Lozano, I. Cruz & I. Tortolero: Pituitary and testicular function in adolescents with varicocele. *Arch Androl* 43, 183-188 (1999)

211. Plymate S.R, C.A. Paulsen & R.I. McLachlan: Relationship between inhibin levels to serum follicle stimulating hormone and sperm production in normal men and men with varicoceles. *J Clin Endocrinol Metab* 74, 859-864 (1992)

212. Nagao R.R, S.R. Plymate, R.E. Berger, E.B. Perin & C.A. Paulsen: Comparison of gonadal function between fertile and infertile men with varicoceles. *Fertil Steril* 46, 930-933 (1986)

213. Mormandi E, O. Levalle, M.G. Ballerini, R. Hermes, R.S. Calandra & S. Campo: Serum levels of dimeric and monomeric inhibins and the degree of seminal alteration in infertile men with varicocele. *Andrologia* 35, 106-111 (2003)

214. Pierik F.H, S.A. Abdesselam, J.T. Vreeburg, G.R. Dohle, F.H. De Jong & R.F. Weber: Increased serum inhibin levels after varicocele treatment. *Clin Endocrinol* 54, 775-780 (2001)

215. Trigo R.V, I. Bergada, R. Rey, M.G. Ballerini, P. Bedecarras, C. Bergada, S. Gottlieb & S. Campo: Altered serum profile of inhibin B, pro-alphaC and anti-Mullerian hormone in prepubertal and pubertal boys with varicocele. *Clin Endocrinol* 60, 758-764 (2004)

216. Bohring C. & W. Krause: Serum levels of inhibin in men with different causes of spermatogenic failure. *Andrologia* 31, 137-141 (1999)

217. Koksal I.T, Y. Ishak, M. Usta, A. Danisman, E. Guntekin, I.C. Bassorgun & A. Ciftcioglu: Varicocele-induced testicular dysfunction may be associated with disruption of the blood-testis barrier. *Arch Androl* 53, 43-48 (2007)

218. Ashkenazi J, D. Dicker, D. Fedlberg, M. Shelef, G.A. Godlman & J Goldman: The impact of spermatic vein ligation on the male factor in *in vitro* fertilization-embryo transfer and its relation to testosterone levels before and after operation. *Fertil Steril* 51, 471-474 (1989)

219. Ishikawa T. & M. Fujisawa: Varicocele ligation on free testosterone levels in infertile men with varicocele. *Arch Androl* 50, 443-448 (2004)

220. Su L.M, M. Goldstein & P.N. Schlegel: The effect of varicocelectomy on serum testosterone levels in infertile men with varicoceles. *J Urol* 154, 1752-1755 (1995)

221. Castro-Magana M, M. Angulo, A. Canas & J. Uy: Leydig cell function in adolescent boys with varicoceles. *Arch Androl* 24, 73-79 (1990)

222. Hudson R.W, R.A. Perez-Marrero, V.A. Crawford & D.E. McKay: Hormonal parameters in incidental

varicoceles and those causing infertility. *Fertil Steril* 45, 692-700 (1986)

223. Fujisawa M, A. Hayashi, O. Imanishi, H. Tanaka, H. Okada, O. Matsumoto & S. Kamidono: The significance of the gonadotropin-releasing hormone test for predicting fertility after varicocelectomy. *Fertil Steril* 61, 779-782 (1994)

224. Segenreich E, S. Israilov, J. Shmulele, E. Niv, J Baniel & P Livne: Evaluation of the relationship between semen parameters, pregnancy rate of wives of infertile men with varicocele, and gonadotropin-releasing hormone test before and after varicocelectomy. *Urology* 52, 853-857 (1998)

225. Lucis O.J & R. Lucis: Distribution of Cadmium 109 and Zinc 65 in mice of inbred strains. *Arch Environ Health* 19, 334-336 (1969)

226. Chellman G.J, Z.A. Shaikh & R.B. Baggs: Decreased uptake and altered subcellular disposition of testicular cadmium as possible mechanisms of resistance to cadmium-induced testicular necrosis in inbred mice. *Toxicology* 30, 157-169 (1984)

227. King L.M, M.B. Anderson, S.C. Sikka & W.J. George: Murine strain differences and the effects of zinc on cadmium concentration in tissues after acute cadmium exposure. *Arch Toxicol* 72, 650-655 (1998)

228. King L.M, W.A. Banks & W.J. George: Differences in cadmium transport to the testes, epididymis, and brain in cadmium-sensitive and –resistant murine strains 129/J and A/J. *J Pharmacol Exp Therapeut* 289, 825-830 (1999)

229. Benoff S: Environmental toxins and varicocele. *Assisted Reproductive Technology/Andrology* IX, 261-284 (1997)

230. Benoff S, M. Barcia, I.R. Hurley, G.W. Cooper, F.S. Mandel, S. Heyner, W.T. Garside, B.R. Gilbert & A. Hershlag: Classifications of male factor infertility relevant to IVF insemination strategies using mannose ligands, acrosome status and anti-cytoskeletal antibodies. *Hum Reprod* 11, 1905-1918 (1996)

231. Vigil P, C. Wohler, E. Bustos-Obregon, F. Comhaire & P. Morales: Assessment of sperm function in fertile and infertile men. *Andrologia* 26, 55-60 (1994)

232. Glazier D.B, J.L. Marmar, S.M. Diamond, M. Gibbs & S.L. Corson: A modified acrosome induction test. *Arch Androl* 44, 59-64 (2000)

233. Fuse H, M. Iwasaki, I. Mizuno & Y. Ikehara-Kawauchi: Evaluation of acrosome reactivity using the Acrobeads test in varicocele patients: findings before and after treatment. *Arch Androl* 49, 1-6 (2003)

234. El Mulla K.F, F.M. Kohn, A.H. El Beheiry & W.B. Schill: The effect of smoking and varicocele on human sperm acrosin activity and acrosome reaction. *Hum Reprod* 10, 3190-3194 (1995)

235. Benoff S, J.I. Rushbrook, I.R. Hurley, F.S. Mandel, M. Barcia, G.W. Cooper & A. Hershlag: Co-expression of mannose-ligand and non-nuclear progesterone receptors identifies an acrosome reaction-inducible subpopulation in fertile donor sperm. *Am J Reprod Immunol* 34,100-115 (1995)

236. Witkin S.S. & A. Toth: Relationship between genital tract infections, sperm antibodies in seminal fluid and infertility. *Fertil Steril* 40, 805-808 (1983)

237. Ozen H, G. Asar, S. Gungor & A.F. Peker: Varicocele and antisperm antibodies. *Int Urol Nephrol* 17, 97-101 (1985)

238. Golomb J, N. Vardinon, Z.T. Homonnai, Z. Braf & I. Yst: Demonstration of antispermatozoal antibodies in varicocele-related infertility with an enzyme-linked immunosorbent assay (ELISA). *Fertil Steril* 45, 397-402 (1986)

