Chapter 2
Origin and Pathophysiology

Testicular Vein Anatomy

In this chapter, we discuss the theories attempting to explain the origin of varicocele and the pathophysiological mechanisms associated with varicocele development.

Testicular Vein Anatomy

Testicular veins emerge from mediastinum of the testis to form the pampiniform plexus, which is composed of three groups of veins, namely, the anterior, middle and posterior groups. The posterior group courses posterior to the spermatic cord and drains into the external pudendal and cremasteric veins. The latter ultimately drains into the inferior epigastric vein at the level of external inguinal ring, as shown in Fig. 2.1a. The middle group courses around the vas deferens to drain into the internal iliac vein. The anterior group courses with the internal spermatic artery. At the superficial inguinal ring, this complex form three or four tributaries that enter the pelvis. These veins eventually converge into two and then into a single internal spermatic vein running in front of the ureter and alongside the testicular artery. It is common for the main venous channel to have medial and lateral components; the lateral branch often terminates into the renal capsular, mesenteric, colonic, or retroperitoneal veins. The right internal spermatic vein enters the inferior vena cava just below the right renal vein. The left internal spermatic vein joins the undersurface of the left renal vein lateral to the vertebral column [32], as shown in Fig. 2.1b.

Variant anatomy is seen in about 20% of cases [32, 33]. Important anomalies include drainage of the right internal spermatic vein into the right renal vein (8–10%) and multiple terminal spermatic veins (15–20%). Valves are present in most but not all internal spermatic veins [33].
Theories of Varicocele Origin

Three theories have been postulated to explain the origin of varicocele, which are not mutually exclusive. The first describes the right-angled insertion of the left testicular vein into the left renal vein, with a consequent increase in the hydrostatic pressure that is ultimately transmitted to the pampiniform plexus [26, 34]. The second relies on congenital incompetent (or absent) venous valves, resulting in retrograde flux and dilatation [18, 34]. This theory has been supported by venographic and color Doppler studies. Based upon the level of these incompetent valves being at or below the communicating veins, which include the internal spermatic, cremasteric, vassal and external pudendal veins, two pathophysiologic subtypes have been described, namely shunt and stop types, as shown in Fig. 2.2a and b. When the incompetent valves are located only above the level of the communicating veins, a stop-type varicocele is present, which constitutes 14% of all varicoceles. The stop-type varicocele is characterized by a brief retrograde flow from the internal spermatic vein towards and into the pampiniform plexus. No orthograde venous blood flow and reflux towards the communicating veins is seen because distal valves are present and are functionally competent. Surgical ligation of the stop-type varicocele shall cure the varicocele by offsetting the reflux-producing incompetent valve.
Theories of Varicocele Origin

against valves from the remaining normal venous drainage system [35]. Conversely, when incompetent venous valves are present below the communicating veins, a shunt-type varicocele is present, which constitutes 86% of all varicoceles [35, 36]. Shunt-type varicocele is characterized by retrograde blood both from the internal spermatic vein into the pampiniform plexus and orthograde reflux into the communicating veins (vasal and cremasteric veins) [37]. Surgical ligation of the shunt-type varicocele would be expected to be less effective because the incompetent valves are most numerous and widely distributed. Mohseni et al. [38] reported in a prospective controlled study involving 74 children and adolescents with varicocele that the shunt-type was associated with a greater risk of testicular hypotrophy compared to the stop-type varicocele. In addition, the authors noted that a higher recurrence rate occurred when the shunt-type varicocele had been repaired by the retroperitoneal approach compared to the inguinal approach.

The third theory involves the so-called nutcracker effect, in which compression of the left renal vein between the superior mesenteric artery and abdominal aorta would partially obstruct the blood flow through the left testicular vein and therefore increased the hydrostatic pressure inside the pampiniform plexus [39]. The nutcracker phenomenon builds up a steadily raised renocaval pressure gradient and reflux down the internal spermatic vein, resulting in the development of collateral venous pathways [40–43]. Evidence supporting this theory was provided by hemodynamic studies in adults and children with varicocele. In adults, Mali et al. [40] reported correlation between the renocaval pressure gradient and renospermatic reflux, thus showing that the severity of left renal vein compression in the upright

Fig. 2.2  a Schematic anatomy of the shunt-type varicocele shows incompetent valves and shunting through communicating veins, whereas in b stop-type varicocele, the reflux in the spermatic vein is stopped by a competent valve. (Reprinted with permission from Mohseni et al. [38])
position determines the velocity of retrograde flow in the left spermatic vein and varicocele size.

