International Urology and Nephrology (2018) 50:583–603
https://doi.org/10.1007/s11255-018-1839-4

UROLOGY - REVIEW

Effect of varicocele repair on sperm DNA fragmentation: a review

Matheus Roque[1] · **Sandro C. Esteves**[2,3,4]

Received: 12 February 2018 / Accepted: 27 February 2018 / Published online: 14 March 2018

Abstract
Varicocele, the leading cause of male infertility, can impair sperm quality and fertility via various oxidative stress mechanisms. An imbalance between excessive reactive oxygen species production and antioxidant protection causes alterations in nuclear and mitochondrial sperm DNA, thus rendering a subset of varicocele men less fertile. In particular, sperm DNA fragmentation is usually elevated in men with clinical varicocele in both abnormal and normal semen parameters by the current World Health Organization criteria. In this review, we discuss the evidence concerning the association between varicocele, oxidative stress, and SDF, and the possible mechanisms involved in infertility. Furthermore, we summarize the role of varicocele repair as a means of alleviating SDF and improving fertility. Lastly, we critically appraise the evidence-based algorithm recently issued by the Society for Translational Medicine aimed at guiding urologists on the use of SDF testing in men with varicocele seeking fertility. Current evidence based on careful review of published studies confirms the effectiveness of varicocelectomy as a means of both reducing oxidatively induced sperm DNA damage and potentially improving fertility. Varicocele repair should be offered as part of treatment option for male partners of infertile couples presenting with palpable varicoceles.

Keywords Male infertility · Oxidative stress · Sperm DNA damage · Sperm DNA fragmentation · Varicocele · Varicocele repair

Introduction

Varicocele, from Latin *varix* (dilated vein) and Greek *kele* (tumor), consists of an abnormal dilatation of the veins of pampiniform plexus. It is commonly seen in the general male population, affecting 15% of individuals at reproductive age, 35% of those with primary infertility, and up to 80% of men with secondary infertility [1–3].

The varicocele diagnosis is primarily based on physical examination alone or combined with imaging studies [4–6]. The Dubin's grading system is the most commonly used criteria to determine its presence and severity. The system categorizes varicoceles on a 1–3 scale, in which a grade 3 (large) varicocele is detected by visual inspection of the scrotum, whereas a grade 2 (moderate) varicocele is readily palpable. In contrast, grade 1 (small) varicoceles are those palpable with the aid of a Valsalva's maneuver [7]. Treatment is usually recommended for infertile men with varicoceles detected during physical examination (any grade) and abnormal semen [5]. The reason stems from the fact that semen parameters and chances of conception, both natural and assisted, are overall increased after varicocele repair in men with palpable (clinical) varicoceles, but not in those with subclinical varicoceles (i.e., solely detected by imaging studies) [7, 8].

The controversy concerning varicocele mainly stems from its unclear pathophysiology that would lead to infertility [5–9]. Furthermore, the reasons why most men with varicocele have no apparent fertility issues remain unclear [10]. Recent studies, however, have shed light on possible

✉ Sandro C. Esteves
s.esteves@androfert.com.br

1 ORIGEN, Center for Reproductive Medicine, Rio de Janeiro, Brazil

2 ANDROFERT, Andrology and Human Reproduction Clinic, Av. Dr. Heitor Penteado, 1464, Campinas, São Paulo 13075-460, Brazil

3 Division of Urology, Department of Surgery, Faculty of Medical Sciences, University of Campinas (UNICAMP), Campinas, Brazil

4 Faculty of Health, Aarhus University, 8000 Aarhus C, Denmark

pathways by showing that reactive oxygen species (ROS) and apoptosis markers are elevated in the semen of infertile men with varicocele [9–13].

An imbalance between ROS production and antioxidant protection leads to oxidative stress (OS), which causes damage to lipids, proteins, and nucleic acids in living sperm [14]. As a consequence, sperm motility and sperm–oocyte fusion are impaired. Moreover, OS can disrupt sperm chromatin structure by inducing breaks in the DNA strands [9, 15, 16], which has been shown to have a negative impact on embryo development and implantation [17–21].

In fact, the role of OS as a central element of varicocele-induced infertility and its association with sperm DNA breaks (so-called sperm DNA fragmentation [SDF]) have gained increased attention [9, 22, 23]. Urologists should be familiar with the evidence data linking varicocele-related infertility to OS and SDF as it has obvious implications for practice. In this review, we briefly discuss the current literature concerning the association between varicocele, oxidative stress, and SDF, and the possible mechanisms involved in infertility. Then, we examine in detail the role of varicocele repair as a means of alleviating SDF and improving fertility. Lastly, we critically appraise the evidence-based algorithm recently issued by the Society for Translational Medicine aimed at guiding urologists on the use of SDF testing in men with varicocele seeking fertility.

Varicocele and oxidative stress

Despite the current debate about varicocele pathophysiology, evidence concerning the role of OS and DNA fragmentation on varicocele-related infertility is increasing steadily. Small quantities of ROS play essential roles in sperm function as ROS are involved in sperm capacitation, acrosome reaction, hyperactivation, and the sperm–oocyte fusion [24]. In contrast, a disproportionate increase in ROS usually leads to OS [9]. The imbalance between ROS production and antioxidant protection causes alterations in nuclear and mitochondrial sperm DNA, including base modification, strand breaks, and chromatin cross-links, and is associated with apoptosis-like processes that affect sperm maturation and nuclear protamination [13–23].