239. Gilbert B.R, S.S. Witkin & M. Goldstein: Correlation of sperm-bound immunoglobulins with impaired semen analysis in infertile men with varicoceles. *Fertil Steril* 52, 469-473 (1989)

240. Jarow J.P. 7 J.J. Sanzone: Risk factors for male partner antisperm antibodies. *J Urol* 148, 1805-1807 (1992)

241. Oshinsky G.S, M.V. Rodriguez & B. Mellinger: Varicocele-related infertility is not associated with increased sperm bound antibody. *J Urol* 150, 871-873 (1993)

242. Knudson G, L. Ross, D. Stuhldreher, D. Houlihan, E. Bruns & G. Prins: Prevalence of sperm bound antibodies in infertile men with varicocele: the effect of varicocele ligation on antibody levels and semen response. *J Urol* 151, 1260-1262 (1994)

243. Djaladat H, A. Mehrsai, M. Rezazade, Y. Djaladat & G. Pourmand: Varicocele and antisperm antibody: fact or fiction. *South Med J* 99, 44-47 (2006)

244. Bohring C. & W. Krause: Differences in the antigen pattern recognized by antipserm antibodies in patients with infertility and vasectomy. *J Urol* 166, 1178-1180 (2001)

245. Buonaguidi A, M. Grasso, C. Lania, M. Castelli, F. Francesca & P. Rigatti: Experiencias sobre la dosificacion de la LDH-X en el plasma seminal como factor diagnostico y pronostico en el varicocele. *Arch Esp de Urol* 46, 35-39 (1993)

246. Morgentaler A, B.C. Stahl & Y. Yin: Testis and temperature: an historical, clinical, and research perspective. *J Androl* 20, 189-195 (1999)

247. Mucahy J.J: Scrotal hyperthermia and the infertile man. *J Urol* 132, 469-470 (1984)

248. Zorgniotti A.W. & A.I. Sealfon: Measurement of intrascrotal temperature in normal and subfertile men. *J Reprod Fertil* 82, 563-566 (1988)

249. Zorgniotti A.W, A.I. Sealfon & A. Toth: Chronic scrotal hypothermia as a treatment for poor semen quality. *Lancet* 1 (8174), 904-906 (1980)

250. Zorgniotti A.W., M.S. Cohen & A.I. Sealfon: Chronic scrotal hyperthermia: results from 90 infertile couples. *J Urol* 135, 944-947 (1986)

251. Murray R.M: Scrotal abnormalities in rams in tropical Queensland with particular reference to ovine brucellosis and its control. *Aust Vet J* 45, 63-7 (1969)

252. Watt D.A: Testicular pathology of Merino rams. *Aust Vet J* 54, 473-478 (1978)

253. Deshpande K.S, J.G. Diwan, C.C. Wakankar & B.R. Deshpande: Varicocele in a Merino ram. *Indian Vet J* 56, 236-239 (1979)

254. Ott R.S, E.H. Heath & A. Bane: Abnormal spermatozoa, testicular degeneration, and varicocele in a ram. *Am J Vet Res* 43, 241-245 (1982)

255. Turner T.T: The study of varicocele through the use of animal models. *Hum Reprod Update* 7, 78-84 (2001)

256. H.M. Nagler H.M, X.Z. Li, E.F. Lizza, A. Deitch & R.D. White: Varicocele: temporal considerations. *J Urol* 134, 411-413 (1985)

257. Turner T.T. & T.J. Lopez: Testicular blood flow in peri-pubertal and older rats with unilateral experimental varicocele and investigation into the mechanism of the bilateral response to a unilateral lesion. *J Urol* 144, 1018-1021 (1990)

258. Snydle F.E. & D.F. Cameron: Surgical induction of varicocele in the rabbit. *J Urol* 130, 1005-1009 (1983)

259. Sofikitis N & I. Miyagawa: Bilateral effect of unilateral varicocele on testicular metabolism in the rabbit. *Int J Fertil* 39, 239-247 (1994)

260. Kay R, N.J. Alexander & W.L. Baugham: Induced varicoceles in Rheus monkeys. *Fertil Steril* 31, 195-199 (1979)

261. Fussell E.N, R.W. Lewis, J.A. Roberts & R.M. Harrison: Early ultrastructural findings in experimentally produced varicocele in the monkey testis. *J Androl* 2, 111-119 (1981)

262. Harrison R.M, R.W. Lewis & J.A. Roberts: Pathophysiology of varicocele in nonhuman primates: long term seminal and testicular changes. *Fertil Steril* 46, 500-551 (1986)

263. Camglio F.S, N. Zampieri, M. Corroppolo, C. Chironi, G. Dipaola, L. Giacomello & A. Ottolenghi: Varicoceles and retrograde adrenal metabolites flow. *Urol Int* 73, 337-342 (2004)

264. Shook T.E, L.M. Nyberg, B.S. Collins & S. Mathur: Pathological and immunological effects of surgically induced varicocele in juvenile and adults rats. *Am J Reprod Immunol Microbiol* 17, 141-144 (1988)

265. Choi H, K.S. Kim & K.M. Kim: The effect of experimental varicocele on the testis of adolescent rats. *J Urol*144, 499-501 (1990)

266. Hurt G.S, S.S. Howards & T.T. Turner: The effects of unilateral, experimental varicocele are not mediated through the ipsilateral testis. *J Androl* 8, 403-408 (1987)

267. Turner T.T. & T.J. Lopez: Effects of experimental varicocele require neither adrenal contribution nor venous reflux. *J Urol* 142, 1372-1375 (1989)

268. Green K.F, T.T. Turner & S.S. Howards: Varicocele: reversal of the testicular blood flow and temperature effects by varicocele repair. *J Urol* 131, 1208-1211 (1984)

269. Hurt G.S, S.S. Howards & T.T. Turner: Repair of experimental varicoceles in the rat. Long-term effects on testicular blood flow and temperature and cauda epididymal sperm concentration and motility. *J Androl* 7, 271-276 (1986)

270. Turner T.T. & S.S. Howards: The venous anatomy of experimental left varicocele: comparison with naturally occurring left varicocele in human. *Fertil Steril* 62, 869-875 (1994)

271. Sofikitis N & I. Miyagawa: Left adrenalectomy in varicocelizedrats does not inhibit the development of varicocele-related physiologic alterations. *Int J Fertil Menopausal Stud* 38, 250-255 (1993)

272. A. Caruso A, R. Walsh, L. Ross, H. Trummer & R. Meacham: Experimental varicocele induces testicular germ cell apoptosis in the rat. *J Urol* 161, 280 (1999)

273. Xia Q, X.B. Zhang & J. Zhang: The apoptosis in damaged testiculartissue caused by varicocele. *Zhonghua Nan Ke Xue* 8, 414-415 (2002)

274. Barqawi A, A. Causo & R.B. Meacham: Experimental varicocele induces germ cell apoptosis in the rat. *J Urol* 171, 501-503 (2004)

275. Cam K, F. Simek, M. Yukel, L. Turkeri, G. Haklar, S. Yalcin & A. Akdas: The role of reactive oxygen species and apoptosis in the pathogenesis of varicocele in a rat model and efficiency of vitamin E treatment. *Int J Androl* 27, 228-233 (2004)

