Seminars in Cell and Developmental Biology xxx (xxxx) xxx

Contents lists available at ScienceDirect

Seminars in Cell and Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

Effect of varicoceles on spermatogenesis

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ARTICLE INFO

Keywords:
Varicocele
Spermatogenesis
Testicular dysfunction
Testosterone deficiency
Oxidative stress
Male infertility

ABSTRACT

Varicoceles are dilated veins within the spermatic cord and a relatively common occurrence in men. Fortunately, the large majority of men are asymptomatic, however, a proportion of men with varicoceles can suffer from infertility and testosterone deficiency. Sperm and testosterone are produced within the testis, and any alteration to the testicular environment can negatively affect the cells responsible for these processes. The negative impact of varicoceles on testicular function occurs mainly due to increased oxidative stress within the testicular parenchyma which is thought to be caused by scrotal hyperthermia, testicular hypoxia, and blood-testis barrier disruption. Management of varicoceles involves ligation or percutaneous embolization of the dilated veins. Repair of varicoceles can improve semen parameters and fertility, along with serum testosterone concentration. In this review, we discuss the pathophysiology of varicoceles, their impact on testicular function, and management.

1. Introduction

Infertility is a global issue that affects approximately 15% of couples, with male factor infertility contributing to up to 50% of the cases [1,2]. Various etiologies for male infertility have been described, including congenital or acquired disorders of spermatogenesis, hormone abnormalities, and reproductive tract structural anomalies. Varicoceles, or abnormally dilated veins within the spermatic cord, including internal spermatic and pampiniform plexus veins, are a common cause of male factor infertility (Fig. 1). Varicoceles were first discovered in the 1st century A.D. by a Greek physician, Celsus, who noted that testis size was smaller due to "swollen and twisted" veins overlying the testicle [3,4]. The term varicocele, however, was not coined until 1843 by T.B. Curling, a British surgeon [4,5]. More recently, varicoceles have been shown to play a role in decreased testicular function, leading to altered spermatogenesis and diminished testosterone levels. This review discusses normal testicular function, varicocele pathophysiology, and effects on testicular function (specifically spermatogenesis and testosterone production), indications for varicocele repair, and options for varicocele management.

2. Epidemiology

The incidence of varicoceles in the general population is approximately 15%, and most men are not affected by the presence of a varicocele [6]. The majority of varicoceles tend to occur after puberty, with almost no varicoceles reported in boys less than nine years of age [6]. As such, evidence suggests that the prevalence is age-dependent, with some studies demonstrating a prevalence of up to 75% in those greater than 80 years of age [7]. The prevalence of varicoceles among men with infertility is high, with up to 35% of men presenting for fertility evaluation with varicoceles [8]. This number is even higher in men with secondary infertility (up to 70–80%) and is the most common cause of secondary infertility [9]. The majority (> 90%) of varicoceles are left-sided due to anatomic circumstances. In a cohort of over 7000 healthy, young men, only 1.1% had bilateral varicoceles, and 0.2% had an isolated right varicocele, and of these the majority were grade 1 (7.4%), followed by grade 2 (5.4%) and grade 3 (2.8%) (Table 1) [10].

3. Varicocele etiologies

There are various proposed etiologies for varicoceles, however, the pathophysiology associated with the formation of varicoceles is not yet fully understood. Anatomic theories suggest that varicoceles arise from

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https://doi.org/10.1016/j.semcdb.2021.04.005
Received 21 December 2020; Accepted 5 April 2021
1084-9521/

impaired gonadal vein drainage. For this reason, left-sided varicoceles are more common (> 90% of cases) because the left gonadal vein is longer and empties into the left renal vein at a 90° angle, compared to the right gonadal vein, which empties directly into the inferior vena cava (IVC) (Fig. 1) [11]. The vertical insertion into the left renal vein creates a hydrostatic column of blood within the left gonadal vein, leading to more turbulent flow and back pressure, resulting in left gonadal vein dilation (Fig. 1) [11]. Men also may present with bilateral varicoceles, which is thought to occur secondary to abnormal anti-reflux valves resulting in backflow and vein dilation [12–14]. In some men with varicoceles, absent, as opposed to abnormal valves, also may contribute to the underlying etiology. A less common etiology for varicoceles are those resulting from the "nutcracker" phenomenon. Varicoceles in this context occur secondary to compression of the renal vein between the superior mesenteric artery and aorta [12–14]. Isolated right-sided varicoceles are less common and may raise suspicion for an underlying retroperitoneal process, pelvic mass, or any other pathologic process causing compression and obstruction of gonadal venous outflow (Fig. 1) [11]. In men with a new finding of right-sided varicocele, radiographic imaging should be obtained to rule out a pathologic process that may be compressing the gonadal vein. Overall, the understanding of various etiologies of varicocele formation has not been fully elucidated, and varicoceles may alternatively result from a combination of the etiologies discussed above.

Table 1
Dubin and Amelar grading system for varicocele.

Grade	Exam findings
0	Subclinical varicocele
1	Varicocele palpated with Valsalva
2	Varicocele palpated without Valsalva
3	Visible varicocele at rest (no Valsalva)

There also exists a genetic component to varicocele pathology. It has been reported that those with first-degree relatives have higher rates of

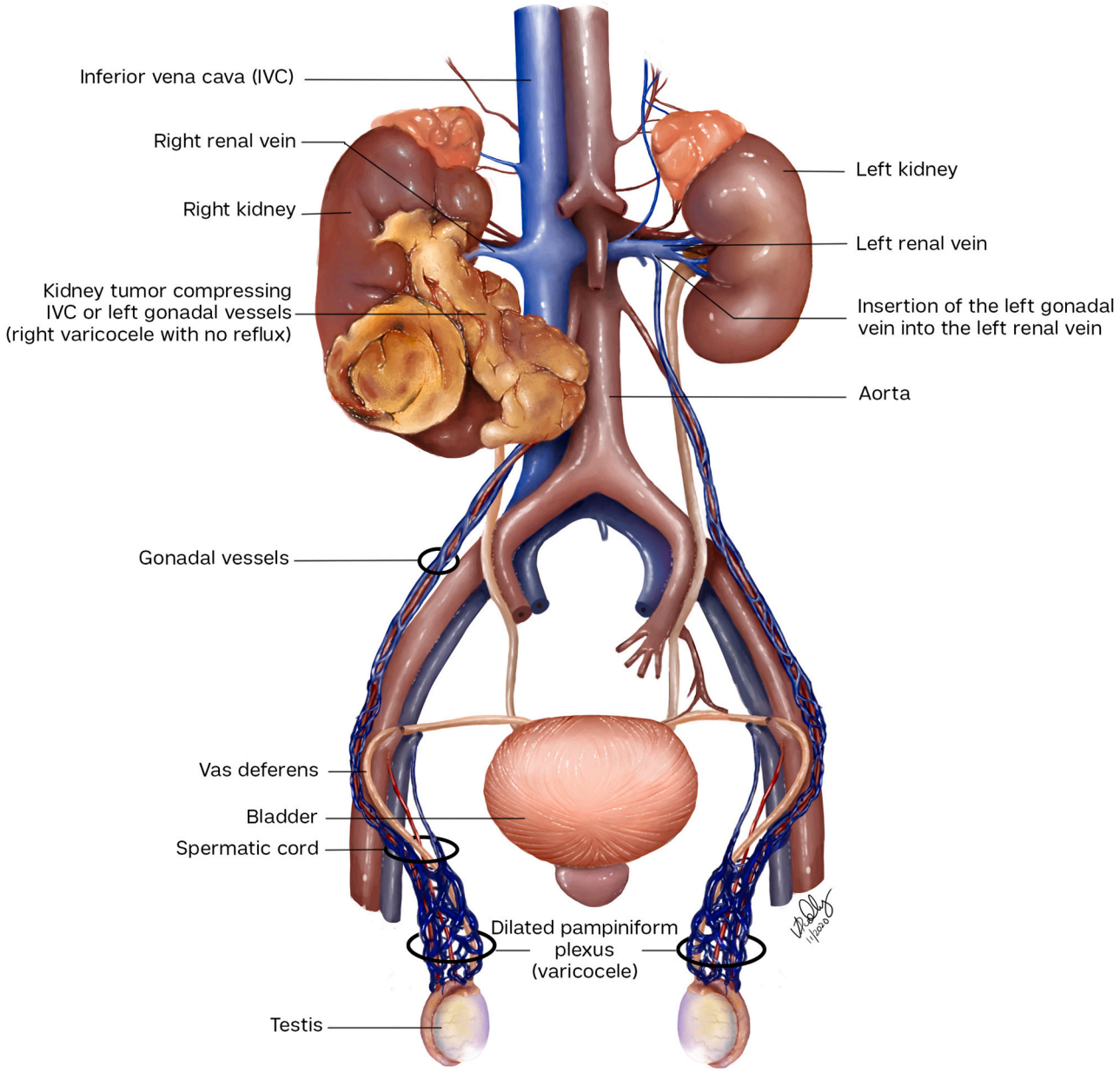

Fig. 1. Varicocele pathophysiology. Right-sided varicocele due to compression of the right gonadal vein or IVC demonstrated (A). Left-sided varicocele due to ninety degree angle insertion of the left gonadal vein into the left renal vein (B).

varicocele, with one study reporting rates in brothers of men with varicocele at greater than 50% [15,16]. However, no genetic factors have been identified that explain the inheritance of varicocele occurrence or grade [16]. Further research is needed to elucidate genetic determinants of varicocele formation.