Selective left renal venography with measurement of the pressure gradient between the left renal vein (LRV) and inferior vena cava (IVC) is the gold standard diagnostic method for assessing the nutcracker effect. Normal length of the left renal vein (LRV) is 6–10 cm and the mean normal LRV diameter is 4–5 mm [7]. The normal pressure gradient between LRV and IVC is 1 mmHg or lower and an elevated gradient >3 mmHg between the LRV and the IVC can be used as a criterion of diagnosis for left renal vein entrapment [44]. Unlu et al. [45] reported using color Doppler ultrasonography that the aortomesenteric angle of men with varicocele ranged between 6–30°, which was significantly different than healthy adult males (25–50°; \( p < 0.05 \)). Such an angle further decreases during the Valsalva maneuver in an erect position, resulting in further compression of the LRV.

Doppler ultrasonography can be used as the first diagnostic test in patients with suspected nutcracker phenomenon [43, 46]. The B-mode sonographic measurement of the diameter of the LRV combined with Doppler sonographic measurement of the peak velocity (PV) of the LRV was used to diagnose LRV entrapment syndrome. It has been suggested that the distal-to-proximal diameter ratios and flow velocity ratios exceeding 5.0 represent nutcracker phenomenon cutoff levels [43, 46, 47]. In one study involving 67 men with varicocele, 55% were demonstrated venographically to have an entrapment phenomenon [48].

The nutcracker phenomenon can be the chief cause of pediatric varicocele. In one report, children with the nutcracker phenomenon had higher grade varicocele and obvious collateral vein formation than did the patients with a lower renocaval pressure gradient [49]. However, the insufficiencies of the internal spermatic vein may be the main cause of renospermatic reflux in patients with a low renocaval pressure gradient [25, 40–42, 50, 51]. In children, the use of Doppler ultrasonography in the diagnosis of nutcracker phenomenon has been limited because the left renal vein sampling area is smaller and the Doppler angle is larger in children than in adults [47, 52].

Lastly, the etiology of varicocele may be a combination of all these mechanisms that are further aggravated by an upright posture. As shown in thin, tall athletic subjects, the incompetence of venous valves and lack of fat support around the left renal vein with narrowing of the aortomesenteric angle may lead to varicocele formation [14].

Is Varicocele a Bilateral Disease?

Historically, 85–90% of all clinical varicoceles are classified as unilateral left-sided. However, recent data indicate that bilateral palpable varicocele is found in >50% of affected men [7, 34]. Such data are in agreement with venographic studies that show bilateral abnormal venous reflux in 84–86% of men with varicocele [53–55]. This finding might explain the occurrence of bilateral testicular damage in such men, and
why there is improvement in semen parameters in only 65% of men after unilateral varicocele repair [56]. In contrast, isolated right-sided varicocele is found in only 2% of patients and may be associated with the presence of an obstructive lesion, such as a retroperitoneal or pelvic compressive mass [55].

**Pathophysiology**

Approximately 80% of men with varicocele are fertile and have normal fecundity [5, 6]. Although the pathophysiology of varicocele has been extensively studied, no conclusive mechanism fully explains why the remaining 15–20% are infertile.

Scrotal hyperthermia, hormonal disturbances, testicular hypoperfusion and hypoxia as well as backflow of toxic metabolites are potential mediators of varicocele-mediated infertility [57]. Recently, oxidative stress has been implicated as an important mediator of varicocele-associated infertility [57]. Nonetheless, the reasons why some patients with varicocele are infertile, whereas the majority of patients are not, remain unclear. Such phenomenon may be partially explained as infertility being a combination of both male and female factors, in which a fully functional female reproductive system can compensate male factor deficiencies and therefore result in a successful conception. Different intrinsic susceptibility must exist among men with varicocele, which culminates in the various effects of varicocele on male fertility [34].