Studies comparing the seminal levels of OS markers among fertile men with and without varicocele have shown increased OS in varicocele men [16, 25, 26]. Likewise, infertile men with varicocele exhibit elevated OS markers. Among these, ROS, nitric oxide, and lipid peroxidation products are common findings [27–29], thus indicating that the presence of a varicocele exacerbates the generation of OS [10]. Along the same lines, infertile men with varicocele have diminished seminal antioxidant capacity when compared to their fertile counterparts [12, 25, 29–31]. Notably, an association between varicocele grade and OS seems to exist, as larger varicoceles are associated with higher levels of seminal OS than smaller ones [31–38].

In varicocele, ROS and nitrogen species are released in the endothelial cells of the dilated pampiniform plexus, testicular cells (germ cells, Leydig cells, macrophages, and peritubular cells), and principal cells of the epididymis [9, 39, 40]. In such condition, excessive ROS negatively affect the sperm membrane and chromatin by causing lipid peroxidation and inducing DNA breaks, respectively [13, 16, 41].

Despite the fact that the mechanisms by which varicocele increases ROS and/or decreases antioxidant capacity are not fully elucidated, the central theory is that ROS generation is related to scrotal hyperthermia, testicular hypoxia, reflux of adrenal/renal metabolites, cadmium accumulation, and epididymal response, as discussed below. Yet, it is still unknown by which mechanisms infertility is prevented in fertile varicocele men. It has been speculated that intrinsic factors either protecting an individual from the deleterious effect of varicocele or exacerbating the harmful effects of oxidation on germ cells modulate the fertility status of men with varicocele [9]. For instance, antioxidant enzymes, such as catalase, superoxide dismutase, vitamin C, and glutathione peroxidase, counteract ROS [9]. In the fertile varicocele population, the equilibrium between oxidants and antioxidants might be more efficient in counteracting the increased ROS levels. Furthermore, other protective mechanisms might exist, including a slowed rate of germ cell apoptosis, enhanced turnover machinery for the oxidized proteins to prevent their aggregation, and reduced cellular signal-transducing effects of ROS [10]. While the disruption of these protective antioxidants can result in OS, it is still unknown which mechanisms exert major protective roles.

Heat stress and SDF

The reflux of abdominal blood through incompetent valves of the internal spermatic and cremasteric veins into the pampiniform plexus leads to scrotal hyperthermia. This change in testicular thermostasis goes against the optimal temperature for spermatogenesis, which is 2.5 °C lower than the body's temperature. Scrotal hyperthermia is the most widely accepted hypothesis to explain OS in varicocele [9, 42, 43]. Heat stress is associated with increased ROS production by cell mitochondria, plasma membrane, cytoplasm, and peroxisomes. Cell damage resulting from hyperthermia occurs in different grades in the various cell compartments [13]. In the testes, spermatogonia B and the developing spermatozoa are highly vulnerable to heat stress. On the contrary, spermatogonia A, as well as Leydig and Sertoli cells, are thermo-resistant [9].

Testicular hypoxia

Infertile men with varicocele can present with signs of ischemia due to the stagnation of blood on the microcirculatory vessels [44]. Arteriolar occlusion by microthrombi, germ cell degeneration, Leydig cell atrophy, and fibrotic thickening of the basement membranes of seminiferous tubules have been observed in testicular biopsy specimens [45]. It seems that ischemia occurs in varicocele patients when the venous hydrostatic pressure of internal testicular vein exceeds the testicular arteriolar pressure [45, 46]. ROS are produced by various sources during this hypoxic state, including activation of hypoxia-inducible factor 1 (HIF-1), mitochondrial dysfunction, xanthine dehydrogenase/oxidase, membrane-associated NAPDH oxidase 5 (NOX5), and phospholipase A2 [9]. Moreover, hypoxia can lead to increases in the expression of leptin and cytokines in testicular tissue, including interleukin (IL)-1 and IL-6, which can induce ROS generation [34, 47–49].

Reflux of adrenal/renal metabolites and cadmium accumulation

The retrograde blood flow through the left testicular vein with adrenal prostaglandins and renal and adrenal metabolites can induce cellular OS [50]. Norepinephrine also contributes to vasospasm and aggravate hypoxia, thus generating more ROS [9]. Cadmium is a natural metal that has been identified in elevated levels in the wall of the internal spermatic veins, testicular tissue biopsy specimens, and the seminal fluid of patients with varicocele [51–53]. It is hypothesized that increased hydrostatic pressure and hypoxia might result in a porous blood–testis barrier that enables cadmium to build up [53]; however, it is still unclear how cadmium affects fertility.

Epididymis dysfunction

Experimental varicoceles have been used to study the epididymal structural and functional changes [9]. In the epididymis, there are three important sources of ROS, namely the luminal fluid from the testis, the endothelial cells layering the rich capillary network around the caput, and the metabolically active principal cells [9]. The initial epididymal segment seems to be the primary site of ROS accumulation. However, cells capable of generating enzymatic and nonenzymatic antioxidants seem to exist in all epididymal sections. Hypoxia and heat stress are the likely triggers underlying the imbalance between ROS and antioxidant defenses in the epididymis. Under these stressful conditions, the principal cells can generate excessive ROS that combined with the impaired production of antioxidants result in oxidative damage to the maturing sperm and epididymal cells [13].