4. Normal testicular function and physiology

The main functions of the testes are to produce mature sperm via spermatogenesis and to produce testosterone. The cell types responsible for both of these processes, Sertoli and Leydig cells, are located in the testis in close proximity, and both may be affected by changes within the testicular environment.

4.1. Testicular structure

The testis is the male gonad, which normally exists as a paired organ within the scrotum. It is responsible for producing mature sperm, the male gamete, and testosterone, the primary hormone responsible for male sex differentiation. The location of the testis within the scrotum is critical because it provides the optimal temperature for Sertoli and Leydig cell function, which occurs several degrees (33–34 °C) lower than average human body temperature (37 °C) [17,18]. The testis may receive arterial blood flow from three sources, the testicular (internal spermatic), cremasteric (external spermatic), and deferential arteries. Venous blood flow from the testis occurs primarily through the pampiniform plexus, a network of venous channels surrounding the testicular artery. As previously discussed, varicoceles generally are considered to be the result of dilated veins within the pampiniform plexus, but other veins in the spermatic cord, including cremasteric and deferential veins, also may become dilated in men with varicoceles. Therefore, varicocele repair involves the ligation of all abnormal veins identified within the spermatic cord. Following varicocele repair, the primary venous outflow from the testicle occurs through remaining normal vasal veins.

The average volume of an adult testis is approximately 20 ml. The inner component, the parenchyma, is comprised mainly of seminiferous tubules and is enveloped by a tough outer layer, the tunica albuginea (Fig. 2). Within each testis, there are approximately 200–300 lobules separated by septa arising from the tunica albuginea, and each of these lobules contains numerous loops of seminiferous tubules. Each seminiferous tubule is one functional unit of the testis and consists of a basement membrane surrounded by peritubular myoid cells, adventitia, and collagen matrix. Sertoli cells promote spermatogenesis, line the basement membrane, and are interspersed with Leydig cells, which produce testosterone (Fig. 2). Tight junctions link adjacent Sertoli cells to form the blood-testis barrier (BTB), which will be discussed later, affording the testis immunoprivilege, or exclusion from immune surveillance.

4.2. Testicular function

4.2.1. Testosterone production and hormonal regulation

The testes produce the majority of circulating testosterone (> 95%) in adult males, and the remainder is produced by the adrenal glands (< 5%) [19]. The hypothalamic-pituitary-testis (HPT) axis is critical for regulating the hormonal milieu required to facilitate spermatogenesis and testosterone production. Initially, gonadotropin-releasing hormone (GnRH) is released from the hypothalamus, stimulating the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary gland [20]. LH stimulates Leydig cells to produce testosterone in a cAMP-dependent manner, while FSH stimulates Sertoli cells to promote spermatogenesis, as well as secrete both inhibin and androgen-binding protein [20,21]. Testosterone is required for normal sperm development, as well as the development of male secondary sex characteristics. Intratesticular testosterone levels are estimated to be 25- to 125-fold higher than that found in the serum [22,23]. This discrepancy is facilitated through the binding of intratesticular testosterone to androgen-binding protein secreted by Sertoli cells [19,22,24–26]. In order for testosterone to facilitate spermatogenesis, it binds to the androgen receptor on Sertoli cells, which initiates various signaling cascades that promote spermatogenesis [22]. Circulating testosterone may be found in the serum as free testosterone (2%) or protein-bound (98%) to albumin or sex-hormone binding globulin. A fraction of serum testosterone is then converted to estradiol in the periphery by the

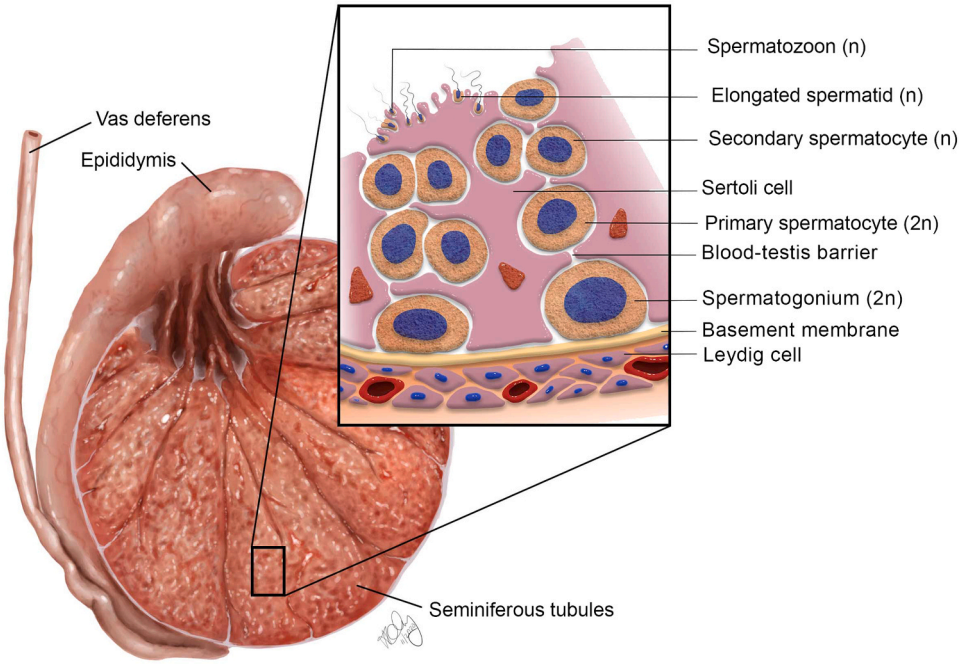

Fig. 2. Testis structure and spermatogenesis. A cross-section of the testis is shown, with various seminiferous tubules per lobule. The box depicts a cartoon representation of spermatogenesis, which occurs in the seminiferous tubule. n, number of chromosomes in a haploid cell (one copy); 2n, number of chromosomes in a diploid cell (two copies).

enzyme aromatase [20]. Estradiol, along with inhibin and testosterone, all negatively regulate the HPT axis to maintain homeostasis and appropriate hormone levels [20].

4.2.2. Spermatogenesis and spermiogenesis

Spermatogenesis is the formation of mature sperm and this intricate biological process occurs in the seminiferous tubules of the testis. It requires both Sertoli cells, which provide a specialized and nurturing microenvironment including the necessary growth factors and nutrients required for the health and maturation of spermatozoa, and Leydig cells, which produce the testosterone necessary for spermatogenesis [22]. Any perturbation of the HPT axis discussed previously and/or testicular factors required for spermatogenesis can lead to abnormal or decreased sperm production.

Spermatogenesis requires approximately 74 days (range 42–76 days) and consists of three phases highlighted in Fig. 2 [27]. The first phase is a mitotic phase and is characterized by the proliferation of spermatogonial stem cells (SSCs). SSCs differentiate into A dark and A pale spermatogonia, and A pale spermatogonia further differentiate into type B spermatogonia [28]. Type B spermatogonia subsequently undergo a series of mitotic events that give rise to primary spermatocytes. The second phase is the meiotic phase. Primary spermatocytes become secondary spermatocytes after completion of meiosis I, and secondary spermatocytes complete meiosis II to become round spermatids [28]. The third and final phase is spermiogenesis. During this phase, round spermatids become elongated spermatids. These spermatids undergo a series of steps, including nuclear condensation, cytoplasmic reduction, acrosome development, and tail elongation prior to eventually becoming a mature spermatozoon [29]. Failure of any of these steps can result in abnormal or decreased sperm development. Once a normal, mature spermatozoon is formed, its structure consists of an acrosome on the anterior surface of the head, a midpiece, and a tail, all of which have critical functions in sperm function. Mature spermatozoa are finally released from the apical surface of Sertoli cells into the lumen of a seminiferous tubule. These sperm subsequently are transported through the male reproductive tract, where they undergo further maturation in the epididymis, along with additional biochemical and morphological changes beyond the scope of this review [30]. Mature sperm are stored within the epididymis until ejaculation [30].

Given the complexity of sperm production and maturation, analysis of ejaculated sperm can provide insight into the cause of a man's fertility issues and provide clues to underlying defects of spermatogenesis. The World Health Organization (WHO) has established the normal semen parameters, including sperm count, concentration, morphology, and motility, based on semen parameters of men with documented fertility [31,32]. However, while abnormal semen parameters do not necessarily prove infertility, grossly abnormal characteristics, such as azoospermia, can provide valuable information and direct further evaluation. Normal semen parameters, per the WHO, along with the definition of associated semen parameter abnormalities, are shown in Table 2 [32].

Table 2
WHO 2010 semen parameters [32].

Semen parameter	WHO 5th Edition (2010) values	Abnormality
Volume (ml)	1.5–6	Aspermia (volume = 0 ml)
Total sperm count (TSC) (million)	39	Azoospermia (TSC = 0) Severe Oligozoospermia (TSC < 5 million)
Concentration (million sperm/ml)	15	Oligozoospermia (TSC < 15 million)
Progressive Motility (%)	32	Asthenospermia
Total Motility (%)	40	
Normal Morphology (%) (strict)	4–14	Teratozoospermia
Vitality (%)	58	
Leukocyte count (million/ml)	< 1	Leukocytospermia (LC > 1 million/ml)

TSC, total sperm count; LC, leukocyte count; ml, milliliters.