276. Onur R, A. Semercioz, I. Orhan & H. Yekeler: The effects of melatonin and the antioxidant defence system on apoptosis regulator proteins (Bax and Bcl-2) in experimentally induced varicocele. *Urol Res* 32, 204-208 (2004)

277. Zhang C.C, A.F. Zhou, M.L. Zhang & J.G. Cao: Effects of experimental varicocele on on the apoptosis of spermatogenic cells in rats. *Zhonghua Nan Ke Xue* 9, 507-509, 511 (2003)

278. Sofikitis N, C. Takahashi, I. Nakamura, S. Hirakawa & I. Miyagawa: Surgical repair of secondary right varicocele in rats with primary left varicocele: effects on fertility, testicular temperature, spermatogenesis, and sperm maturation. *Arch Androl* 28, 43-52 (1992)

279. Kilinc F, F. Kayaselcuk, C. Aygun, S. Guvel, T. Egilmez & H. Ozkardes: Experimental varicocele induces hypoxia inducible factor-1alpha, vascular endothelial growth factor expression and angiogenesis in rat testis. *J Urol* 172, 1188-1191 (2004)

280. Kilinc F, S. Guvel, F. Kayaselcuk, C. Aygun, T. Egilmez & H. Ozkardes: p53 Expression and apoptosis in varicocele in the rat testis. *J Urol* 172, 2475-2478 (2004)

281. De Stefani S, V. Silingardi, S. Micali, A. Mofferdin, M.C. Sighinolfi, A. Celia, G. Bianchi, S. Giulini, A. Volpe, F. Giusti & A. Maiorana: Experimental varicocele in the rat: early evaluation of the nitric oxide levels and histological alterations in testicular tissue. *Andrologia* 37, 115-118 (2005)

282. Kisa U, M.M. Basar, M. Ferhat, E. Yilmaz, H. Basar, O. Caglayan & E. Batislam: Testicular tissue nitric oxide and thiobarbituric acid reactive substance levels: evaluation with respect to the pathogenesis of varicocele. *Urol Res* 32, 196-199 (2004)

283. Koksal I.T, T. Ergogru, H. Gulkesen, C. Sezer, M. Usta, A. Ciftcioglu and M. Baykara: The potential role of inducible nitric oxide synthase (iNOS) activity in the testicular dysfunction associated with varicocele: an experimental study. *Int Urol Nephro*l 36, 67-72 (2004)

284. Ozdamar A.S, A.G. Soylu, M. Culha, M. Ozden & A. Gokalp: Testicular oxidative stress. Effects of experimental varicoceles in adolescent rats. *Urol Int* 73, 343-347 (2004)

285. Celik-Ozenci C, Z. Bayram, G. Akkoyunlu, E.T. Korgun, T. Erdogru, Y. Seval, I. Ustenel, M. Baykara & R. Demir: Localization of NGF and nNOS in varicocele-induced rat testis. *Acta Histochem* 107, 435-442 (2006)

286. Suzuki N & N. Sofikitis: Protective effects of antioxidants on testicular function in varicocelized rats. *Yonago Acta Med* 42, 87-94 (1999)

287. Celik-Ozenci C, Z. Sahin, I. Ustunel, G. Akkoyunlu, T. Erdogru, E.T. Korgun, M. Baykara and R. Demir: The Fas system may have a role in male reproduction. *Fertil Steril* 85 Suppl 1, 1168-1178 (2006)

288. Koksal T, T. Ergogru, B. Toptas, K. H. Gulkesen, M. Usta, A. Baykal & M. Baykara: Effect of experimental varicocele in rats on testicular oxidative stress status. *Andrologia* 34, 242-247 (2002)

289. Sweeney T.E, J.S. Rozym & R.W. Gore: Alteration of testicular microvascular pressures during venous pressure elevation. *Am J Physiol* 269, H37-H45 (1995)

290. Raitsina S.S, A.V. Kurnosov, I.M. Iarovaia, N.S. Gladkova & A.I. Davydova: Autoimmune nature of spermatogenesis disorders in varicocele models of rats. *Biull Eksp Bio Med* 87, 44-48 (1979)

291. SalamavN, A. Bergh & J.-E. Damber: The changes in testicular vascular permeability during progression of the experimental varicocele. *Eur Urol* 43, 84-91 (2003)

292. Turner T.T, C.E. Jones & M.S. Roddy: Experimental varicocele does not affect the blood-testis barrier, epididymal electrolyte concentrations, or testicular blood gas concentration. *Biol Reprod* 36, 926-932 (1987)

293. Turner T.T, L.A. Caplis & C.P. Rhoades: Testicular vascular permeability: effects of experimental lesions associated with impaired testis function. *J Urol* 155, 1078-1082 (1996)

294. Ogi S, N. Tanji, T. Iseda & M. Yokoyama: Expression of heat shock proteins in developing and degenerating rat testes. *Arch Androl* 43, 163-171 (1999)

295. Sahin Z, Z. Bayram, C. Celik-Ozenci, G. Akkoyunlu, Y. Seval, T. Erdogru, I. Ustunel, M. Baykara & R. Demir: Effect of experimental varicocele on the expressions of notch 1, 2, and 3 in rat testes: an immunohistochemical study. *Fertil Steril* 83, 86-94 (2005)

296. Ruas J.L, Lendahl & L. Poellinger: Modulation of vascular gene expression by hypoxia. *Curr Opin Lipidol* 18, 508-514 (2007)

297. Hayashi T, Y. Kageyama, K. Ishizaka, G. Xia, K. Kihara & H. Oshima: Requirement of Notch 1 and its ligand jagged 2 expressions for spermatogenesis in rat and human testes. *J Androl* 22, 999-1011 (2001)

298. Hayashi T, T. Yamada, Y. Kageyama & K. Kihara: Expression failure of the notch signaling system is associated with the pathogenesis of testicular germ cell tumor. *Tumour Biol* 25, 99-105 (2004)

299. Khan V.R. & I.R. Brown: The effect of hyperthermia on the induction of cell death in brain, testis, and thymus of the adult and developing rat. *Cell Stress Chaperones* 7, 73-90 (2002)

300. Lue Y.H, B.L. Lasley, L.S. Laughlin, R.S. Swerdloff, A.P. Hikim, A. Leung, J.W. Overstreet & C. Wang: Mild testicular hyperthermia induces profound transitional spermatogenic suppression through increased germ cell

apoptosis in adult cynomolgus monkeys (Macaca fascicularis). *J Androl* 23, 799-805 (2002)

301. Sinha Hikim A.P, Y. Lue, M. Diaz-Romero, P.H. Yen, C. Wang & R.S. Swerdloff: Deciphering the pathways of germ cell apoptosis in the testis. *J Steroid Biochem Mol Biol* 85, 175-182 (2003)