Varicocele and sperm DNA fragmentation

Sperm DNA integrity is critical to the development of a healthy embryo [54, 55]. Damage to sperm DNA is a complex process involving multiple, non-mutually exclusive, causative mechanisms that generate a variety of insults to DNA [39]. Among DNA lesions, two main types are of utmost clinical importance: single-strand DNA breaks (SS-DB) and double-strand DNA breaks (DS-DB) [55]. SDF usually refers to either SS-DB or DS-DB, or both, and is more common in infertile men than in fertile counterparts. Several etiological factors have been implicated in the impairment of sperm DNA content, including varicocele [17, 43, 56, 57].

A variety of assays have been developed to measure the proportion of sperm with SDF [42, 57]. Probes or dyes are used to identify the existence of DNA breaks in specimens examined by fluorescence and optical microscopy or flow cytometry [12, 17, 56, 58, 59]. The sperm chromatin structure assay (SCSA), terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), sperm chromatin dispersion test (SCD), and single gel electrophoresis (Comet) are the most commonly used methods to measure SDF [60, 61].

Men with high levels of SDF in semen have difficulties to impregnate their partners, both naturally and assisted [62]. Among those establishing a pregnancy unassisted, the time-to-pregnancy is longer in couples whose male partners have high SDF [63]. SDF has also been associated with poor intrauterine insemination and assisted reproductive technology (ART) outcomes [64]. Although sperm with fragmented DNA may fertilize an egg with apparently similar efficiency as sperm without DNA fragmentation [65, 66], the negative impact of a damaged paternal chromatin to the integrity of embryonic genome is usually observed after implantation [67] and is often manifested by early pregnancy loss [19, 20]. However, massive SDF can also promote embryonic arrest [17, 18]. It has also been speculated that SDF might lead to a higher risk of congenital disabilities in the offspring [20, 68].

In men with varicocele, SDF is probably one of the critical consequences of OS via ROS as depicted in Fig. 1. This fact is supported by the usual observation of a concomitant impairment in sperm DNA integrity and altered oxidative stress markers in such men [13]. In the sections below, we discuss the clinical evidence of the OS-induced SDF in men with varicocele and the effect of interventions.

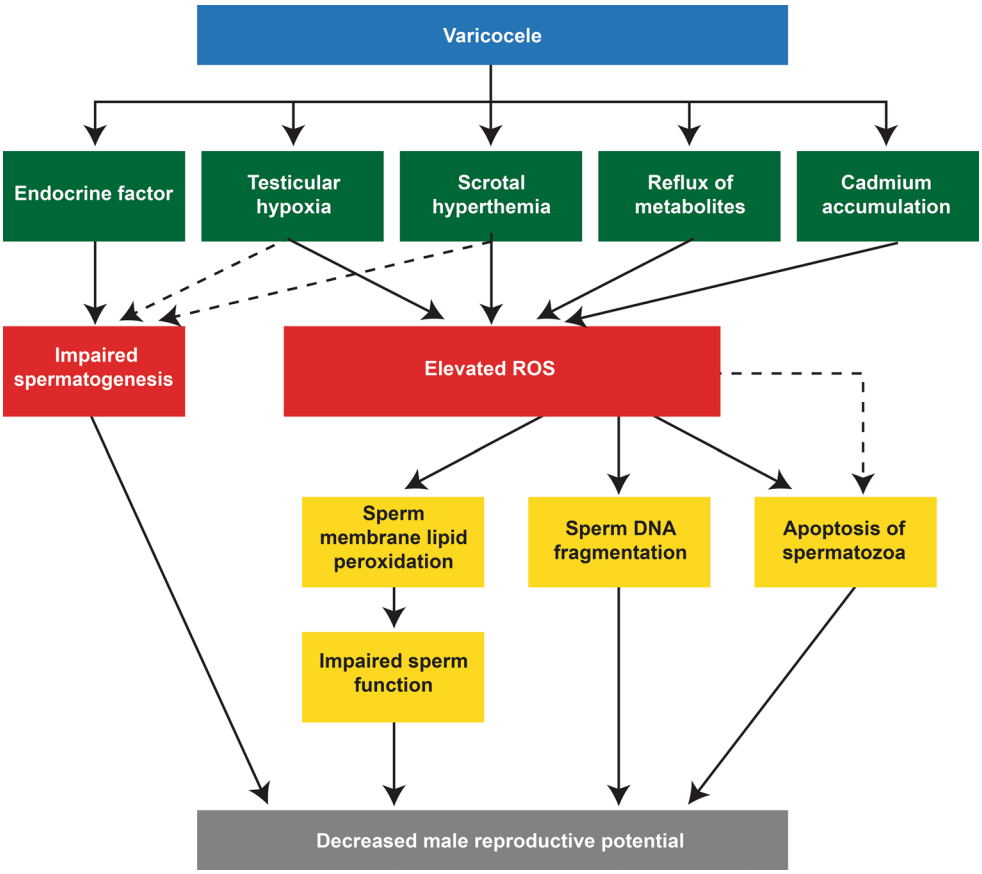

Fig. 1 Pathophysiology of varicocele and its association with reactive oxygen species and sperm DNA fragmentation (solid lines and dotted lines indicate direct and indirect effects, respectively). Reprint from Cho et al. Novel insights into the pathophysiology of varicocele and its association with reactive oxygen species and sperm DNA fragmentation. Asian J Androl. 2016 Mar–Apr; 18(2):186–193, under the terms of the Creative Commons Attribution-Non-Commercial-ShareAlike License, which permits non-commercial use, distribution and reproduction in any medium, provided the original work is properly cited