5. Varicocele and spermatogenesis

Varicoceles were first linked to male factor infertility at the end of the 19th century when a patient was shown to have improved sperm quality after surgical repair of a varicocele [3]. However, a definitive link between varicoceles and decreased spermatogenesis continues to be controversial. Some studies have reported an association between varicoceles and decreased spermatogenesis, whereas other studies have not [33–35]. Most men with varicoceles will be fertile, whereas others may be azoospermic [36]. Regardless of the conflicting data, the general guidelines and practice statements from national medical societies, including the American Urological Association (AUA), European Association of Urology (EAU), and the American Society for Reproductive Medicine (ASRM) recommend that, if present, palpable varicoceles should be repaired in individuals with semen parameter abnormalities whether or not they are attempting to conceive a child [37–39].

Both observational and randomized studies have evaluated changes in semen parameters or examined pregnancy rates following varicocele repair to determine the clinical impact on spermatogenesis. In one observational study, sperm concentration increased by 12 million/ml along with a concomitant 11% increase in motility following varicocele repair in infertile men [40]. Improvements were demonstrated by two randomized controlled trials (RCTs), which compared pregnancy rates in couples where men had palpable varicoceles and abnormal semen parameters, along with negative female evaluation (no female infertility factor detected), where they demonstrated improved pregnancy rates following varicocele repair [41,42]. In these studies, the natural pregnancy rate ranged from 33% to 60% after varicocele repair compared with 10–14% in the unrepaired group [41,42]. A recent meta-analysis of infertile men reinforced the improvements observed following microsurgical varicocelectomy such that men with abnormal semen parameters had improved odds of natural conception after varicocele repair (OR 4.15, 95%CI 2.31–7.45) [43]. One study found improvement in semen parameters and testis volume (increase in size from 14.6 ± 0.5 ml to 15.8 ± 0.6 ml after 12 months) after varicocele repair, but no difference in natural pregnancy rates compared to non-repaired controls [44]. Overall, these data suggest that varicocele treatment improves semen quality and possibly testicular function and pregnancy rates. This improvement in semen quality after varicocele repair may obviate the need for assisted reproductive technology (ART) and is an essential component of patient counseling prior to formulating fertility treatment plans [45]. For example, men with varicocele and severe oligozoospermia (total sperm count less than 5 million/ml) who may initially have required in vitro fertilization and intracytoplasmic sperm injection (IVF-ICSI) may only require intrauterine insemination (IUI), which typically requires total sperm count greater than 5 million/ml, following varicocele repair. Furthermore, men with varicocele and oligozoospermia who may have required IUI prior to varicocele repair may have semen parameter improvements that permit natural conception [45].

The main factors that lead to decreased fertility in varicoceles are thought to be scrotal hyperthermia and testicular tissue hypoxia. These abnormalities are thought to bring about changes in the vasculature and parenchyma that alter molecular architecture in the testis that increases the susceptibility of testicular cells (Sertoli, Leydig, and spermatogenic cells) to oxidative stress and increased apoptosis. Some of the molecular mechanisms thought to drive oxidative stress, and increased apoptosis in the testes of men with varicocele are altered heat shock protein (HSP) and factor (HSF) expression, decreased expression of cold-induced RNA-binding protein (CIRP), increased expression of hypoxic factors like hypoxia-inducible factor-1 (HIF-1) and vascular endothelial growth factor (VEGF), leukocytospermia, and toxin (e.g., cadmium) accumulation. Additional damage to the testicular parenchyma may occur secondary to BTB disruption, allowing the formation of anti-sperm

antibodies (ASA). Lastly, the presence of reactive oxygen species (ROS) may result in decreased sperm DNA quality. Here we review the evidence linking varicoceles to decreased spermatogenesis or sperm function and discuss potential etiologies for subfertility or infertility in men with varicoceles (Fig. 3).

5.1. Oxidative stress and apoptosis

Oxidative stress is an imbalance between the accumulation and removal of ROS in tissues [46]. ROS include superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen, all of which are produced predominantly by mitochondria during both normal physiological and abnormal pathological processes [46]. Enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) decrease intracellular ROS by catalyzing reactions to convert ROS into non-harmful molecules [46]. Cellular processes, including protein phosphorylation, transcription factor activation, apoptosis, and cellular differentiation, may be perturbed when ROS levels are too high [46,47]. While ROS are generally perceived as harmful, some oxidative stress is required during normal cellular processes, including fertilization, specifically in the process of sperm capacitation [48]. In this case, low levels of oxidative stress are thought to be protective because GPx, an antioxidant enzyme, promotes sperm chromatin cross-linking, which is protective against DNA damage [49].

Men with varicoceles have been shown to have higher levels of ROS in their seminal fluid, which may indicate oxidative stress occurring in the testis [50]. Markers of oxidative stress within the human testis include malondialdehyde (MDA) and 4-hydroxy-2-nonenal (4-HNE)--modified proteins, which also have been implicated in cardiovascular, pulmonary, gastrointestinal, and neurologic disease [50,51]. 4-HNE is a major byproduct of lipid peroxidation. It covalently binds to cysteine, lysine, and histidine residues of proteins, resulting in protein malfunction or cellular apoptosis [50,52]. An increase in 4-HNE-modified proteins is observed not only in the testis of patients with varicoceles but also in those with reproductive tract obstruction, and idiopathic infertility [53]. This increase in oxidative stress coincides with a decrease in PCNA (proliferating cell nuclear antigen) expression, which is a known marker of spermatogenic cell proliferation and spermatogenesis [50]. This phenomenon appears to occur in a grade-dependent manner, with higher levels of 4-HNE-modified observed in men with higher varicocele grade [51,52]. Staining of 4-HNE in testis samples from men with varicoceles demonstrated increased 4-HNE staining in Sertoli cells, spermatogonia, and pre-leptotene and leptotene spermatocytes [50]. Furthermore, 4-HNE also upregulates p53 expression, resulting in impaired germ cell proliferation [50]. These findings suggest that increased oxidative stress results in decreased proliferation or increased apoptosis of spermatogenic cells, which may manifest clinically as decreased sperm numbers in men with varicoceles.

Another marker of cellular stress is upregulation of p53, a protein critical in the regulation of cell cycle progression or initiation of apoptosis in the setting of DNA damage [54]. p53 upregulation can induce cell cycle arrest in the G1 phase or initiate apoptosis [50]. A rat varicocele model demonstrated significantly elevated p53 expression in the testis compared with controls, which contributed to apoptosis of both spermatogonia and primary spermatocytes [54,55]. In addition to increased p53-mediated apoptosis, cellular stress also results in decreased PCNA levels, indicating decreased spermatogenesis [50]. In humans, p53 is expressed within the testis in spermatogonia but not associated with apoptotic markers, suggesting that upregulation of p53 in spermatogonia results in cell cycle arrest [50]. Further work is needed to shed light on the precise role of p53 in the human spermatogenic cycle.

Oxidative stress can result in apoptosis, and this link has been made in several non-urologic diseases [56,57]. The precise mechanism of apoptosis in human germ cells is not clear. However, studies in rats have demonstrated that apoptosis of spermatids and spermatocytes associated with increased scrotal temperature proceeds via the mitochondrial pathway, which is mediated by cytochrome C, apoptotic protease activating factor-1 (Apaf-1), and caspase-9 [58]. Additional research is needed to determine the apoptotic pathways involved in human spermatogenic cell death.

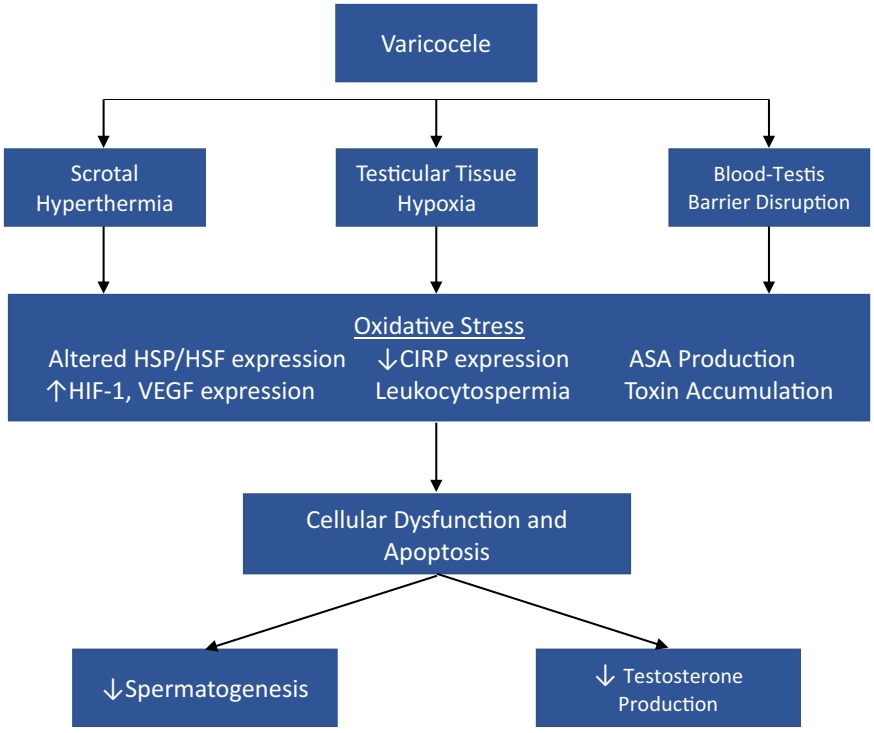

Fig. 3. Schematic of proposed mechanisms for decreased spermatogenesis in men with varicoceles.