**Oxidative Stress**

Reactive oxygen species (ROS) are byproducts of oxygen metabolism and energy production that act as regulators of vital physiological intracellular processes. In the male reproductive tract, small quantities of ROS have important roles on sperm function—regulating capacitation, acrosome reaction, hyperactivation and the fusion of spermatozoa with the oocyte [58]. By contrast, natural intracellular and extracellular antioxidants (enzymatic and non-enzymatic) scavenge and neutralize the harmful effects stemming from increases in ROS levels. When ROS levels disproportionately increase compared with the neutralizing capacity of antioxidants, or when a reduction in the antioxidant capacity has occurred, oxidative stress usually follows.

An imbalance between ROS production and decreased total antioxidant capacity (TAC) has been implicated as the result of acidification of spermatozoa cytosol and seminal plasma in men with varicocele [59]. Oxidative stress via ROS, especially lipid peroxidation, not only damages membrane function in sperm head and mid-piece altering morphology and impairing motility, but also leads to a decrease in intracellular pH. The ideal pH for ROS scavenging activity by the enzymatic antioxidant systems ranges from neutral to slightly alkaline, being markedly depressed in
acidic states. Impairment of TAC may reflect as a further decrease in sperm motility [60]. These effects, however, have been speculated to vary from one subject to another according to their capacity to counteract the deleterious effects of membrane dysfunction and oxidative DNA damage. This may help understand the variable effect of varicocele on male infertility.

In a meta-analysis of studies involving oxidative stress markers in men with varicocele, we observed that oxidative stress markers were significantly increased in varicocele patients compared with normal sperm donors [60]. In one of the included studies, Mitropoulos et al. [61] evaluated oxidative stress in the peripheral blood samples of subfertile men with varicocele. The authors found an elevated level of oxidative stress due to the release of nitric oxide synthase and xanthine oxidase within the dilated spermatic vein. This led to a dramatic increase in the levels of nitric oxide, peroxynitrite, and S-nitrosothiols, all of which are biologically active. They suggested that peroxynitrite production from the reaction of nitric oxide and superoxide might be responsible for an impaired sperm function in patients with varicocele. In another study, Allamaneni et al. [62] reported that semen ROS levels correlated positively with varicocele grade. The authors showed that men with larger varicoceles had significantly higher semen ROS levels than men with small varicoceles. Similarly, Koksal et al. [63], evaluating malondialdehyde in testicular biopsy specimens, found significantly higher levels of this oxidative stress marker in infertile men with large varicoceles compared to men with small or moderate varicoceles. These findings indicate that the larger the varicocele, the higher the levels of oxidative stress. Interestingly, surgical treatment of varicocele has been shown to reduce seminal oxidative stress in such patients [64–66].

An elevated production of ROS in the reproductive tract disrupts not only the fluidity of the sperm plasma membrane, but also the integrity of DNA in the sperm nucleus. It has been shown that infertile men with varicoceles have high levels of sperm DNA damage [67]. In one study, Chen et al. [68] reported that patients with varicocele had increased levels of 8-hydroxy-2’-deoxyguanosine, a marker of oxidative DNA damage. Sperm DNA damage could also result from aberrant chromatin packaging during spermatogenesis or be a consequence of the triggering of an apoptotic-like process by ROS overproduction. Sadek et al. [69] assessed the rate of chromatin condensation using aniline-blue staining in infertile men with varicocele and showed significant improvement in DNA packing following surgical correction of large varicose veins. Excessive levels of DNA damage have been associated with a reduction in many fertility indices, including fertilization, embryo development and implantation, as well as pregnancy and live birth rates. Furthermore, DNA damage can have other significant clinical implications because in vitro fertilization using spermatozoa containing damaged DNA may lead to paternal transmission of defective genetic material with adverse consequences for embryonic development. Fortunately, this damage may be reversible, as shown by Zini and Libman, who recently reported that sperm DNA integrity was significantly improved in infertile men 6 months after surgical varicocele repair [70].