Clinical evidence of the association between varicocele and SDF

Following the confirmation of a consistent association between conventional semen parameters and varicocele, the majority of recent studies addressing varicocele and sperm quality have focused on sperm function markers and genetic defects. These range from markers of oxidative stress, mitochondrial activity, chromatin compaction, DNA methylation, and DNA fragmentation (reviewed by Agarwal et al. [69]).

As for SDF, infertile men with varicocele often present with elevated DNA damage in semen. In an early study involving 55 infertile men with clinical varicocele and 25 normozoospermic donors, elevated SDF (defined as the mean of the control group plus 2 SD) was seen in 49% varicocele patients with normal semen profile and 58% of those with abnormal semen parameters [70]. In another study involving 593 men with various etiologies attending infertility clinics, including a control group of semen donors, SDF rates (by SCD) were highest in both varicocele patients (35.7 ± 18.3%) and those with subclinical genital infection (41.7 ± 17.6%) [22]. Notably, two distinct sperm populations with fragmented DNA were identified, namely standard DNA fragmentation and degraded DNA fragmentation (DDS). Spermatozoa with standard fragmented DNA exhibited either the absence or presence of a small halo of chromatin dispersion around a compact nucleoid, whereas spermatozoa with degraded DNA showed a ghost-like morphology owing to massive SS-DB and DS-DB, as well as nuclear protein damage. In the study mentioned above, the proportion of sperm with degraded DNA was eightfold higher in varicocele patients than donors. Moreover, although the presence of sperm with degraded DNA was not pathognomonic of varicocele, it was possible to identify varicocele patients by computing the index of sperm with degraded DNA with 94% accuracy [22].

The observations mentioned above were corroborated by two systematic reviews. In one report, Zini and Dohle assessed 16 case–control studies evaluating SDF in fertile and infertile men with and without varicocele [12]. The authors found that in four out of nine studies, SDF rates were overall higher in infertile men with varicocele than infertile counterparts without varicocele. Moreover, the group of patients with varicocele had poorer seminal parameters than the group of infertile patients without varicocele. The remaining seven studies specifically included fertile men with varicocele. In six studies, SDF rates were higher in men with varicocele (and no history of infertility) than in fertile men or sperm donors without varicocele [12]. Another systematic review followed by meta-analysis compiled the data

from seven studies including 240 patients with varicocele and 176 normal healthy controls without varicocele [71]. In this study, SDF was higher in varicocele men than controls without varicocele (mean difference: 9.84%; 95% CI 9.19–10.49, $P < 0.00001$).

In fact, confirmatory data concerning the association between varicocele and elevated SDF have increased steadily [72, 73]. Furthermore, it has been shown that other essential markers of sperm function, including epididymal neutral α-glucosidase and sperm PLCζ levels, are also reduced in men with high SDF and varicocele [72]. Despite that, the impact of varicocele grade on SDF levels remains poorly studied as does the effect of subclinical varicocele on sperm DNA integrity.

Collectively, objective evidence indicates that SDF is overall increased in men with palpable varicocele, particularly in those with abnormal semen parameters, and that such increase is usually accompanied by alterations in markers of oxidative stress and sperm function.

Effect of varicocele treatment on SDF

Surgical repair has been used as the treatment for infertile men with varicocele for over a century [74]. Indeed, such intervention has been associated with significant improvements to various biomarkers of male infertility, such as semen parameters and pregnancy rates [8, 75–78]. Recently, varicocele repair has been used as an attempt to alleviate oxidatively induced SDF and protect against the progressive nature of varicocele and its consequent upregulation of systemic OS [10].

In fact, over 20 studies accounting for more than 1200 treated subjects were published in the last 12 years addressing the effect of varicocelectomy on SDF (Tables 1, 2, 3) [12, 29, 79–97]. The overwhelming majority of studies included men with clinical varicocele and abnormal semen parameters according to the WHO criteria. Despite using different SDF assays, heterogeneous design, and variable sample size, all studies reported a significant decrease in SDF rates after varicocele repair in a follow-up period ranging from 3 to 12 months (Tables 1, 2, 3). Yet, the exact percentage of men who benefit from surgery concerning SDF remains poorly reported. In a retrospective small cohort study including 37 men, Moskovtsev et al. reported improvements in SDF rates in 78% of the treated patients [81]. In another report, Werthman et al. studied 11 men with clinical varicocele and observed that 90% of the patients showed a significant decrease in the rates of SDF 3–6 months after varicocelectomy [80] (Table 1).