5.2. Altered expression of heat shock proteins and factors

HSPs act as molecular chaperones to assist misfolded proteins secondary to cellular stress and are thus regarded as cytoprotective, anti-apoptotic factors [59]. Normally, HSPs are involved in various developmental processes but more commonly are induced by cellular heat stress and regulated by heat shock factors [59,60]. HSPs belong to six general families, HSP 110, HSP 90, HSP 70, HSP 60, small molecular weight HSPs, and immunophilins [59]. HSP 60 is expressed in Leydig cells of neonatal and prepubertal rats [60]. HSP 70 and 90 are expressed in germ cells, Sertoli cells, Leydig cells in the developing testis, and in spermatocytes and round spermatids after puberty [60]. Increased scrotal temperatures in rats demonstrated an increase in expression of HSPs, mainly in spermatocytes and spermatids [60]. In men with varicocele and infertility, HSP 70 and 90 were significantly upregulated, as determined by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS), western blot, and immunocytochemistry [61]. Furthermore, several heat shock factors, specifically *HSPA4*, *HSF1*, and *HSF2*, were upregulated in sperm collected from men with varicocele and oligozoospermia, demonstrating ongoing cellular stress of the ejaculated sperm [59]. Upregulation of HSPs and HSFs in men with varicoceles may be a cellular response to scrotal hyperthermia in the setting of varicocele, but also may suggest attempted self-correction of spermatogenic impairment. *Hsf1* and *Hsf2* are known to play a role in normal mature sperm production, and mice lacking *Hsf1* and *Hsf2* had abnormal sperm morphology and increased germ cell apoptosis, respectively [62]. However, more research is needed to elucidate the precise function of HSPs and HSFs in human sperm.

5.3. Decreased expression of cold-inducible RNA-binding protein

CIRP is a constitutively-expressed protein within the testis, primarily in spermatocytes. Expression of *CIRP* is induced by lower temperatures (32 °C) [53]. *CIRP* has been described as a proto-oncogene, and involved in processes that affect protein translation, antioxidant activity, cellular proliferation, and mRNA stability within spermatocytes [63]. CIRP-bound mRNA exhibits increased stability, and CIRP may play a role in both mitotic and meiotic processes within spermatogenic cells [53]. In men with varicoceles, lower levels of CIRP expression were observed in the testis [53]. In mice, *Cirp* expression was observed in spermatocytes and not spermatogonia, and scrotal hyperthermia resulted in decreased *Cirp* levels, and mice lacking *Cirp* were observed to have increased levels of germ cell apoptosis and damage via activation of various MAP kinase (MAPK) pathways [53,63]. Because meiosis and mitosis are tightly regulated in spermatogenesis, alteration of CIRP levels may result in diminished sperm numbers. However, more research is needed to determine the exact role and/or effect of CIRP in the human testis.

5.4. Increased expression of hypoxic factors

In varicoceles, hypoxia can occur secondary to vascular changes and scrotal hyperthermia [64]. Local ischemia can result in deprivation of not only oxygen but also nutrients and growth factors [64]. Elevations in venous pressure beyond arterial pressure have been shown to lead to testicular ischemia [65]. Tissue hypoxia has been shown to induce increased expression of *HIF-1* and *VEGF*, resulting in damage of seminiferous tubules [64,66]. Some evidence suggests that *VEGF* can inhibit spermatogonial proliferation and promote the production of ROS [64]. Additionally, a hypoxic environment may lead to increased cytokine expression, primarily interleukin 6 (IL-6), which correlates with elevated ROS levels [67]. Reflux of toxic adrenal metabolites, such as norepinephrine, also may result in ROS generation [11]. Catecholamines like norepinephrine may cause vasospasm and further potentiate hypoxia in testicular tissue, as described above [6,68].

5.5. Leukocytospermia

Leukocytospermia, or the presence of high numbers of leukocytes in semen, has been shown in men with varicoceles and infertility [32,69]. Although leukocytes can normally be found in semen (normal threshold is 1 million leukocytes/ml), the presence and increased number of leukocytes within semen may indicate infection or inflammation of the male reproductive organs and/or tract [69]. Antibiotics are typically administered to clear any infection that may be impacting sperm production or fertility. However, it is important to note that the administration of certain antibiotics may negatively affect spermatogenesis and/or sperm function [70]. It is thought that leukocytes negatively affect semen parameters due to the generation of ROS [11,71–73]. One possible mechanism is the presence of leukocytes overwhelms normal cellular defense antioxidant mechanisms, with subsequent higher levels of ROS [71]. Another possible mechanism is the direct production of ROS by leukocytes present in semen [72]. ROS can directly affect sperm within the reproductive tract by lipid peroxidation (LPO), a process where ROS attack lipids on the outer membrane of sperm [73]. LPO then allows for ROS to enter sperm and disrupt ATP genesis by destroying mitochondrial DNA [72]. A meta-analysis of eleven studies found that in men with leukocytospermia and infertility, antibiotics improved sperm parameters and pregnancy rates [69]. Additional research is needed to determine how leukocytes affect sperm function and spermatogenesis in men with varicoceles, and whether anti-microbial or anti-inflammatory medications may improve sperm count and function in this cohort.

5.6. Toxin accumulation

Accumulation of harmful substances, like cadmium and carbon monoxide, can result in the apoptosis of testicular cells [74]. Cadmium is a metal ion that causes oxidative stress in cells resulting in the induction of apoptosis [74]. Cadmium is thought to traverse the BTB, accumulate in the interstitium of the testis, reduce sperm production, and alter cytoskeletal scaffolding [75]. The resultant increased oxidative stress and apoptosis in germ cells may explain lower sperm counts in men with varicoceles [75]. Some evidence has suggested that increased cadmium levels reduce seminal zinc (an antioxidant) concentrations and subsequently allow for increases of ROS [76]. Some men exhibit deletions in L-VDCC, L-type calcium channel alpha 1 sub-unit expressed in testis, which results in altered calcium channel function and increased cadmium accumulation in the testis [75]. Men with L-VDCC deletions and varicoceles who underwent varicocele repair had lower postoperative sperm counts than men with normal L-VDCC who underwent varicocele repair [75]. These data suggest that accumulation of toxins, like cadmium, can decrease sperm count even further in men with varicoceles and that genetic determinants may exacerbate the infertility phenotype in these patients.

Carbon monoxide also may act as a cellular protectant and antioxidant in the setting of oxidative stress, however, high levels of carbon monoxide may be harmful to cells and trigger apoptosis [52,77]. Heme oxygenase 1 (*HO-1*) is a stress-inducible member of the heme oxygenase family, and degrades heme generating antioxidants (carbon monoxide, biliverdin, and iron) in the process [52,77]. In the setting of cadmium exposure, *HO-1* expression was upregulated in Leydig cells resulting in high levels of carbon monoxide generation triggering apoptosis of spermatogenic cells [77]. This data suggests that accumulation of carbon monoxide is damaging to spermatogenic cells, and may be one mechanism by which cadmium toxicity diminishes sperm count.

5.7. Blood-testis barrier disruption

The BTB, comprised of myoid cells, basement membrane, and Sertoli cell tight junctions, exists to create a highly specialized microenvironment for developing germ cells that is exempt from immune surveillance [78,79]. E-cadherin is a crucial component of Sertoli cell tight junctions

and is required for the formation of the BTB [78]. The α-, β-, and γ-catenins are intracellular proteins that link E-cadherin to the actin cytoskeleton [78]. E-cadherin also may play a role in anchoring germ cells to Sertoli cells, as expression of E-cadherin has been demonstrated in germ cells near the basement membrane [80]. Examination of the BTB in patients with varicoceles demonstrates reduced E-cadherin and α-catenin expression in Sertoli cell tight junction complexes suggesting that disruption of the BTB may play a role in varicocele pathophysiology [78]. Breakdown of the BTB may allow for the production of ASA, and there is evidence that infertile men have a higher incidence of ASA than fertile men [78,81]. Although the link between ASA and male infertility is controversial, the WHO recommends testing for ASA in the presence of sperm agglutination, and there is some evidence that the presence of ASA may result in negative effects on semen parameters [31,82]. More research is needed to determine whether decreased BTB integrity results in decreased spermatogenesis.

5.8. Oxidative stress and sperm DNA damage

In addition to the impact of varicoceles on spermatogenesis, there also have been reported changes to the quality of sperm DNA. These changes are thought to occur secondary to oxidative stress from ROS. Numerous studies have reported that sperm DNA quality is negatively affected by the presence of varicoceles, and is subsequently improved following varicocele repair [83]. Therefore, in men with varicoceles who have normal semen parameters, additional tests such as DNA fragmentation analysis (using assays such as terminal deoxynucleotide transferase-mediated dUTP nick-end labeling (TUNEL assay), single-cell gel electrophoresis (Comet assay), sperm chromatin dispersion (SCD, Halo assay), or sperm chromatin structure assay (SCSA™)) may provide useful information for determining etiology of male infertility and patient counseling [36]. To date, indications for DNA fragmentation assessment remain controversial, but is recommended in situations such as recurrent pregnancy loss, failed ART, unexplained infertility, and in men with subfertility, varicocele, and normal semen parameters [84].