Recent findings reported by Blumer et al. [71] confirmed previous reports of a negative correlation between sperm morphology and the percentage of sperm with
high DNA fragmentation ($r = -0.450$) in men with varicocele. Although an increase in oxidative stress as determined by the rise in malondialdehyde, which is the major product of lipid peroxidation, was not observed in the aforementioned study, a decrease in mitochondrial activity and acrosome integrity was documented. In a study involving men with palpable varicocele and oligozoospermia, Smit et al. showed significant improvement in the DNA fragmentation index (DFI) 3 months after varicocelectomy (pre-op. 35.2% ± 13.1%; post-op. 30.2% ± 14.7%, $p=0.019$) [72, 73]. A difference could also be noted between couples who conceived naturally or with assisted reproductive technology (ART) compared to those who failed (DFI%: 26.6% ± 13.7% versus 37.3% ± 13.9%, $p=0.013$). Notwithstanding, these authors demonstrated that not all patients had a decrease in sperm DNA damage after varicocele repair. In a recent work by Dada et al. [74] studying 11 men with clinical varicocele, surgical repair resulted in rapid (1 month) significant decline in free radical levels followed by slow (3–6 months) decline in DNA damage assessed by the Comet assay. On the basis of their findings, the authors of the aforementioned study recommended that infertile couples whose male partner had varicocele repair should wait 6 months after surgery before attempting to conceive. Not surprisingly, Smith et al. [72] found that high levels of sperm DNA damage were associated with varicocele even when semen analysis results were within the reference range. Of note, semen analysis as routinely performed is limited in its validity as surrogate for the assessment of male fertility potential. For this reason, it has been suggested that sperm function tests, such as sperm DNA integrity, are better indicators of male fertility potential and should be included in the semen evaluation [75, 76].

**Scrotal Hyperthermia**

An elevated testicular temperature has been demonstrated in men with varicocele and impaired sperm quality. Along the same lines, reduction in testicular temperature was shown to follow varicocele repair [77–81]. Because spermatogenesis is optimally at a temperature 2.5 °C lower than the core temperature, heat stress can lead to a deterioration in sperm production. However, given that most men with varicocele are fertile, and such individuals also have higher scrotal temperature than healthy men, the sole contribution of the heat stress to the infertility problem cannot entirely explain varicocele-related infertility.

The primary question is to determine whether heat stress can generate oxidative stress in the testes. Indeed, in vitro and in vivo studies have shown a direct, temperature-dependent relationship between heat exposure and generation of ROS. For instance, the exposure of in vitro cultures of mouse and rabbit spermatozoa to successive temperature elevation, ranging from 34 to 40 °C, but kept at constant oxygen concentrations, resulted in a concordant rise in the level of malondialdehyde [82]. Similarly, heat stress has been shown to induce increased mitochondrial, plasma membrane, cytoplasmic and peroxisomal ROS production in various human cell lines [83, 84]. Spermatagonia A, Sertoli and Leydig cells are considered
thermotolerant cells as they have been previously exposed to higher temperatures in the uterus. In contrast, spermatogonia B and developing spermatozoa, particularly pachytene spermatocytes and early spermatids, are highly vulnerable to heat stress [85, 86].

**Venous Hypertension and Reflux of Toxic Metabolites**

Testicular venous hypertension is characterized by an excessive hydrostatic pressure column that is transmitted over the already incompetent gonadal venous valves. It is associated with a reflux of toxic adrenal and renal metabolites into the testis, including epinephrine, urea and prostaglandins E and F2α, which result in chronic vasoconstriction of testicular arterioles [87]. This phenomenon leads to persistent hypoperfusion, stasis and hypoxia, and subsequent dysfunction of the spermatogenic process [88, 89]. Microscopic evaluation of spermatic vein fragments has revealed alterations in the longitudinal muscle layers, in addition to a decrease in the number of nerve elements and “vasa vasorum” in the vessel wall. These findings suggest a defective contractile mechanism of blood transport through the pampiniform plexus. Nonetheless, a five-fold increase in hydrostatic pressure has been documented during vasography studies of the varicose spermatic veins [54], which reverses the pressure gradient and thereby lead to a hypoxic state [26, 54].