Of the few studies providing pregnancy outcomes, postoperative SDF rates were overall lower in men from couples who achieved pregnancy success than those who did not. In one report, Smit et al. prospectively evaluated 49 men with clinical varicocele, oligozoospermia, and at least 1-year infertility duration subjected to varicocelectomy. These authors observed improvements in SDF rates 3 months after varicocelectomy (preoperative 35.2 ± 13.1%; postoperative 30.2 ± 14.7%, $P = 0.019$; SCSA). In their study, couples that conceived naturally or with ART exhibited lower postoperative SDF levels (26.6 ± 13.7%) than those who did not (37.3 ± 13.9%, $P = 0.013$) [82]. In another study, Ni et al. [93] evaluated 42 subfertile patients with clinical varicocele grades 2 and 3 and altered seminal parameters subjected to microsurgical varicocelectomy. SDF was measured by SCSA, and the preoperative results were compared to a control group of semen donors. The SDF levels were significantly higher preoperatively in the patient group than in the control group. After 3–6 months postoperatively, SDF decreased overall (preoperative: 28.4%; postoperative: 22.4%; $P = 0.018$), despite remaining higher than controls. Notably, SDF levels in patients who achieved pregnancy naturally after varicocele repair (20.6 ± 3.5%) were not significantly different than controls (11.5 ± 3.9%), but were lower than both preoperative values (27.4 ± 6.3%; $P < 0.01$) and non-pregnant patients (24.7 ± 6.5%; $P < 0.010$) [93]. Recently, Mohammed et al. prospectively evaluated 75 infertile men with clinical varicocele and abnormal semen parameters and found that couples with positive pregnancy outcome at 1-year follow-up had had significantly lower DFI (16.4 ± 6.4%) than those who did not (24.2 ± 4.1%, $P = 0.04$) [94]. Notwithstanding, contrary results were reported by Baker et al. who retrospectively evaluated data from a small group of 24 infertile men with clinical varicocele who underwent microsurgical varicocele repair and had pre- and postoperative SDF results [89]. The authors observed that despite a significant decrease in SDF rates from a preoperative mean of 40.8% to a postoperative mean of 24.5% ($P = 0.001$), DFI results in pregnant and non-pregnant couples did not differ (22.2 ± 14.4 vs. 25.7 ± 14.5%, respectively).

Several studies evaluating the impact of varicocelectomy on SDF also assessed oxidative stress markers, sperm chromatin compaction, or other advanced sperm function characteristics. Decreases in such markers were noticeable in most studies, thus underscoring the association among varicocele, OS, and SDF (Tables 2, 3) [87–97]. Yet, although these studies unequivocally reported significant reductions in SDF after varicocelectomy, some studies have failed to demonstrate reduction in OS markers after surgery [87, 89], rendering it unclear as to why not all men with signs of OS improve after varicocele repair.

The published literature on varicocelectomy and SDF contains a few controlled studies, comprised of either healthy fertile men with normal semen parameters (WHO criteria) and without varicocele, infertile men with clinical

Table 1 Studies evaluating the effect of varicocelectomy on sperm DNA fragmentation, not including controls or concomitant assessment of oxidative stress markers

Study	Design	Patients	SDF assay	Surgical technique	Main results
Zini et al. [79]	Retrospective cohort	37 men (mean age 37.5 years) with clinical varicocele subjected to varicocelectomy (left: 26 patients; bilateral: 11 patients)	SCSA	Microsurgical subinguinal	Mean (±SE) SDF rates before and after microsurgical varicocelectomy (6-month interval) were 27.7 ± 2.9% versus 24.6 ± 2.7% ($P = 0.04$)
Werthman et al. [80]	Retrospective cohort	11 men with clinical varicocele (8 left: 8 patients; bilateral: 3 patients) and high levels of SDF (DFI > 27%) subjected to varicocelectomy	SCSA	Microsurgical subinguinal	90% of the patients showed a significant decrease in the rates of SDF 3–6 months after varicocelectomy The average percent change was 24% DNA fragmentation index. Seven of the eleven patients showed decreases in DFI to normal levels
Moskovtsev et al. [81]	Retrospective cohort	37 patients with clinical varicocele treated with oral antioxidants only and 9 men subjected to varicocelectomy and given oral antioxidants	SCSA	Microsurgical subinguinal	Improvements in SDF were observed in 78% of the patients subjected to the combination of varicocelectomy and antioxidants (pre: 44.7 ± 12.8%; post: 28.4 ± 9.5% ($P < 0.03$) Patients treated with antioxidants alone showed no improvement in SDF rates (pre: 45.3 ± 13.7%; post: 42.5 ± 16.7%)
Smit et al. [82]	Prospective cohort	49 men (mean age: 34 years) with clinical varicocele and oligozoospermia subjected to varicocelectomy	SCSA	High inguinal ligation ($N = 36$) and microsurgical ($N = 8$)	Improvements in SDF were observed in the treated subjects (pre: 35.2%; post: 30.2%; $P = 0.019$) The mean postoperative DNA fragmentation index was significantly higher (37.5 ± 13.3%) in couples who did not conceive naturally or with assisted reproductive technique (36.9 ± 15.6%) than those who did (30.1 ± 12.2% and 21.3 ± 14.7%, respectively) ($P = 0.033$). After varicocelectomy, 37% of the couples conceived naturally and 24% achieved pregnancy with ART
Zini et al. [12]	Prospective cohort	25 men with clinical varicocele and abnormal semen parameters subjected to varicocelectomy	SCSA	Microsurgical subinguinal	Improvements in SDF were observed in the treated subjects (pre: 18 ± 11%; post (4 months): 10 ± 5% ($N = 25$ subjects; $P = 0.0009$); post (6 months): 7 ± 3%; ($N = 19$ subjects; $P = 0.0021$)
Kadioglu et al. [83]	Retrospective cohort	92 infertile men (mean age: 33.8 years) with clinical varicocele (left and altered semen analysis subjected to varicocelectomy	TUNEL	Microsurgical subinguinal	The DFI decreased from 42.6% to 20.5% 6 months after surgery ($P < 0.001$) Higher preoperative DFI was associated with a larger decrease in postoperative DFI