302. Vera Y, M. Diaz-Romero, S. Rodriguez, Y. Lue, C. Wang, R.S. Swerdloff & A.P. Sinha Hikim: Mitochondria-dependent pathway is involved in heat-induced male germ cell death: lessons from mutant mice. *Biol Reprod* 1534-1540 (2004)

303. Somwaru L, S. Li, L. Doglio, E. Goldberg & B.R. Zirkin: Heat-induced apoptosis of mouse meiotic cells is suppressed by ectopic expression of testis-specific calpastatin. *J Androl* 25, 506-513 (2004)

304. Lue Y, A.P. Sinha Hikim, C. Wang, A. Leung & R.S. Swerdloff: Functional role of inducible nitric oxide synthase in the induction of male germ cell apoptosis, regulation of sperm number, and determination of testes size: evidence from null mutant mice. *Endocrinology* 144, 3092-3100 (2003)

305. Yin Y, K.L. Hawkins, W.C. Dewolf & A. Morgentaler: Heat stress cause testicular germ cell apoptosis in adult mice. *J Androl* 18, 159-165 (1997)

306. Kumagai A, H. Kodama, J. Kumagai, J. Fukuda, K. Kawamura, H. Tanikawa, N. Sato & T. Tanaka: Xanthine oxidase inhibitors suppress testicular germ cell apoptosis induced by experimental cryptorchidism. *Mol Hum Reprod* 8, 118-123 (2002)

307. Okubo K, H. Nakamura, J. Watanabe, T. Kamoto, J. Yodoi, O. Ogawa & H. Nishiyama: Over expression of thioredoxin-1 in transgenic mice attenuates germ cell apoptosis. *J Urol* 172, 2479-2482 (2004)

308. Ishii T, S. Matsuki, Y. Iuchi, F. Okada, S. Toyosaki, Y. Tomita, Y. Ikeda & J. Fujii: Accelerated impairment of spermatogenic cells in SOD1-knockout mice under heat stress. *Free Radic Res* 39, 697-705 (2005)

309. Zini A, J. Abitol, D. Schulsinger, M. Goldstein & P. Schlegel: Restoration of spermatogenesis after scrotal replacement of experimentally cryptorchid rat testis: assessment of germ cell apoptosis and eNOS expression. *Urology* 53, 223-227 (1999)

310. DeFoor W.R, C.Y. Kuan, M. Pinkerton, C.A. Sheldon & A.G. Lewis: Modulation of germ cell apoptosis with a nitric oxide synthase inhibitor in a murine model of congenital cryptorchisism. *J Urol* 172, 1731-1735 (2004)

311. Ishikawa T, Y. Kondo, K. Goda & M. Fujisawa: Overexpression of endothelial nitric oxide synthase in transgenic miceaccelerates testicular germ cell apoptosis induced by experimental cryptorchidism. *J Androl* 26, 281-288 (2005)

312. Kondo Y, T. Ishikawa, K. Yamaguchi, T. Yada & M. Fujisawa: Oral administration of tetrahydrobiopterin attenuates testicular damage by reducing nitric oxide synthase activity in a cryptorchid mouse model. *J Androl* 2007 Oct 31 (Epub ahead of print)

313. Xu J, Z. Xu, Y. Jiang, X. Qian & Y. Huang: Cryptorchidism induces mouse testicular germ cell apoptosis and changes in bcl-2 and bax protein expression. *J Environ Pathol Toxicol Oncol* 19, 25-33 (2000)

314. Dundar M, I. Kocak, N, Culhaci & H. Erol: Determination of apoptosis through bax expression in cryptochid testis: an experimental study. *Pathol Oncol Res* 11, 170-173 (2005)

315. Zhang L.C, X.M. Zheng, Z.W. Yang & S.W. Li: Expression of Bcl-2 and Bax in the impairment of contraleteral testes of unilateral cryptorchidism in rats. *Zhonghua Nan Ke Xue* 12, 652-354 (2006)

316. Li Y.C, X.Q. Hu, L.J. Xiao, Z.Y. Hu, J. Guo, K.Y. Zhang, X.X. Song & Y.X. Liu: An oligonucleotide microarray study on gene expression profile in mouse testis of experimental cryptorchidism. *Front Biosci* 11, 2465-2482 (2006)

317. Yin Y, W.C. Deqolf & A. Morgentaler: Experimental cryptorchidism induces testicular germ cell apoptosis by p53-dependent and –independent pathways in mice. *Biol Reprod* 58, 492-496 (1998)

318. Yin Y, B.C. Stahl, W.C. Dewolf & A. Morgentaler: p53 and Fas are sequential mechaisms of testicular germ cell apoptosis. *J Androl* 23, 64-70 (2002)

319. Fukui N: Action of body temperature on the testicle. *Jpn Med World* 3, 160 (1923)

320. Ohta Y, A. Nishikawa, Y. Fukazawa, H. Urushitani, A. Mtsuzawa, Y. Nishina & T. Iguchi: Apoptosis in adult mouse testis induced by experimental cryptorchidism. *Acta Anat* 157, 195-204 (1996)

321. Kazusa K, Y. Namiki, A. Asano, Y. Kon, D. Endoh & T. Agui: Differences in spermatogenesis in cryptorchid testes among various strains of mice. *Comp Med* 54, 179-184 (2004)

322. Sinha Hikim A.P, C. Wang, Y. Kue, L. Johnson, X.-H. Wang & R.S. Swerdloff: Spontaneous germ cell apoptosis in humans: evidence for ethnic differences in the susceptibility of germ cells to programmed cell death. *J Clin Endocrinol Metab* 83, 152-156 (1998)

323. Goda K, M. Fujisawa, T. Shirakawa, M. Dobashi, G. Shiota, Z.J. Zhang, A. Gotoh & S. Kamidono: Adenoviral-mediated HGF expression inhibits germ cell apoptosis in rats with cryptorchidism. *J Gene Med* 6, 869-876 (2004)

324. Zhang K, Y. Shang, S. Liao, W. Zhang, H. Nian, Y. Liu, Q. Chen & C. Han: Uncoupling protein 2 protects

testicular germ cells from hyperthermia-induced apoptosis. *Biochem Biophys Res Commun* 360, 327-332 (2007)

325. Benoff S, K.J. Auborn, D.-Z. Chen and J.L. Marmar & I.R. Hurley: Chronic exposure to environmentally-relevant, low-doses of cadmium (Cd) in a rat model mimcs effect on spermatogenesis observed in infertile men with varicoceles. Sixty-Third Annual Meeting of the American Society for Reproductive Medicine, Abstract no. O-60, pS23 (2007)

326. S. Benoff S, K. Auborn, J. Marmar and I. Hurley: Link between low-dose environmentally-relevant cadmium exposures and Leydig cell dysfunction in varicocele-associated infertility. Thirty-Third Annual Meeting of the American Society of Andrology, Abstract no. 47, p.43 (2008)

327. Gunn S.A, T.C. Gould & W.A.D. Anderson: Strain differences in susceptibility of mice and rats to cadmium-induced testicular damage. *J Reprod Fertil* 10, 273-275 (1965)