Venographic studies have shown that reversal of venous blood flow within a left-sided varicocele is common. As such, renal and adrenal metabolites can gain access to endothelial cells of the left internal spermatic vein and testicular tissue [91, 92]. These substances are known to induce cellular oxidative stress in various human cell cultures *in vitro* [93, 94]. For instance, exposure to supraphysiological levels of urea can inhibit urea transporters that mediate its cellular efflux, resulting in the carbamylation of proteins and a reduction in the level of intracellular glutathione. Carbamylation is a post-translational modification of proteins resulting from the non-enzymatic reaction between isocyanic acid and specific free functional groups. This reaction alters protein structure and therefore their functional properties. PGF-2α can induce ROS production in a variety of cell lines, whereas PGE can inhibit ROS generation. An elevated level of PGE can be attributed to endothelial cells overproduction in response to oxidative stress induced by PGF-2α. Norepinephrine can contribute to vasospasm and perpetuate hypoxia, thus aggravating ROS-mediated oxidative stress.

**Apoptosis and DNA Damage**

It is well known that varicocele is associated with sperm DNA damage, which has been associated with decreased fertility [67, 95, 96]. High levels of DNA damage have also been associated with elevated ROS levels in patients with varicocele.
when compared with normal controls [23]. Interestingly, these differences were found in men with varicocele irrespective of impairment of semen parameters.

Varicocele is also associated with an increase in intratesticular apoptosis [89, 97]. Many apoptosis-inducing factors have been linked to varicocele-associated male infertility such as cadmium accumulation, androgen deprivation, heat stress and interleukin-6 [89, 98].

**Recent Discoveries**

Although an exact pathway for varicocele-induced infertility has not been completely elucidated, there is a plethora of novel studies documenting multiple derangements in the setting of varicocele. Briefly, abnormal expression of leptin receptors, glial cell-derived neurotrophic factor specific receptor GFR-a1 on germ cells [99, 100], and increased expression of heme oxygenase on Leydig cells are some of them [101]. In one study, Nicotina et al. [102] showed an increased expression of aquaporin receptor-1 (AQP-1) on venular endothelial cell membranes as well as Sertoli cell, diploid germ cells, and haploid cells membranes of patients with varicocele. Aquaporins are a family of transcellularmembrane proteins that mediate water transport across the cell membrane. This may indicate that in the presence of a varicocele, the testis is attempting to overcome a fluid imbalance in both tubular and interstitial compartments. In another study, Ozen et al. [103] reported a novel effect of varicocele on vas deferens motility using a rat model. The authors revealed a decline in the contractile response in the ipsilateral vas deferens compared with the contralateral vas deferens in rats with surgically-induced varicocele. Such findings suggest that other pathways, in addition to testicular damage, may take place in the presence of varicocele.

In summary, current evidence suggests that there is a multitude of mechanisms implicated in the pathophysiology of varicocele. Oxidative stress seems to be a central element contributing to infertility in such men, whose testis respond by way of, for instance, heat stress, ischemia or production of vasodilators. These responses have their own implications in exacerbating the underlying oxidative stress. The principal cells in the epididymis, the endothelial cells in the dilated pampiniform plexus and the testicular cells (developing germ cells, Leydig cells, macrophages and peritubular cells) are the three main sites of ROS production, which include nitrogen reactive species. Varicocele-associated cell stressors induce ROS generation by distinct sperm biochemical pathways. In the mitochondria, heat and hypoxic stress can directly activate complex III of the electron transport chain to release ROS. NO, generated from testicular and endothelial cells in the testis with varicocele, can nitrosylate complexes I and IV to promote excessive release of ROS by complex III. In the sperm tail, where glycolytic units are present, NO can nitrosylate glyceraldehyde-3-phosphate dehydrogenase, contributing to intracellular acidification [59] through reducing the NADH to NAD⁺ ratio and reducing the production of lactate, as shown in Figs. 2.3 and 2.4.
Fig. 2.3 Reactive oxygen and nitrogen species generation in infertile men with varicocele. Three components can release ROS in men with varicocele under heat and hypoxic stress: the principal cells in the epididymis, the endothelial cells in the dilated pampiniform plexus and the testicular cells (developing germ cells, Leydig cells, macrophages and peritubular cells). ROS reactive oxygen species
Fig. 2.4 Varicocele-induced sperm biochemical pathways of ROS generation. In the mitochondria, heat and hypoxic stress can directly activate complex III of the electron transport chain to release ROS. NO, generated from testicular and endothelial cells in the testis with varicocele, can nitrosylate complexes I and IV to promote excessive release of ROS by complex III. In the sperm tail, where glycolytic units are present, NO can nitrosylate glyceraldehyde-3-phosphate dehydrogenase, contributing to intracellular acidification through reducing the NADH to NAD⁺ ratio and reducing the production of lactate. ROS reactive oxygen species
Why Is It That Not All Men with Varicocele Are Infertile?