Table 1 (continued)

Study	Design	Patients	SDF assay	Surgical technique	Main results
Telli et al. [84]	Prospective cohort	72 infertile men (mean age: 29.3 years) with clinical varicocele (unilateral: 66; bilateral: 6 patients) and oligozoospermia subjected to varicocelectomy Left sided grade 1, grade 2, and grade 3 varicocele were found in 19, 26, and 21 patients	Acridine orange assay using fluorescence microscopy	Inguinal macroscopic	The mean DFI was 34.5 ± 3.3% and 28.2 ± 3.5% before and after varicocelectomy ($P = 0.024$) with a follow-up of 6.2 ± 2.4 months
Su et al. [85]	Randomized controlled trial	358 infertile men with left clinical and right subclinical varicocele subjected to either unilateral left varicocele repair ($n = 179$) or bilateral varicocele repair ($n = 179$) Mean age 32.1 ± 6.1 years in the bilateral group and 31.8 ± 5.6 years in the unilateral group	SCSA	Subinguinal microsurgical	DFI was significantly reduced in both varicocelectomy groups at 1 year follow-up (unilateral: 21.6 ± 7.1% preop. versus 11.8 ± 6.0% postop.; Bilateral: 23.0 ± 8.1% preop. versus 12.1 ± 6.8% postop. (P value not specified) No differences were observed in preoperative and postoperative DFI results between groups, despite greater postoperative improvements in sperm count, motility and morphology in the bilateral group than unilateral group
Zaazaa et al. [86]	Randomized controlled trial	80 infertile men with clinical varicocele (grades 2 or 3) and DFI > 30% subjected to (i) varicocelectomy and (ii) varicocelectomy followed with 1 mg ketotifen twice daily for 3 months, and 40 men with clinical varicocele (grades 2 or 3) and DFI > 30% treated with oral ketotifen (mast cell stabilizer) 1 g twice daily for 3 months	SCD	Subinguinal microsurgical	Sperm DFI was improved compared with the pre-treatment data in all groups 3 months after surgery ($P < 0.05$): (i) varicocelectomy (34.6 vs. 28.3%), (ii) mast cell (MC) stabilizer only (33.4 vs. 27.8%), and (iii) varicocelectomy followed by MC stabilizer (34.3 vs. 25.1%) DFI improvement percentages showed the highest improvement in men subjected to varicocelectomy followed by MC stabilizer (26.8%) compared with varicocelectomy alone (18.2%; $P = 0.04$) and MC stabilizer alone (16.8%; $P = 0.02$) Sperm DFI improvement percentages showed significant increases ($P < 0.05$) in the infertile patients with varicocele grade 3 (19.7, 18.5, 27.1%) compared to varicocele grade 2 (13.1, 16.4, 25.5%) in varicocelectomy alone, MC stabilizer alone, and varicocele repair + MC stabilizer groups, respectively

ART assisted reproductive technology, *DFI* DNA fragmentation index, *MC* mast cell, *SCSA* sperm chromatin structure assay, *SCD* sperm chromatin dispersion assay, *SDF* sperm DNA fragmentation

or subclinical varicocele and normal semen parameters, or infertile men without varicocele. Despite limitations concerning confounding factors and design, SDF is shown to be significantly higher in varicocele patients than controls [88, 92–97] (Tables 2 and 3). Of the controlled studies assessing OS markers and other sperm functional characteristics, the overwhelming majority report higher levels of oxidative stress, DNA decondensation, and SDF in infertile men with clinical varicocele than healthy fertile counterparts without varicocele and men with subclinical varicocele (Table 3). Notably, such markers seem to be elevated in both patients with normal and abnormal semen parameters according to the WHO criteria.

In contrast, repair of subclinical varicoceles concerning SDF does not seem beneficial, but the evidence is based on a single study [60]. In this report, Garcia-Peiró et al. evaluated 60 infertile patients with varicocele using several SDF methods (TUNEL, SCD, and SCSA). While SDF rates decreased after repairing clinical varicoceles, there were no improvements in SDF rates in infertile patients with subclinical varicocele subjected to surgery [98].