328. Taylor B.A, H.J. Heiniger & H. Meier: Genetic analysis of resistance to cadmium-induced testicular damage in mice. *Proc Soc Exp Biol Med* 143, 629-633 (1973)

329. Dalton T.P, M.L. Miller, X. Wu, A. Menon, E. Cianciolo, R.A. McKinnon, P.W. Smith, L.J. Robinson & D.W. Nebert: Refining the mouse chromosomal location of Cdm, the major gene asscoaited with susceptibility to cadmium-induced testicular necrosis. *Pharmacogenetics* 10, 141-151 (2000)

330. King L.M, W.A. Banks & W.J. George: Differential zinc transport into testis and brain of cadmium-sensitive and –resistant murine strains. *J Androl* 21, 656-663 (2000)

331. Dalton T.P, L. He, B. Wang, M.L. Miller, L. Jin, K.F. Stringer, X. Chang, C.S. Baxter & D.W. Nebert: Identification of mouse SLC39A8 as the transporter responsible for cadmium-induced toxicity in the testis. *Proc Nat Acad Sci USA* 102, 3401-3406 (2005)

332. B. Wang B, S.N. Schnieder, N. Dragin, K. Girijashanker, T.P. Dalton, L. He, M.L. Miller, S.K. Stringer, M. Soleimani, D.D. Richardson & D.W. Nebert: Enhanced-cadmium-induced testicular necrosis and renal proximal tubular damage caused by gene-dose increase in a Slc39a8-transgenic mouse line. *Am J Physiol Cell Physiol* 292, 1523-1535 (2007)

333. Kaji T, A. Mishima, E. Koyanagi, C. Yamamoto, M. Sakamoto & H. Kozuka: Possible mechanism for zinc protection against cadmium cytotoxicity in cultured vascular endothelial cells. *Toxicology* 76, 257-270 (1992)

334. Martin L.J., H. Chen, X. Liao, H. Allayee, D.M. Shih, G.S. Lee, D.N. Hovland Jr, W.A. Robbins, K. Carnes, R.A. Hess, A.J. Lusis & M.D. Collins: FK506, a calcineurin inhibitor, presents cadmium-induced testicular toxicity in mice. *Toxicol Sci* 100, 474-485 (2007)

335. Elinder C.G, C. Edling, E. Lindberg, B. Kagedal & O. Vesterberg: B2-microglobulinuria among workers previously exposed to cadmium: follow-up and dose-response analysis. *Am J Ind Med* 8, 553-564 (1985)

336. Brinkworth M.H, G.F. Weinbauer, S. Schlatt & E. Nieschlag: Identification of germ cells undergoing apoptosis in adult rats. *J Reprod Fertil* 105, 25-33 (1995)

337. Henriksen K, H. Hakovirta & M. Parvinen: Testosterone inhibits and induces apoptosis in rat semininferous tubules in a stage-specific manner: *in situ* quantification in squash preparations after administration of ethane dimethane sulfonate. *Endocrinology* 136, 3-4 (1995)

338. Sinah Hikim A.P, C. Wang, A. Leung & R.S. Swerdloff: Involvement of apoptosis in the induction of germ cell degeneration in adults rats after gonadotropin-releasing hormone antagonist treatment. *Endocrinology* 136, 2770-2775 (1995)

339. Sinha Hikim A.P, T.B. Rajavashisth, I. Sinha Hikim, Y. Lue, J.J. Bonavera, A. Leung, C. Wang & R.S. Swerdloff: Significance of apoptosis in the temporal and stage-specific loss of germ cells in the adult rat after gonadotropin deprivation. *Biol Reprod* 57, 1193-1201 (1997)

340. Vera Y, K. Erkkila, C. Wang, C. Nunez, S. Kyttanen, Y. Lue, L. Dunkel, R.S. Swerdloff & A.P. Sinha Hikim: Involvement of p38 mitogen-acitivated protein kinase and inducible nitric oxide synthase in apoptotic signaling of murine and human male germ cells after hormone deprivation. *Mol Endocrinol* 20, 1597-1609 (2006)

341. Lenzi A, F. Culasso, L. Gandini, F. Lombardo & F. Dondero: Placebo-controlled, double-blind, cross-over trial of glutathione therapy in male infertility. *Hum Reprod* 8, 1657-1662 (1993)

342. Tripodi L, A. Tripodi, C. Mammi, C. Pulle & F. Cremonesi: Pharmacological action and therapeutic effects of glutathione on hypokinetic spermatozoa for enzymatic-dependent pathologies and correlated genetic aspects. *Clin Exp Obstet Gynecol* 30, 130-136 (2003)

343. Fraga C.G, P.A. Motchnik, M.K. Shigenaga, H.J. Hlebrock, R.A. Jacob & B.N. Ames: Ascorbic acid protects against endogenous oxidative DNA damage in human sperm.*Proc Nat Acad Sci USA* 88, 11003-11006 (1991)

344. Mostafa T, T.H. Anis, S. Ghazi, A.R. El-Nashar, H. Imam & I.A. Osman: Reactive oxygen species and antioxidant relationship in the internal spermatic vein blood of infertile men with varicocele. *Asian J Androl* 8, 451-454 (2006)

345. Rolf C, T.G. Cooper, C.H. Yeung & E. Nieschlag: Antioxidant treatment of patients with asthenozoospermia or moderate oligoasthenozoospermia with high-dose vitamin C and vitamin E: a randomized, placebo-controlled, double-blind study. *Hum Reprod* 14, 1028-1033 (1999)

346. Ozturk H, B. Tander, A. Aydin, Z. Okumus & S. Cetinkursun: The effects of chemical sympathectomy on testicular injury with varicocele. *BJU Int* 87, 232-234 (2001)

347. Powell S.R: The antioxidant properties of zinc. *J Nutr* 130, 1447S-1454 (2000)

348. Takihara H, M.J. Consention & A.T.K. Cockett. Zinc sulfate therapy for infertile men with or without varicocele. *Urology* 29, 638-641 (1987)

349. Ando S, A. Carpino, M. Buffone, M. Maggiolini & D. Sisci: The evaluation of free-L-carnitine, zinc and fructose in the seminal plasma of patients with varicocele and normozoospermia. *Andrologia* 21, 155-60 (1989)

350. Ando S, A. Carpino, M. Buffone, M. Maggiolini, C. Giacchetto & F. Seidita: Fructose, prostatic phosphatase and zinc levels in the seminal plasma of varicoceles. *Int J Fertil* 35, 249-52 (1990)

351. Marmar J.L, S. Katz, D.E. Praiss & T.J. DeBenedictis: Semen zinc levels in infertile and postvasectomy patients and patients with prostatitis. *Fertil Steril* 26, 1057-1063 (1975)

352. Cavallini G, G. Biagiotti, A.P. Ferraretti, L. Gianaroli & G Vitali G: Medical therapy of oligoasthenospermia associated with left varicocele. *BJU Int* 91, 513-518 (2003)