High levels of oxidative stress may impact DNA by causing direct and indirect changes to the sperm genome. ROS impact both mitochondrial and sperm DNA [57]. Mitochondrial DNA is more susceptible to damage because sperm DNA is more tightly packed secondary to protamine [85]. Mechanistically, ROS disrupt DNA in a two-step fashion, first by impairing protamine packaging of sperm DNA, followed by direct DNA damage once sperm DNA has been unpacked [11,86]. Oxidative stress from heat stress is one of the main sources of ROS and subsequent DNA damage in men with varicoceles, in addition to toxic metals such as cadmium described above.

6. Varicocele and testosterone production

As previously discussed, the presence of a varicocele impairs venous blood outflow from the testicle, resulting in increased scrotal temperature and decreased levels of oxygenated blood flow into the testicular parenchyma. Therefore, not only are spermatogenic and Sertoli cells affected, resulting in impaired spermatogenesis and infertility, but testicular Leydig cells also may be affected, resulting in reduction of serum testosterone concentrations [87]. Therefore, it is not surprising that men with varicoceles have issues with fertility, along with testosterone deficiency. In fact, lower testosterone levels are observed in men with varicoceles at all ages compared with age-matched, fertile men without varicoceles [88]. Low serum testosterone levels may subsequently result in various sexual (decreased libido, erectile dysfunction (ED)), psychological (depressed mood, fatigue, impaired cognition), and metabolic (decreased bone mineral density, decreased muscle mass increased body fat, insulin resistance, anemia) derangements that can affect the overall health of an individual along with his quality of life [89]. Thus, varicocele is a risk factor for testosterone deficiency, and low testosterone levels should be considered an indication for surgical repair [90]. Importantly, although 15% of all men have varicoceles, only 20% of them are grade 3, which is approximately 3% of all men. These men are the most at risk for impaired testosterone function and may benefit from early varicocele repair [91].

Various prospective and retrospective studies performed on men with varicoceles undergoing repair have demonstrated improvement in serum testosterone levels following varicocele repair [88,90,92,93]. The most considerable benefit is seen in patients with lower serum testosterone levels prior to varicocele repair, and up to 70% of men can have postoperative improvement in their testosterone levels [90,92]. Some studies report an improvement in serum testosterone of approximately 90 ng/dl after varicocele repair, however, whether the improvement in testosterone after varicocele repair results in clinically meaningful symptom improvement is unclear [92–94]. Studies have found that ED, a common symptom of testosterone deficiency, was more likely to be present in men with varicocele, although this finding did not reach statistical significance. However, after varicocele repair, those with ED had an approximate 2-point improvement in their ED symptoms based on the International Index of erectile function (IIEF-5) validated symptom questionnaire [93].

Mechanisms by which Leydig cells are affected by varicoceles likely mirror that seen in various other testicular cell types, including spermatogenic cells and Sertoli cells. Leydig cells are likely sensitive to hypoxia, hyperthermia, gonadotoxins, and oxidative stress, all of which may be derived from impaired venous drainage due to varicocele. Examination of testis biopsies from men with varicoceles revealed Leydig cell changes in both testicles despite most subjects with unilateral varicocele [95–97]. Two studies found Leydig cell hyperplasia, whereas one study showed no difference in Leydig cell density [95–97]. A separate study found that Leydig cells harvested from the testes of men with varicoceles had decreased testosterone biosynthetic capabilities [98]. In rats with surgically-induced varicoceles, the testosterone synthesis pathway was examined, and impairment of 17,20-desmolase and 17 α-hydroxylase was noted [99]. Another study found that human testicular C17,20-lyase activity may be inhibited in men with varicocele. Thus, varicoceles may affect Leydig cells by targeting various enzymes within the testosterone biosynthetic pathway, resulting in low serum testosterone concentrations. Importantly, lower serum and intratesticular testosterone levels can then worsen any spermatogenic defects caused by varicoceles. In men with testosterone deficiency and spermatogenic dysfunction, varicocele repair may improve both conditions.

7. Varicocele pathology

Testis histology obtained from men with varicoceles demonstrate sloughing and germ cell degeneration at various stages of maturation arrest in the seminiferous tubule lumen [100]. No consistent changes have been demonstrated with respect to meiotic abnormalities [101]. Leydig cells may atrophy or display hyperplasia [3,95–97]. The vasculature may show degenerative changes, including endothelial hypertrophy, basement membrane thickening, and/or narrowing of the vessel lumen [102]. Other changes include sloughing of Sertoli cells and thickening of the seminiferous tubule basement membrane [102]. From these studies, it is clear that the histopathology of a testis affected by varicocele is variable, likely due to the numerous effects that varicoceles have on the testicular parenchyma.

8. Clinical evaluation and management of varicoceles

8.1. Presentation

Varicoceles often are discovered incidentally at the time of physical exam during an infertility evaluation. While the majority of men with varicoceles are symptom-free, a subset of men will present with varicocele-related symptoms such as testicular pain or discomfort. Etiologies for varicocele-associated discomfort include reflux of toxic

metabolites, increased hydrostatic pressure, testicular tissue hypoxia, abnormal testicular temperature from vein dilation and/or compression of neural fibers from dilated pampiniform plexus veins [103]. Pain may be felt in the testicle but also in the scrotum or groin [38]. Others may present for cosmetic reasons, as the appearance of abnormal veins may be distressing to patients and/or their partners.

8.2. Evaluation

Providers should review focused infertility, sexual, and symptom-related history during the initial evaluation of a man with varicoceles. Additional focus should be given to signs and symptoms of hypogonadism. A focused physical exam of the male genital region, including the penis, scrotum, and spermatic cord, should be performed. Grading of varicoceles (Table 1) should be documented, as well as the size and consistency of the testicles. For men with anatomically challenging exams (i.e., obesity, presence of hydrocele) a scrotal ultrasound with doppler may be obtained. Laboratory tests, such as total serum testosterone concentration, can be measured in men with suspected testosterone deficiency. Semen analysis, and additional sperm tests (i.e., anti-sperm antibodies) as appropriate, can be performed in men presenting for infertility evaluation. Assays examining sperm DNA fragmentation may be performed in men with grade 2 or 3 varicoceles and normal semen parameters, or men with grade 1 varicoceles and borderline or abnormal semen parameters [84].

8.3. Varicocele examination

Since varicocele assessment relies heavily on physical exam, an appropriately completed examination is paramount. Patients should be seated in a quiet environment and a heating pad placed on the scrotum to relax the cremasteric and dartos muscles. Examination should be completed in both supine and standing positions with and without Valsalva maneuvers (i.e., bearing down or cough). Visualized and dilated veins should be documented, and if the varicocele is not visible, the spermatic cord should be examined by isolating the cord between the fingers to palpate for any change in fullness (i.e., impulse) in the veins with Valsalva maneuvers. Examination should be performed on both the left and right sides. As mentioned previously, if physical exam findings are equivocal, scrotal ultrasound may be obtained to evaluate the size of and flow in the paratesticular veins. The sonographer should measure the size of the paratesticular veins in the standing and supine positions with and without Valsalva maneuvers and document the presence or absence of reversal of flow with Valsalva maneuvers. Typically, varicocele is present if the veins are greater than 2–3 mm in diameter, there is a serpiginous appearance to the veins, or there is reversal of flow with Valsalva.

8.4. Indications for treatment

Treatment for varicocele may occur for a variety of reasons. There is evidence that varicocele repair results in improvements in semen parameters, including concentration and sperm motility, as well as improved DNA fragmentation [104]. The AUA, EAU, and ASRM recommend that palpable varicoceles be repaired in individuals with semen abnormalities whether or not they are actively trying to conceive, and in children with ipsilateral testis atrophy, typically a greater than 20% decrease in size. Early repair of varicoceles in children with delayed testicular growth may permit testicular growth of the repaired side to equal the non-repaired, normal contralateral testis [104]. Other indications include symptomatic varicoceles (i.e., pain or discomfort), with reported resolution of pain in up to 90%, for cosmesis, and in some cases low testosterone [91,104].

8.5. Varicocele management

General management options for varicocele include observation, percutaneous embolization by interventional radiology, and surgical intervention (Fig. 4, Table 3). In men who are attempting to conceive, another option is sperm retrieval for ART. Sperm retrieval can be attempted with procedures performed in the clinic or operating room, and procedures can range from percutaneous sperm aspiration to microsurgical sperm extraction from the epididymis or testicle. Informed discussion of each of the procedures is recommended prior to proceeding with the treatment plan.

Observation may be reserved for those with no impact on fertility, normal testosterone levels, appropriate testicular growth, and minimal symptoms related to varicocele. Since varicoceles themselves are benign processes, there is no urgent indication for repair. However, it is important to discuss potential impacts on fertility and testosterone production in the setting of varicoceles, as varicoceles may progress and impact future testicular function.

Percutaneous embolization is an option for patients who desire avoidance of general anesthesia or have significant medical comorbidities that place them at high risk for anesthetic complications. The procedure includes sedation and sharp entry into the jugular or femoral vein to gain access to the spermatic venous vessels. The dilated veins are identified using angiography, and tiny coils are deployed to occlude the abnormal veins. Complications and outcomes are described in Table 3.