As for the role of other treatment modalities for decreasing SDF in varicocele-related infertility, the published literature is very scarce. In a small cohort non-controlled study, 20 patients with grade 1 varicocele were treated with oral antioxidants (1500 mg L-Carnitine, 60 mg vitamin C, 20 mg coenzyme Q10, 10 mg vitamin E, 200 µg vitamin B9, 1 µg vitamin B12, 10 mg zinc, 50 µg selenium) daily for 3 months [99]. The relative reductions in SDF and the percentage of highly degraded sperm cells—assessed by the SCD assay—were 22.1% ($P=0.02$) and 31.3% ($P=0.07$), suggesting a possible role for oral antioxidants in men with clinical varicocele and SDF. In a recent prospective trial involving 80 infertile men with clinical varicocele (grades 2 or 3) and DFI > 30%, the patients were randomized to (1) microsurgical subinguinal varicocelectomy, (2) varicocelectomy followed with 1 mg ketotifen (mast cell stabilizer) twice daily for 3 months, and (3) oral ketotifen 1 g twice daily for 3 months [86]. The percent improvement in sperm DFI after treatment was significant ($P<0.05$) but not different between varicocelectomy alone versus oral therapy, whereas the highest percent improvement was seen with the combination of surgery and medication (Table 1). Despite these results, the evidence is too limited to draw any definite conclusions about the potential role of antioxidants as a treatment for SDF-infertility in men with clinical varicocele.

In conclusion, the existing evidence is reassuring as to the effectiveness of varicocele surgical repair as a means of alleviating oxidatively induced sperm DNA damage. Given the current observations, urologists should advise male partners of infertile couples presenting with palpable varicoceles of the connection with SDF and oxidative stress, and discuss varicocele repair as a way of both decreasing SDF and potentially improving fertility.

Clinical practice guidelines on SDF testing in varicocele patients

While the essential role of sperm DNA integrity in human reproduction has been extensively studied, the clinical indication of SDF testing is less clear. In the context of varicocele, current guidelines issued by major professional societies recommend varicocelectomy to be considered in infertile men with clinical varicocele and abnormal semen analysis [100, 101]. These guidelines also recommend that varicocele treatment should not be offered to patients with normal semen quality. However, it is well-established that conventional semen analysis alone is not enough to assess semen quality [102–104]. Reference ranges and interpretation vary according to the World Health Organization (WHO) edition utilized for the examination of human semen [14]. Moreover, a routine semen analysis cannot identify abnormalities affecting the sperm chromatin. Assessment of sperm DNA integrity in the context of varicocele has been proposed as complementary to conventional semen analysis. Indeed, SDF can be present even in men with semen parameters within normal ranges as per the WHO criteria [56].

A 2017 clinical practice guidelines (CPG) issued by the Society for Translational Medicine (STM) provides evidence-based recommendations for SDF testing in male infertility scenarios, including varicocele [105]. The guidelines recommend testing to patients with varicocele grades 2 or 3 with normal conventional semen parameters as per the WHO criteria. SDF testing was also prescribed to patients with grade 1 varicocele with borderline/abnormal traditional semen parameter results (Table 4). The reasoning of these recommendations relies on the previously discussed association between the presence of palpable varicoceles and increased SDF, and the overall positive effect of varicocelectomy on sperm DNA damage. Notably, the CPG mentioned above propose the utilization of SDF testing results for clinical decision-making in varicocele clinical scenarios in which treatment is not warranted by itself (Fig. 2). It has been postulated that identification of the affected individuals might allow urologists to better select varicocele candidates for early surgical interventions and potentially halt further deterioration of semen and fertility [57]. Moreover, SDF test results can be used to monitor the effectiveness of varicocelectomy [10].

Interestingly, results of a 2017 cross-sectional questionnaire-based survey involving 65 participants with expertise in male infertility, mostly urologists, indicated that while SDF testing is commonly utilized (61.2%) in infertile men with high-grade varicocele and "normal" semen parameters

Table 2 Studies evaluating the effect of varicocelectomy on sperm DNA fragmentation, including controls or concomitant assessment of oxidative stress/sperm function markers

Study	Design	Patients	Controls	SDF assay	Oxidative stress and/or other sperm function markers	Surgical technique	Main results
Lacerda et al. [87]	Prospective cohort	21 adolescents between 15 and 19-year-old with grades 2 or 3 varicocele subjected to varicocelectomy	NA	Comet	Determination of mitochondrial activity and thiobarbituric acid-reactive substances (TBARS) levels	Microsurgical subinguinal	Comet class I cells (undamaged DNA) increased after varicocelectomy (49.6 ± 23.1% to 64.5 ± 13.6%; $P = 0.011$) Percentage of sperm with mostly inactive mitochondria (diaminobenzidine [DAB] class III) decreased after varicocelectomy (20.2 ± 4.9–17.1 ± 3.2; $P = 0.013$). The TBARS levels remained unaltered
Li et al. [88]	Not specified	19 infertile men (mean age: 33.1 years) with clinical varicocele (left: 19 patients; bilateral: 2 patients) subjected to varicocelectomy	19 normozoospermic controls	SCSA	Not assessed	Microsurgical subinguinal	SDF was higher in men with varicocele (28.4 ± 15.6%) than controls (DFI: 17.4 ± 5.3%; $P = 0.007$) DFI decreased from 28.4 ± 15.6% before surgery to 22.4 ± 12.9% 3 months postoperatively ($P = 0.018$) and postoperative DFI in varicocele patients was similar to controls

Table 2 (continued)