353. Cavallini G, A.P. Ferraretti, L. Bianaroli, G. Biagiotti & G. Vitali: Cinnoxicam and L-carnitine/acetyl-L- carnitine treatment of idiopathic and varicocele-associated oligoasthenospermia. *J Androl* 25, 761-770 (2004)

354. Gattuccio F, A.F. De Rose and M.A. Latteri, eds.: Varicocele 2000. Palermo, Italy: Cofese Editore (2000)

355. Lenzi A, F. Lombardo, P. Sgro, P. Salacone, L. Caponecchia, F. Dondero & L. Gandini: Use of carnitine therapy in selected cases of male factor infertility: a double-blind crossover trial. *Fertil Steril* 79, 292-300 (2003)

356. Lenzi A, P. Sgro, P. Salacone, D. Paoli, B. Gilio, F. Lombardo, M. Santulli, A. Agarwal & L. Gandini: A placebo-controlled double-blind randomized trial of the use of combine l-carnitine and l-acetyl-carnitine treatment in men with asthenozoospermia. *Fertil Steril* 81, 1578-1584 (2004)

357. Sigman M, S. Glass, J. Campagnone & J.L. Pryor: Carnitine for the treatment of idiopathic asthenospermia: a randomized, double-blinded, placebo-controlled trial. *Fertil Steril* 85, 1409-1414 (2006)

358. Bandhauer K. & H.U. Meili: Varicocele: spermiogram, testicular biopsy, plasma testosterone. Results of therapy. *Urologe A* 16, 154-157 (1977)

359. Check J.H: Improved semen quality in subfertile males with varicocele-associated oligospermia following treatment with clomiphene citrate. *Fertil Steril* 33, 423-426 (1980)

360. Check J.H: Treatment of male infertility. *Clin Exp Obstet Gynecol* 34, 201-206 (2007)

361. Netto N.R Jr., G.C. Lemos & J.F. Claro: Varicocele: medical and surgical treatment. *Int J Fertil* 32, 432-435 (1987)

362. Unal D, E. Yeni, A. Verit & O.F. Karatas: Clomiphene citrate versus varicocelectomy in treatment of subclinical varicocele: a prospective randomized study. *Int J Urol* 8, 227-230 (2001)

363. Krause W, H. Holland-Moritz and P. Schramm: Treatment of idiopathic oligospermia with tamoxifen – a randomized controlled study. *Int J Androl* 15, 507-508 (1992)

364. Kadioglu T.C, IT Koksal, M. Tunc, I. Nane & S. Tellaloglu: Treatment of idiopathic and postvaricocelectomy oligospermia with oral tamoxifen citrate. *BJU Int* 83, 646-648 (1999)

365. Yamamoto Y, H. Hibi, S. Katsuno & M. Miyake: Human chorionic gonadotropin adjuvant therapy for patients with Leydig cell dysfunction after varicocelectomy. *Arch Androl* 35, 49-55 (1995)

366. Sen Gupta R, E. Sen Gupta, B.K. Dhakal, A.R. Thakur & J. Ahnn: Vitamin C and Vitamin E protect the rat testes from cadmium-induced reactive oxygen species. *Mol Cells* 17, 132-139 (2004)

367. Oteiza P.I, V.N. Adonayio & C.L. Keen: Cadmium-induced testes oxidative damage in rats can be influenced by dietary zinc intak. *Toxicology* 137, 13-22 (1999)

368. Amara S, H. Abdelmelek, C. Garrel, P. Guiraud, T. Douki, J.L. Ravanat, A. Favier, M. Sakly & K. Ben Rhouma: Preventive effect of zinc against cadmium-induced oxidative stress in the rat testis. *J Reprod Dev* 54, 129-134 (2007)

369. Karbownik M, E. Gitto, A. Lewinski & R.J. Reiter: Induction of lipid peroxidation in hamster organs by the carcinogen cadmium: amelioration by melatonin. *Cell Biol Toxicol* 17, 33-40 (2001)

370. Sen Gupta R, J. Kim, C. Gomes, S. Oh, J. Park, W.-B. Im, J.Y. Seong, R.S. Ahn, H.-B. Kwon & J. Soh: Effect of ascorbic acid supplementation on testicular steroidogenesis and germ cell death in cadmium-treated male rats. *Mol Cell Endocrinol* 221, 57-66 (2004)

371. Al-Bader A, A.E. Omu & H. Dashti: Chronic cadmium toxicity to sperm of heavy cigarette smokers: immunomodulation by zinc. *Arch Androl* 43, 135-140 (1999)

372. Ripa S. & R. Ripa: Zinc cellular traffic: physiopathological considerations. *Minerva Med* 86, 37-43 (1995)

373. Hectenberg S, T. Schafer, J. Benters & D. Beyersmann: Effects of cadmium on cellular calcium and proto-oncogene expression. *Ann Clin Lab Sci* 26, 512-521 (1996)

374. Martelli A, E. Rousselet, C. Dycke, A. Bouron & J.M. Moulis: Cadmium toxicity in animal cells by interference with essential metals. *Biochimie* 88, 1807-1814 (2006)

375. Beyersmann D. & S. Hechtenberg: Cadmium, gene regulation, and cellular signaling in mammalian cells. *Toxicol Appl Pharmacol* 144, 247-261 (1997)

376. Bridges C.C. & R.Z. Zalups: Molecular and ionic mimicry and the transport of toxic metals. *Toxicol Appl Pharmacol* 204, 274-308 (2005)

377. Jones M.M, C. Xu & P.A. Ladd: Selenite suppression of cadmium-induced testicular apoptosis. *Toxicology* 116, 169-175 (1997)

378. Rodriguez-Rigau L.J, K.D. Smith & E. Steinberger: Varicocele and the morphology of spermatozoa. *Fertil Steril* 35, 54-57 (1981)

379. Dubin L. & R. Amelar: Varicocelectomy: twenty-five years of experience. *Int J Fertil* 33, 226-235 (1988)

380. Marks J.L, R. McMahon & L.I. Lipshultz: Predictive parameters of successful varicocele repair. *J Urol* 136, 609-12 (1986)

381. O'Brien J, B. Bowles, K.K. Kamal, K. Jarvi & A. Zini: Does the gonadotropin-releasing hormone stimulation test predict clinical outcomes after microsurgical varicocelectomy? *Urology* 63, 1143-1147 (2004)

382. Yoshida K, S. Kitahara, K. Chiba, S. Horiuchi, H. Horimi, S. Sumi & H. Moriguchi: Predictive indicators of successful varicocele repair in men with infertility. *Int J Fertil Womens Med* 45, 279-284 (2000)

383. Poulakis V, N. Ferakis, R. de Vries, U. Witzsch & E. Becht: Induction of spermatogenesis in men with azoospermia or severe oligoteratoasthenospermia after antegrade internal spermatic vein sclerotherapy for treatment of varicocele. *Asian J Androl* 8, 613-619 (2006)

384. Matthews G.J, E.D. Matthews & M. Goldstein: Induction of spermatogenesis and achievement of pregnancy after microsurgical varicocelectomy in men with azoospermia and severe oligospermia. *Fertil Steril* 70, 71-75 (1998)