There are a number of surgical options for varicocele repair. Varicocelectomy involves ligation of the internal spermatic, external spermatic, and cremasteric veins using metal clips or suture ties. Rarely, vasal veins also can be abnormally dilated, and any abnormal vasal venous vessels also may be ligated during varicocelectomy. The gold standard in the surgical management of varicoceles is microsurgical varicocelectomy. This approach occurs through either a sub-inguinal or inguinal incision. Using a sub-inguinal incision, the spermatic cord is identified at the level of the external ring after dissection through Camper's and Scarpa's fascia (Fig. 4). The cord is located, isolated, and secured for easy visualization and manipulation using a Penrose drain. The external and internal spermatic fascia are opened to reveal the contents of the spermatic cord. The vas deferens and vasal vessels lie within a fascial compartment outside of the internal spermatic fascia and can typically be easily avoided during the procedure. A micro-doppler is used to identify any internal spermatic (testicular) arteries and also can be used to identify the cremasteric artery. The arteries may be isolated using a vessel loop to be easily identifiable while veins are being ligated. Any small caliber veins or bleeding should be managed with bipolar cautery. Lymphatic channels should be preserved to minimize the risk of

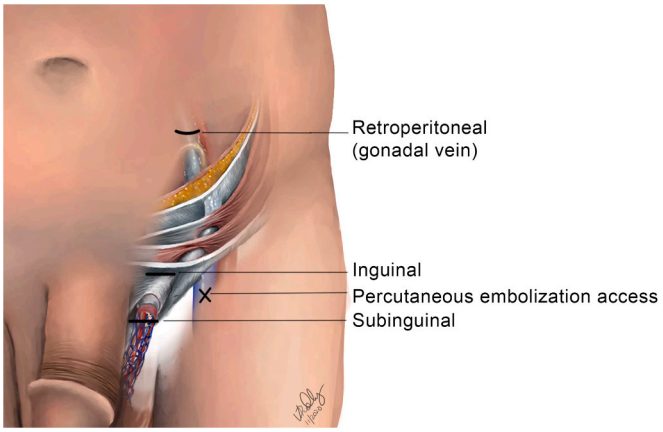

Fig. 4. Location of vein ligation for various varicocelectomy approaches, and site of access for percutaneous embolization procedure. (Image is a draft – still working on minor edits).

Table 3
Recurrence, complication, and pregnancy rates of various treatment options for varicocele [38,103,104].

Technique	Recurrence/ persistence	Hydrocele formation	Spontaneous pregnancy	Other complications
Embolization (Antegrade and Retrograde)	3–11%	–	33.2%	Reaction to contrast media, thrombophlebitis, hematoma, infection, bleeding, vascular injury, coil migration, retroperitoneal hemorrhage, ureteric obstruction.
Open/Non-microsurgical	2.6%	7%	36%	Arterial injury, scrotal hematoma, higher likelihood of missing an abnormal vein, wound infection, pain, bleeding
Open/Microsurgical	0–2%	0.4%	42%	Arterial injury, scrotal hematoma, wound infection, pain, bleeding
Retroperitoneal	9–45%	8%	38%	Arterial injury, scrotal hematoma, wound infection, pain, bleeding
Laparoscopic/Robotic	3–15%	2.8%	30%	Testicular artery injury or ligation, pulmonary embolism, peritonitis, bleeding, pain, pneumo-scrotum, wound infection, visceral organ injury

hydrocele formation. The testicle may be delivered to evaluate for and ligate any abnormal gubernacular veins. An inguinal approach requires a higher groin incision and opening of the external oblique aponeurosis (Fig. 4) to identify the spermatic cord. One benefit of performing an inguinal varicocelectomy is that the venous vasculature is typically simpler as one progresses proximally (toward the abdomen) on the spermatic cord. After ligation of the veins, the external oblique aponeurosis should be reapproximated and sutured. Both approaches are well-tolerated and performed as outpatient procedures.

Other surgical options include inguinal or sub-inguinal approaches without the use of a standard operating microscope. As an open procedure, varicocelectomy is performed in the same fashion as described above and may be completed with or without the use of surgical loupes. Alternatively, varicocelectomy may be performed using a laparoscopic or robotic approach. After transperitoneal ports are placed, laparoscopic or robotic tools are used to sharply incise the peritoneum overlying the gonadal vessels proximal to the internal inguinal ring. An attempt to spare the artery should be made while the abnormal venous vessels are isolated and ligated, typically with metal clips. Ligation of the gonadal vein(s) proximal to the internal inguinal ring also may be completed retroperitoneally using an open or retroperitoneoscopic technique. The open approach, known as the Palomo technique, requires a Gibson incision followed by identifying the internal spermatic vein, which is isolated between the renal vein and anterior superior iliac spine and is ligated (Fig. 4). Using a retroperitoneoscopic approach an incision is made below the 12th rib, and once access is obtained into the retroperitoneum, the spermatic vessels are isolated and veins ligated. Complications and outcomes of the various surgical options are described in Table 3.

9. Conclusions

Varicoceles are a common finding in men. The majority of men with varicoceles are asymptomatic. However, the 3% of men with grade 3 varicoceles are at greatest risk for impaired testicular dysfunction and, in those men, conservative management should be microsurgical repair in order to preserve testicular function. Men with varicoceles should have a complete evaluation, and following review of treatment indications, offered observation or varicocele repair. While precise mechanisms of effects on testicular function remain inconclusive, varicoceles appear to have negative impacts on spermatogenesis and testosterone production, which clinically manifest as male infertility and testosterone deficiency. More research is needed to shed light on mechanisms of varicocele-induced testicular dysfunction, so that new targeted therapies and treatments may be developed for men with varicoceles.

Funding

CK and NP are supported by the Frederick J. and Theresa Dow Wallace Fund of the New York Community Trust.

Conflict of interest

The authors declare there is no conflict of interest.

Acknowledgments

The authors would like to acknowledge Vanessa Dudley for her outstanding illustrations.

References

[1] M.E. Thoma, A.C. McLain, J.F. Louis, R.B. King, A.C. Trumble, R. Sundaram, G. M. Buck Louis, Prevalence of infertility in the United States as estimated by the current duration approach and a traditional constructed approach, Fertil. Steril. 99 (2013), 1324-31.e1.
[2] K. Jarvi, K. Lo, E. Grober, V. Mak, A. Fischer, J. Grantmyre, A. Zini, P. Chan, G. Patry, V. Chow, T. Domes, The workup and management of azoospermic males, Can. Urol. Assoc. J. 9 (2015) 229–235.
[3] S.J. Skoog, K.P. Roberts, M. Goldstein, J.L. Pryor, The adolescent varicocele: what's new with an old problem in young patients? Pediatrics 100 (1997) 112–121.
[4] A. Marte, The history of varicocele: from antiquity to the modern ERA, Int. Braz. J. Urol. 44 (2018) 563–576.
[5] H.D. Noske, W. Weidner, Varicocele–a historical perspective, World J. Urol. 17 (1999) 151–157.
[6] M.L. Eisenberg, L.I. Lipshultz, Varicocele-induced infertility: newer insights into its pathophysiology, Indian J. Urol. 27 (2011) 58–64.
[7] U. Levinger, M. Gornish, Y. Gat, G.N. Bachar, Is varicocele prevalence increasing with age? Andrologia 39 (2007) 77–80.
[8] C.K. Naughton, A.K. Nangia, A. Agarwal, Pathophysiology of varicoceles in male infertility, Hum. Reprod. Update 7 (2001) 473–481.
[9] M.A. Witt, L.I. Lipshultz, Varicocele: a progressive or static lesion? Urology 42 (1993) 541–543.
[10] J. Damsgaard, U.N. Joensen, E. Carlsen, J. Erenpreiss, M. Blomberg Jensen, V. Matulevicius, B. Zilaitiene, I.A. Olesen, A. Perheentupa, M. Punab, A. Salzbrunn, J. Toppari, H.E. Virtanen, A. Juul, N.E. Skakkebæk, N. Jørgensen, Varicocele is associated with impaired semen quality and reproductive hormone levels: a study of 7035 healthy young men from six European countries, Eur. Urol. 70 (2016) 1019–1029.
[11] C.L. Cho, S.C. Esteves, A. Agarwal, Novel insights into the pathophysiology of varicocele and its association with reactive oxygen species and sperm DNA fragmentation, Asian J. Androl. 18 (2016) 186–193.
[12] S.W. Leslie, H. Sajjad, L.E. Siref, Varicocele, StatPearls, Treasure Island (FL), 2020.
[13] P.D. Kantartzi, Ch.D. Goulis, G.D. Goulis, I. Papadimas, Male infertility and varicocele: myths and reality, Hippokratia 11 (2007) 99–104.
[14] N.S. El-Saeity, P.S. Sidhu, Scrotal varicocele, exclude a renal tumour. Is this evidence based? Clin. Radiol. 61 (2006) 593–599.
[15] G. Mokhtari, F. Pourreza, S. Falahatkar, A.N. Kamran, M. Jamali, Comparison of prevalence of varicocele in first-degree relatives of patients with varicocele and male kidney donors, Urology 71 (2008) 666–668.
[16] J.D. Raman, K. Walmsley, M. Goldstein, Inheritance of varicoceles, Urology 65 (2005) 1186–1189.
[17] P.V. Bach, B.B. Najari, M. Goldstein, Varicocele – a case for early intervention, F1000Res 5 (2016) 1792.
[18] C. Wang, 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 (2007) 3292–3304.
[19] I. Masliukaite, J.M. Hagen, K. Jahnukainen, J.B. Stukenborg, S. Repping, F. van der Veen, M. van Wely, A.M.M. van Pelt, Establishing reference values for age-related spermatogonial quantity in prepubertal human testes: a systematic review and meta-analysis, Fertil. Steril. 106 (2016) 1652–1657, e2.
[20] A.A. Dabaja, P.N. Schlegel, Medical treatment of male infertility, Transl. Androl. Urol. 3 (2014) 9–16.