Although seminal markers of oxidative stress are elevated in fertile men with varicocele, this does not necessarily result in deterioration of fertility potential [104–106]. As aforementioned, about 80% of men with varicocele are fertile. As such, it is reasonable to speculate that certain protective mechanisms are activated to counteract the oxidative stress in order to protect sperm from damage. Variation in genetic transcriptional responses to oxidative stress might explain why most men with varicocele are fertile. Studies involving eukaryotic cells have shown that the genetic response to oxidative stress varies both among different cell lines and in response to different ROS subtypes and concentrations [107].

Unfortunately, human studies exploring the genomic and proteomic germ-cell response to oxidative stress are lacking. However, varicocele-associated cellular stressors (such as heat and hypoxia) might illicit similar and dissimilar genetic responses in germ, Sertoli, Leydig, epididymal principal and endothelial cells.

Heat and hypoxia induce damage to and/or alterations in sperm genetic material and other sperm cell organelles. Electron microscopy of spermatozoa from infertile men with varicocele revealed a high incidence of disintegrated plasma membrane, reacted or absent acrosome, abnormal nuclear shapes with disrupted chromatin and deranged axonemal and periaxonemal cytoskeletal structures [108]. Fluorescent in situ hybridization also revealed a higher frequency of aneuploidy due to meiotic segregation errors, resulting in more disomies and diploidies in spermatozoa from infertile men with varicocele than in fertile controls [108]. Conflicting reports, however, suggest that oxidative stress resulting from heat and hypoxia can induce specific cellular genetic responses manifested by increases in mRNA that counteract the harmful effects of ROS, therefore, conferring cellular adaptation to such stressors [109]. As an example of mammalian cellular responses to oxidative stress, it has been shown that in response to exogenous H₂O₂ exposure, except for heme oxygenase (HO), and thioredoxin reductase (TRXR), the cell antioxidant system is not inducible and is constitutive in nature [110]. With regards to varicocele, only heme oxygenase has been studied [101, 111]. Enhanced heme oxygenase expression in Leydig cells in the testes of men with varicocele is associated with the protection of these cells, maintenance of an intact testosterone milieu and process of sperm production [101]. By contrast, lower seminal levels of heme oxygenase among infertile men with varicocele are significantly correlated with the severity of sperm count reduction observed in these men (p=0.001) [111]. Currently, the mechanisms by which nuclear and/or mitochondrial genes are regulated or repressed in response to varicocele-associated cellular stressors are still unknown. We speculate that in addition to constitutively-expressed cellular antioxidants, the functional genetic response to oxidative stress is a key element for cellular survival. According to our hypothesis, germ cells can compensate for the elevated levels of oxidative stress markers measured in fertile men with varicocele, thereby protecting sperm from damage. In infertile men with varicocele, these adaptive genetic responses might be overwhelmed, culminating in sperm dysfunction and cell death.
**Key Points**

• No mechanism has conclusively explained infertility in men with varicocele.
• Scrotal hyperthermia, hormonal disturbances, testicular hypoperfusion and hypoxia as well as backflow of toxic metabolites are potential mediators of varicocele-mediated infertility.
• Oxidative stress has been implicated as the central mediator of varicocele-associated infertility.
• Variation in genetic transcriptional response to oxidative stress might explain why most men with varicocele retain their reproductive potential.