385. Kim E.D, B.B. Leibman, D.M. Grinblat & L.I. Lipshultz: Varicocele repair improves semen parameters in azoospermic men with spermatogenic failure. *J Urol* 162, 737-740 (1999)

386. Kadioglu T.C, A. Tefekli, S. Cayan, E. Kandirali, F. Erdemir & S. Tellaloglu: Microsurgical varicocele repair in azzospermic men. *Urology* 57, 328-333 (2001)

387. Pasqualotto F.F, A.M. Lucon, J. Hallak, P.M. Goes, L.B. Saldana & S. Arap: Induction of spermatogenesis in azoospermic men after varicocele repair. *Hum Reprod* 18, 108-112 (2003)

388. Pasqualotto F.F, A.M. Lucon, P.M. De Goes, J. Hallak, B.P. Sobreiro, E.B. Pasqualotto & S. Arap: Testicular growth, sperm concentration, percent motility, and pregnancy outcome after varicocelectomy based on testicular histology. *Fertil Steril* 83, 362-366 (2005)

389. Cakan M & U. Altug: Induction of spermatogenesis by inguinal varicocele repair in azoospermic men. *Arch Androl* 50, 145-150 (2004)

390. Esteves S.C. & S. Glina: Reovery of spermatogenesis after microsurgical subinguinal varicocele repair in azoospermic men based on testicular histology. *Int Braz J Urol* 31, 541-547 (2005)

391. Schlegel P.N. & J. Kaufmann: Role of varicocelectomy in men with nonobstructive azoospermia. *Fertil Steril* 81, 1585-1588 (2004)

392. Hadziselimovic F, B. Leibundgut, D. Da Rugna & M.W. Buser: The value of testis biopsy in patients with varicocele. *J Urol* 135, 707-710 (1986)

393. Benoff S, K. Provoncha, C. Millan, I.R. Hurley, B. Napolitano and J.L. Marmar: Testicular cadmium (Cd) more informative than histology for varicocele prognosis. Fifty-Ninth Annual Meeting of the American Society for Reproductive Medicine, Abstract No. P-351, S237 (2003)

394. P. Brackin P, J.L. Marmar, C. Millan & S. Benoff: Identification of heat shock protein 70-1 (HSP 70-1) in testis tissue of men with varicocele. Ninety-Eighth Annual Meeting of the American Urological Association, ID# 1552 (2003)

395. Benoff S, I.R. Hurley, L. Yuan, H. Xu & J.L. Marmar: The protective effects of a heat shock response among infertile men with varicoceles. Sixty-Second Annual Meeting of the American Society for Reproductive Medicine, Abstract no. O-250, pS106-S107 (2006)

396. Benoff S, I.R. Hurley, G.W. Cooper, A. Jacob & B.R. Gilbert: Human sperm mannose receptor expression (MR) and seminal plasma (SP) metal levels: potential biomarkers for the outcome of varicocele repair. American Society for Reproductive Medicine, 54th Annual Meeting, Abstract No. O-193 (1998)

397. K. Okubo K, K. Nagahama, T. Kamoto, H. Okuno, O. Ogawa & H. Nishiyama: GSTT1 and GSTM1 polymorphisms are associated with improvement in seminal findings after varicocelectomy. *Fertil Steril* 83, 1579-1580 (2005)

398. Rajfer J, T.T. Turner, F. Rivera, S.S. Howards & S.C. Sikka: Inhibition of testicular testosterone biosynthesis following experimental varicocele in rats. *Biol Reprod* 36, 933-937 (1987)

399. Atikeler K, E. Yeni, A. Semercioz, O. Yalcin & C. Baydinc: The value of the gonadotropin-releasing hormone test as a prognostic factor in infertile patients with varicocele. *Br J Urol* 78, 632-634 (1996)

400. Fisch H, G. Hyun & T.W. Hensle: Testicular growth and gonadotropin response associated with varicocele repair in adolescent males. *BJU Int* 91, 75-78 (2003)

401. Muttukrishna S, H. Yussoff, M. Naidu, J.Barua, K. Arambage, H. Suharjono & M. Sathanandan: Serum anti-Mullerian hormone and inhibin B disorders of spermatogenesis. *Fertil Steril* 88, 516-518 (2007)

402. Romeo C, T. Arrigo, P. Impellizzeri, A. Manganaro, P. Antonuccio, G. Di Pasquale, M.F. Messina, Marseglia, I. Formica & B. Zuccarello: Altered serum inhibin B levels in adolescents with varicocele. *J Pediatr Surg* 42, 390-394 (2007)

403. Fujisawa M, M. Dobashi, T. Yamasaki, M. Kanzaki, H. Okada, S. Arakawa & S. Kamidono: Significance of serum inhibin B concentration for evaluating improvement in spermatogenesis after varicocelectomy. *Hum Reprod* 16, 1945-1949 (2001)

404. Turkyilmaz Z, R. Karabulut, K. Sonmez, B. Demirogullari, O. Ozen, S. Moralioglu, C. Karakus, C. Basaklar & N. Kale: Inhibin B levels in peripheral vein do not correlate with inhibin B levels in the spermatic vein in adolescents with varicocele. *Arch Andol* 52, 325-328 (2006)

405. Yoshida K.-I, M. Yano, K. Chiba, M. Honda & S. Kitahara: CAG repeat length in the androgen receptor gene is enhanced in patients with idiopathic azoospermia. *Urology* 54, 1078-1081 (1999)

406. Yong E.L, C.J. Loy and K.S. Sim: Androgen receptor gene and male infertility. *Hum Reprod Update* 9, 1-7 (2003)

407. M.V. Meng M.V, L.D. Black, I. Cha, B.M. Ljung, R.A. Pera & P.J. Turek: Impaired spermatogenesis in men with congential absence of the vas deferens. *Hum Reprod* 16, 529-533 (2001).

408. Wennemuth G, A. Meinhardt, C. Mallidis, M. Albrecht, W. Krause, H. Renneberg & G. Aumuller. Assessment of fibronectin as a potential new clinical tool in andrology. *Andrologia* 33, 43-46 (2001)

409. Christova M: Ultrastructual peculiarities of the endothelium of the testicular vein and vasa vasis in idiopathic varicocele. *Folia Medica* 30, 18-24 (1988)

410. Hienz H.A, J. Voggenthaler & L. Weisback: Histological findings in testes with varicocele during childhood and therapeutic consequences. *Eur J Pediatr* 133, 139-146 (1980)

411. Nagler H.M, E.F. Lizza, S.D. House, P. Tomashefsky & H.H. Lipowsky: Testicular hemodynamic changes after the surgical creation of a varicocele in the rat. Intravital microscopic observations. *J Androl* 8, 292-298 (1987)

412. Michiels C, N. Bouaziz & J. Remacle: Role of the endothelium and blood stasis in the development of varicose veins. I*nt Angiol* 21 (2 Suppl 1), 18-25 (2002)