[21] W.L. Miller, Steroidogenesis: unanswered questions, Trends Endocrinol. Metab. 28 (2017) 771–793.
[22] L.B. Smith, W.H. Walker, The regulation of spermatogenesis by androgens, Semin. Cell Dev. Biol. 30 (2014) 2–13.
[23] S. Maddocks, T.B. Hargreave, K. Reddie, H.M. Fraser, J.B. Kerr, R.M. Sharpe, Intratesticular hormone levels and the route of secretion of hormones from the testis of the rat, guinea pig, monkey and human, Int. J. Androl. 16 (1993) 272–278.
[24] B.R. Zirkin, R. Santulli, C.A. Awoniyi, L.L. Ewing, Maintenance of advanced spermatogenic cells in the adult rat testis: quantitative relationship to testosterone concentration within the testis, Endocrinology 124 (1989) 3043–3049.
[25] I.A. Kopera, B. Bilinska, C.Y. Cheng, D.D. Mruk, Sertoli-germ cell junctions in the testis: a review of recent data, Philos. Trans. R. Soc. Lond. B Biol. Sci. 365 (2010) 1593–1605.
[26] R.W. Holdcraft, R.E. Braun, Androgen receptor function is required in Sertoli cells for the terminal differentiation of haploid spermatids, Development 131 (2004) 459–467.
[27] L.M. Misell, D. Holochwost, D. Boban, N. Santi, S. Shefi, M.K. Hellerstein, P. J. Turek, A stable isotope-mass spectrometric method for measuring human spermatogenesis kinetics in vivo, J. Urol. 175 (2006) 242–246.
[28] F.T. Neto, P.V. Bach, B.B. Najari, P.S. Li, M. Goldstein, Spermatogenesis in humans and its affecting factors, Semin. Cell Dev. Biol. 59 (2016) 10–26.
[29] L. O'Donnell, Mechanisms of spermiogenesis and spermiation and how they are disturbed, Spermatogenesis 4 (2014), e979623.
[30] G. van Der Horst, J.V. Seier, A.C. Spinks, S. Hendricks, The maturation of sperm motility in the epididymis and vas deferens of the vervet monkey, Cercopithecus aethiops, Int. J. Androl. 22 (1999) 197–207.
[31] S.S. Vasan, Semen analysis and sperm function tests: how much to test? Indian J. Urol. 27 (2011) 41–48.
[32] T.G. Cooper, E. Noonan, S. von Eckardstein, J. Auger, H.W.G. Baker, H.M. Behre, T.B. Haugen, T. Kruger, C. Wang, M.T. Mbizvo, K.M. Vogelsong, World Health Organization reference values for human semen characteristics, Hum. Reprod. Update 16 (2010) 231–245.
[33] The influence of varicocele on parameters of fertility in a large group of men presenting to infertility clinics. World Health Organization, Fertil. Steril., 57, 1992, p. 1289–1293.
[34] F. Pasqualotto, A. Lucon, P. Degoes, B. Sobreiro, J. Hallak, E. 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 (2005) 74–77.
[35] J. Zargooshi, Sperm count and sperm motility in incidental high-grade varicocele, Fertil. Steril. 88 (2007) 1470–1473.
[36] A.W. Pastuszak, R. Wang, Varicocele and testicular function, Asian J. Androl. 17 (2015) 659–667.
[37] Report on varicocele and infertility, Fertil. Steril., 90, 2008, pp. S247–S249.
[38] A. Shridharani, G. Lockwood, J. Sandlow, Varicocelectomy in the treatment of testicular pain: a review, Curr. Opin. Urol. 22 (2012) 499–506.
[39] A. Jungwirth, A. Giwercman, H. Tournaye, T. Diemer, Z. Kopa, G. Dohle, C. Krausz, European Association of Urology guidelines on male infertility: the 2012 update, Eur. Urol. 62 (2012) 324–332.
[40] A. Baazeem, E. Belzile, A. Ciampi, G. Dohle, K. Jarvi, A. Salonia, W. Weidner, A. Zini, Varicocele and male factor infertility treatment: a new meta-analysis and review of the role of varicocele repair, Eur. Urol. 60 (2011) 796–808.
[41] I. Madgar, R. Weissenberg, B. Lunenfeld, A. Karasik, B. Goldwasser, Controlled trial of high spermatic vein ligation for varicocele in infertile men, Fertil. Steril. 63 (1995) 120–124.
[42] T.A. Abdel-Meguid, A. Al-Sayyad, A. Tayib, H.M. Farsi, Does varicocele repair improve male infertility? An evidence-based perspective from a randomized, controlled trial, Eur. Urol. 59 (2011) 455–461.
[43] K.H. Kim, J.Y. Lee, D.H. Kang, H. Lee, J.T. Seo, K.S. Cho, Impact of surgical varicocele repair on pregnancy rate in subfertile men with clinical varicocele and impaired semen quality: a meta-analysis of randomized clinical trials, Korean J. Urol. 54 (2013) 703–709.
[44] E. Nieschlag, L. Hertle, A. Fischedick, K. Abshagen, H.M. Behre, Update on treatment of varicocele: counselling as effective as occlusion of the vena spermatica, Hum. Reprod. 13 (1998) 2147–2150.
[45] E.W. Kirby, L.E. Wiener, S. Rajanahally, K. Crowell, R.M. Coward, Undergoing varicocele repair before assisted reproduction improves pregnancy rate and live birth rate in azoospermic and oligospermic men with a varicocele: a systematic review and meta-analysis, Fertil. Steril. 106 (2016) 1338–1343.
[46] G. Pizzino, N. Irrera, M. Cucinotta, G. Pallio, F. Mannino, V. Arcoraci, F. Squadrito, D. Altavilla, A. Bitto, Oxidative stress: harms and benefits for human health, Oxid. Med. Cell. Longev. 2017 (2017) 1–13.
[47] T.M. Buttke, P.A. Sandstrom, Oxidative stress as a mediator of apoptosis, Immunol. Today 15 (1994) 7–10.
[48] R.J. Aitken, M.A. Baker, B. Nixon, Are sperm capacitation and apoptosis the opposite ends of a continuum driven by oxidative stress? Asian J. Androl. 17 (2015) 633–639.
[49] R.J. Aitken, T.B. Smith, M.S. Jobling, M.A. Baker, De, G.N. Iuliis, Oxidative stress and male reproductive health, Asian J. Androl. 16 (2014) 31–38.
[50] K. Shiraishi, 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 (2007) 1012–1017.
[51] I.T. Köksal, 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 (2000) 549–552.
[52] K. Shiraishi, K. Naito, Increased expression of Leydig cell haem oxygenase-1 preserves spermatogenesis in varicocele, Hum. Reprod. 20 (2005) 2608–2613.
[53] H. Nishiyama, S. Danno, Y. Kaneko, K. Itoh, H. Yokoi, M. Fukumoto, et al., Decreased expression of cold-inducible RNA-binding protein (CIRP) in male germ cells at elevated temperature, Am. J. Pathol. 152 (1998) 289–296.
[54] F. Kilinc, S. Guvel, F. Kayaselcuk, C. Aygun, T. Egilmez, H. Ozkardes, p53 expression and apoptosis in varicocele in the rat testis, J. Urol. 172 (2004) 2475–2478.
[55] I.Y. Chang, J.H. Kim, K.H. Park, S.P. Yoon, Experimental varicocele induces p53-dependent germ cell apoptosis through activation of γ-H2AX, Urol. Int. 85 (2010) 216–220.
[56] E. Radi, P. Formichi, C. Battisti, A. Federico, Apoptosis and oxidative stress in neurodegenerative diseases, J. Alzheimers Dis. 42 (Suppl. 3) (2014) S125–S152.
[57] K. Sinha, J. Das, P.B. Pal, P.C. Sil, Oxidative stress: the mitochondria-dependent and mitochondria-independent pathways of apoptosis, Arch. Toxicol. 87 (2013) 1157–1180.
[58] A.P. Sinha Hikim, 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 (2003) 175–182.
[59] A. Ferlin, E. Speltra, C. Patassini, M.A. Pati, A. Garolla, N. Caretta, C. Foresta, Heat shock protein and heat shock factor expression in sperm: relation to oligozoospermia and varicocele, J. Urol. 183 (2010) 1248–1252.
[60] S. Ogi, N. Tanji, T. Iseda, M. Yokoyama, Expression of heat shock proteins in developing and degenerating rat testes, Arch. Androl. 43 (1999) 163–171.
[61] C.C. Chan, G.H. Sun, H.A. Shui, G.J. Wu, Differential spermatozoal protein expression profiles in men with varicocele compared to control subjects: upregulation of heat shock proteins 70 and 90 in varicocele, Urology 81 (2013) 1379, e1-8.
[62] W. Widlak, N. Vydra, The role of heat shock factors in mammalian spermatogenesis, Adv. Anat. Embryol. Cell Biol. 222 (2017) 45–65.
[63] Z. Xia, X. Zheng, H. Zheng, X. Liu, Z. Yang, X. Wang, Cold-inducible RNA-binding protein (CIRP) regulates target mRNA stabilization in the mouse testis, FEBS Lett. 586 (2012) 3299–3308.
[64] L. Jankovic Velickovic, V. Stefanovic, Hypoxia and spermatogenesis, Int. Urol. Nephrol. 46 (2014) 887–894.
[65] Y. Gat, Z. Zukerman, J. Chakraborty, M. Gornish, Varicocele, hypoxia and male infertility. Fluid Mechanics analysis of the impaired testicular venous drainage system, Hum. Reprod. 20 (2005) 2614–2619.
[66] J.D. Lee, 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 (2006) 1045–1048.
[67] K.P. Nallella, S.S. Allamaneni, 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 (2004) 1010–1013.
[68] M. Roque, S.C. Esteves, Effect of varicocele repair on sperm DNA fragmentation: a review, Int. Urol. Nephrol. 50 (2018) 583–603.
[69] J.H. Jung, M.H. Kim, J. Kim, S.K. Baik, S.B. Koh, H.J. Park, J.T. Seo, Treatment of leukocytospermia in male infertility: a systematic review, World J. Mens Health 34 (2016) 165–172.
[70] P.N. Schlegel, T.S. Chang, F.F. Marshall, Antibiotics: potential hazards to male fertility, Fertil. Steril. 55 (1991) 235–242.
[71] R.K. Sharma, A.E. Pasqualotto, D.R. Nelson, A.J. Thomas Jr., A. Agarwal, Relationship between seminal white blood cell counts and oxidative stress in men treated at an infertility clinic, J. Androl. 22 (2001) 575–583.
[72] S. Pentyala, J. Lee, S. Annam, J. Alvarez, A. Veerraju, N. Yadlapalli, S.A. Khan, Current perspectives on pyospermia: a review, Asian J. Androl. 9 (2007) 593–600.
[73] A. Ayala, M.F. Muñoz, S. Argüelles, Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal, Oxid. Med. Cell. Longev. 2014 (2014) 1–31.
[74] A.M. Hassanin, H.H. Ahmed, A.N. Kaddah, A global view of the pathophysiology of varicocele, Andrology 6 (2018) 654–661.
[75] S. Benoff, 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 (2005) 622–634.
[76] S. Benoff, 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 (1997) 336–347.
[77] N. Ozawa, N. Goda, N. Makino, T. Yamaguchi, Y. Yoshimura, M. Suematsu, Leydig cell-derived heme oxygenase-1 regulates apoptosis of premeiotic germ cells in response to stress, J. Clin. Investig. 109 (2002) 457–467.
[78] I.T. Koksal, Y. Ishak, M. Usta, A. Danisman, E. Guntekin, I.C. Bassorgun, A. Ciftcioglu, Varicocele-induced testicular dysfunction may be associated with disruption of blood-testis barrier, Arch. Androl. 53 (2007) 43–48.
[79] M. Dym, D.W. Fawcett, The blood-testis barrier in the rat and the physiological compartmentation of the seminiferous epithelium, Biol. Reprod. 3 (1970) 308–326.
[80] J.C. Wu, C.W. Gregory, R.M. DePhilip, Expression of E-cadherin in immature rat and mouse testis and in rat Sertoli cell cultures, Biol. Reprod. 49 (1993) 1353–1361.
[81] A. Barbonetti, C. Castellini, S. D'Andrea, G. Cordeschi, R. Santucci, S. Francavilla, F. Francavilla, Prevalence of anti-sperm antibodies and relationship of degree of