413. Somers P & M. Knaapen.: The histopathology of varicose vein disease. *Angiology* 57, 546-555 (2006)

414. Naoum J.J, G.C. Hunter, K.J. Woodside & C. Chen: Current advances in the pathogenesis of varicose veins. *J Surg Res* 141, 311-316 (2007)

415. Bergan J.J, L. Pascarella & G.W. Schmid-Schonbein: Pathogenesis of primary venous disease: Insights from animal models of venous hypertension. *J Vasc Surg* 47, 183-192 (2008)

416. Jacob T, A. Hingorani & E. Ascher: Overexpression of transforming growth factor-beta1 correlates with increased synthesis of nitric oxide synthase in varicose veins. *J Vasc Surg* 41, 523-530 (2005)

417. Ascher E, T. Jacob, A. Hingorani, B. Tsemekhin & Y. Gunduz: Expression of molecular mediators of apoptosis and their role in the pathogenesis of lower-extremity varicose veins. *J Vasc Surgery* 33, 1080-1086 (2001)

418. Urbanek T, B. Skop, R. Wiaderkiewicz, T. Wilczok, K. Zaida, T. Lebda-Wyborny & K. Pawlicki: Smooth muscle cell apoptosis in primary varicose veins. *Eur J Vasc Endovasc Surg* 28, 600-611 (2004)

419. Urbanek T, B. Skop, K. Zaida, T. Wilczok, R. Wiaderkiewicz, A. Palasz, U. Mazurek & E. Wielgus: Sapheno-femoral junction pathway: molecular mechanisms of saphenous vein incompetence. *Clin Appl Thromb Hemost* 10, 311-321 (2004)

420. Ducasse E, K. Giannakakis, F. Speziale, D. Midy, E. Sbarigia, J.C. Baste & T. Faraggiana: Association of primary varicose veins with dysregulated vein wall apoptosis. *Eur J Vasc Endovasc Surg* 35, 224-229 (2008)

421. Kim D.I, H.S. Eo & J.H. Joh: Identification of differentially expressed genes in primary varicose veins. *J Surg Res* 123, 222-226 (2005)

422. Bagchi D, C.K. Sen, M. Bagchi & M. Atalay: Anti-angiogenic, anti-oxidant, and anti-carcinogenic properties of a novel antrhocyanin-rich berry extract formula. *Biochemistry* (Mosc.) 69, 75-80 (2004)

423. Tilki D, E. Kilic, R. Tauber, D. Pfeiffer, C.G. Stief, R. Tauber & S. Ergün: The complex structure of the smooth muscle layer of spermatic veins and its potential role in the development of varicocele testis. *Eur Urol* 51, 1402-1409 (2007)

424. Lee J.D, S.Y. Jeng & T.H. Lee: Bcl-2 overexpression in the internal spermatic vein of patients with varicocele. *J Formos Med Assoc* 106, 308-312 (2007)

425. Haider S.G, D. Passia, G. Servos & H. Hettwer: Electron microscopic evidence for deep invaginations of the lamina propria towards the seminiferous tubule lumen in a patient with varicocele. *Int J Androl* 9, 27-37 (1986)

426. Santamaria L, R. Martin, M. Nistal & R. Paniagua: The peritubular myoid cells in the testes from men with varicocele: an ultrastructural, immunohistochemical and quantitative study. *Histopathology* 21, 423-433 (1992)

427. Santoro G, C. Romeo, P. Impellizzeri, C. Gentile, G. Anatasi and A. Santoro: Ultrastructural and immunohistochemical study of basal lamina of the testis in adolescent varicocele. *Fertil Steril* 73, 699-705 (2000)

428. Dobashi M, M. Fujisawa, I. Naito, T. Yamazaki, H. Okada & S. Kamidono: Distribution of type IV collagen in human testes and their association with spermatogenesis. *Fertil Steril* 80 Suppl 2, 755-760 (2003)

429. Hollingsworth S.J, G. Powell, S.G. Barker & D.G. Cooper: Primary varicose veins: altered transcription of VEGF and it receptors (KDR, flt-1, soluble flt-1) with sapheno-femoral junction incompetence. *Eur J Vasc Endovasc Surg* 27, 259-268 (2004)

430. Zhang Y.Q, X.Z. He, J.S. Zhang, R.A. Wang, J. Zhou & R.J. Xu: Stage-specific localization of transforming growth factor beta1 and beta3 and their receptors during spermatogenesis in men. *Asian J Androl* 6, 105-109 (2004)

431. Dobashi M, M. Fujisawa, T. Yamazaki, H. Okada & S. Kamidono: Distribution of intracellular and extracellular expression of transforming growth factor-beta1 (TGF-beta1) in human testis and their association with spermatogenesis. *Asian J Androl* 4, 105-109 (2002)

432. Krege J.H, S.W. John, L.L. Langenbach, J.B. Hodgin, J.R. Hagaman, E.S. Bachman, J.C. Jennette, D.A. O'Brien & O. Smithies: Male–female differences in blood pressure and fertility in ACE-deficient mice. *Nature* 375, 146-148 (1995)

433. Köhn F.M, I. Dammshäuser, C. Neukamm, H. Renneberg, W.E. Siems, W.B. Schill & G. Aumüller: Ultrastructural localization of angiotensin-converting enzyme in ejaculated human spermatozoa. *Hum Reprod* 13, 604-610 (1998)

434. Shibahara H, M. Kamata, J. Hu, H. Nakagawa, H. Obara, N. Kondoh, H. Shima & I. Sato: Activity of testis angiotensin converting enzyme (ACE) in ejaculated human spermatozoa. *Int J Androl* 24, 295-299 (2001)

435. Asci R, S. Sarikaya, R. Buyukalpelli, A. Yilmaz & A. Bedir: Somatic angiotensin converting enzyme in varicocele. *Arch Androl* 52, 329-334 (2006)

436. Cotton L.M, M.K. O'Bryan & B.T. Hinton: Cellular signaling by fibroblast growth factors (FGFs) and their receptors (FGFRs) in male reproduction. *Endocr Rev* 2008 Jan 23 (Epub ahead of print)

437. Schultz K, B.L. Fanburg & D. Beasley: Hypoxia and hypoxia-inducible factor-1alpha promote growth factor-induced proliferation of human vascular smooth muscle cells. *Am J Physiol Heart Circ Physiol* 290, H2528-H2534 (2006)

438. Siu M.K. & C.Y. Cheng: Interactions of proteases, protease inhibitors, and the beta1 integrin/laminingamma3 protein complex in the regulation of ectoplasmic specialization dynamics in the rat testis. *Biol Reprod* 70, 945-964 (2005)

Key Words: Varicoceles, Etiology, Animal Models, Medical Therapies, Outcome Predictor Variables, Review

Send correspondence to: Susan Benoff, Fertility Research Laboratories, Center for Oncology and Cell Biology, The Feinstein Institute for Medical Research, North Shore-Long Island Jewish Health System, 350 Community Drive, Manhasset, New York, 11030?, Tel: 516-562-1121, Fax: 516-562-1030, E-mail: SBenoff@optonline.net

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