sperm auto-immunization to semen parameters and post-coital test outcome: a retrospective analysis of over 10 000 men, Hum. Reprod. 34 (2019) 834–841.
[82] D. Cui, G. Han, Y. Shang, C. Liu, L. Xia, L. Li, S. Yi, Antisperm antibodies in infertile men and their effect on semen parameters: a systematic review and meta-analysis, Clin. Chim. Acta 444 (2015) 29–36.
[83] O. Telli, H. Sarici, M. Kabar, B.C. Ozgur, B. Resorlu, S. Bozkurt, Does varicocelectomy affect DNA fragmentation in infertile patients? Indian J. Urol. 31 (2015) 116–119.
[84] A. Agarwal, C.L. Cho, A. Majzoub, S.C. Esteves, The Society for Translational Medicine: clinical practice guidelines for sperm DNA fragmentation testing in male infertility, Transl. Androl. Urol. 6 (2017) S720–S733.
[85] D.E. Sawyer, S.D. Roman, R.J. Aitken, Relative susceptibilities of mitochondrial and nuclear DNA to damage induced by hydrogen peroxide in two mouse germ cell lines, Redox Rep. 6 (2001) 182–184.
[86] G.N. De Iuliis, L.K. Thomson, L.A. Mitchell, J.M. Finnie, A.J. Koppers, A. Hedges, B. Nixon, R.J. Aitken, DNA damage in human spermatozoa is highly correlated with the efficiency of chromatin remodeling and the formation of 8-hydroxy-2'-deoxyguanosine, a marker of oxidative stress, Biol. Reprod. 81 (2009) 517–524.
[87] K. Shiraishi, H. Takihara, H. Matsuyama, Elevated scrotal temperature, but not varicocele grade, reflects testicular oxidative stress-mediated apoptosis, World J. Urol. 28 (2010) 359–364.
[88] P.N. Schlegel, M. Goldstein, Alternate indications for varicocele repair: non-obstructive azoospermia, pain, androgen deficiency and progressive testicular dysfunction, Fertil. Steril. 96 (2011) 1288–1293.
[89] J.P. Mulhall, L.W. Trost, R.E. Brannigan, E.G. Kurtz, J.B. Redmon, K.A. Chiles, D. J. Lightner, M.M. Miner, M.H. Murad, C.J. Nelson, E.A. Platz, L.V. Ramanathan, R.W. Lewis, Evaluation and management of testosterone deficiency: AUA guideline, J. Urol. 200 (2018) 423–432.
[90] C. Tanrikut, M. Goldstein, J.S. Rosoff, R.K. Lee, C.J. Nelson, J.P. Mulhall, Varicocele as a risk factor for androgen deficiency and effect of repair, BJU Int. 108 (2011) 1480–1484.
[91] J. Steckel, A.P. Dicker, M. Goldstein, Relationship between varicocele size and response to varicocelectomy, J. Urol. 149 (1993) 769–771.
[92] R.P. Hayden, C. Tanrikut, Testosterone and varicocele, Urol. Clin. N. Am. 43 (2016) 223–232.
[93] W. Zohdy, S. Ghazi, M. Arafa, Impact of varicocelectomy on gonadal and erectile functions in men with hypogonadism and infertility, J. Sex. Med. 8 (2011) 885–893.
[94] F. Li, H. Yue, K. Yamaguchi, K. Okada, K. Matsushita, M. Ando, K. Chiba, M. Fujisawa, Effect of surgical repair on testosterone production in infertile men with varicocele: a meta-analysis, Int. J. Urol. 19 (2012) 149–154.
[95] J.J. Sirvent, R. Bernat, M.A. Navarro, J. Rodriguez Tolra, R. Guspi, R. Bosch, Leydig cell in idiopathic varicocele, Eur. Urol. 17 (1990) 257–261.
[96] S. Francavilla, B. Bruno, M. Martini, S. Moscardelli, G. Properzi, F. Francavilla, V. Santiemma, A. Fabbrini, Quantitative evaluation of Leydig cells in testicular biopsies of men with varicocele, Arch. Androl. 16 (1986) 111–117.
[97] D.B. Weiss, 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 (1978) 305–312.
[98] D.B. Weiss, L. Rodrigues-Rigau, K.D. Smith, E.H. Grotjan Jr., E. Steinberger, Leydig cell density and function and their relation to gonadotropins in infertile oligospermic men with varicocele, Isr. J. Med. Sci. 15 (1979) 556–563.
[99] J. Rajfer, T.T. Turner, F. Rivera, S.S. Howards, S.C. Sikka, Inhibition of testicular testosterone biosynthesis following experimental varicocele in rats, Biol. Reprod. 36 (1987) 933–937.
[100] A. Etriby, S.M. Girgis, H. Hefnawy, A.A. Ibrahim, Testicular changes in subfertile males with varicocele, Fertil. Steril. 18 (1967) 666–671.
[101] Y.X. Wang, C. Lei, S.G. Dong, A.C. Chandley, M. MacIntyre, T.B. Hargreave, Study of bilateral histology and meiotic analysis in men undergoing varicocele ligation, Fertil. Steril. 55 (1991) 152–155.
[102] H.A. Hienz, J. Voggenthaler, L. Weissbach, Histological findings in testes with varicocele during childhood and their therapeutic consequences, Eur. J. Pediatr. 133 (1980) 139–146.
[103] S. Paick, W.S. Choi, Varicocele and testicular pain: a review, World J. Mens Health 37 (2019) 4–11.
[104] A. Mehta, M. Goldstein, Microsurgical varicocelectomy: a review, Asian J. Androl. 15 (2013) 56–60.