Study	Design	Patients	Controls	SDF assay	Oxidative stress and/or other sperm function markers	Surgical technique	Main results
Baker et al. [89]	Retrospective cohort	22 men with clinical varicocele subjected to varicocelectomy	NA	TUNEL	Measurement of ROS and TAC levels	Microsurgical subinguinal	DFI decreased from a preoperative mean of 40.8% to a postoperative mean of 24.5% (mean % change: − 16.2; 95% CI − 7.3 to − 25.2; $P = 0.001$. A higher preoperative DFI was associated with a larger decrease in postoperative DFI ($r2 = 0.53$; $P = 0.01$) DFI results in pregnant and non-pregnant couples did not differ (22.2 ± 14.4 vs. 25.7 ± 14.5%, respectively) The mean TAC decreased from 2292 µM preoperatively to 1885 uM postoperatively ($P = 0.03$) and the percentage of patients with a TAC above the normal value (1420 µM) decreased from 86% preoperatively to 71% postoperatively ;however, postoperative TAC remained above the normal reference value for the majority of subjects. There was no statistically significant change in ROS levels after surgery

Table 2 (continued)

Study	Design	Patients	Controls	SDF assay	Oxidative stress and/or other sperm function markers	Surgical technique	Main results
Pourmand et al. [90]	Randomized controlled trial	100 infertile men with clinical left varicocele ($N=78$) or subclinical ($N=22$)) varicocele subjected to varicocelectomy alone (group 1) or varicocelectomy plus 750-mg L-carnitine orally daily for 6 months (group 2)	NA	TUNEL	Protamine damage	Not specified	There were improvements in DFI from before to 6 months after surgery in both groups (group 1: 14.0% vs. 9.5%, $P=0.02$; group 2: 13.9 vs. 8.5%, $P<0.001$), but results were not different between groups There was a significant improvement in protamine damage from before to 6 months after surgery in group 2 only (44.9 vs. 33.7%, $P<0.001$)
Tavalaee et al. [91]	Not specified	23 infertile men (mean age: 31.3 years) with grades 2 or 3 left varicocele subjected to varicocelectomy	NA	TUNEL	Protamine deficiency (chromomycin A3), oxidative stress (DCFH-DA staining), and global DNA methylation (immunostaining)	Not specified	%DFI (15.9 ± 1.2% preop. vs. 10.8 ± 1.1% postop., $P<0.001$), % sperm with protamine deficiency (46.7 ± 2.6% preop. vs. 39.4 ± 2.6% postop., $P=0.02$), and %sperm with OS (47.6 ± 6.6% preop. vs. 36.6 ± 3.8% postop., $P=0.03$) improved 3 months after surgery Percentage of sperm exhibiting global DNA methylation and intensity of DNA methylation also improved after surgery, although the differences were not significant – except in the group of oligozoospermic patients ($P=0.03$) when compared with preoperative results

DCFH-DA: 2′, 7′-dichlorodihydrofluorescein diacetate; DFI: DNA fragmentation index; MDA: malondialdehyde; NA: not applicable; NR: not reported; OS: oxidative stress; ROS: reactive oxygen species; SCSA: sperm chromatin structure assay; SCD: sperm chromatin dispersion assay; SDF: sperm DNA fragmentation; TAC: total antioxidant capacity

Table 3 Studies evaluating the effect of varicocelectomy on sperm DNA fragmentation, including both controls and concomitant assessment of oxidative stress/sperm function markers

Study	Design	Patients	Controls	SDF assay	Oxidative stress and/or other sperm function markers	Surgical technique	Main results
Sakamoto et al. [29]	Retrospective cohort	30 infertile men with grades 2 or 3 varicocele (15 oligozoospermic and 15 normozoospermic) subjected to varicocele repair	15 age-matched healthy controls without varicocele and with normal semen characteristics 15 oligozoospermic infertile men without varicocele	TUNEL	Nitric oxide (NO), 8-hydroxy-2′-deoxyguanosine (8-OHdG), hexanoyl-lysine (HEL), superoxide dismutase (SOD) activity, interleukin (IL)-6, IL-8 and tumor necrosis factor-alpha in seminal plasma	Microsurgical subinguinal	The percentage of TUNEL-positive sperm 6 months after surgery was significant lower than before (postop: 27.5 ± 19.4%; preop: 79.6 ± 13.6%; $P < 0.001$). TUNEL results of controls not provided; Seminal plasma NO concentration and SOD activity of normozoospermic patients with varicocele were significantly higher than that of controls ($P < 0.05$). In oligozoospermic patients, the NO. IL-6, and HEL levels in seminal plasma in men with varicocele were significantly higher than in those without There was a significant reduction in the level of NO, HEL, 8-OHdG and SOD activity after surgery
La Vignera et al. [92]	Not specified	30 Men (mean age: 26.5 years) with oligoasthenoteratozoospermia and grade 3 left varicocele subjected to varicocelectomy	30 normozoospermic controls without varicocele	TUNEL	Mitochondrial membrane potential (MMP), phosphatidylserine externalization (Annexin V/PI assay), and chromatin compactness	Microsurgical subinguinal	SDF rates significantly decreased after surgery (4 months) from 5.0 ± 3.0% to 2.1 ± 0.4% ($P < 0.05$), and these postoperative results were similar to that of healthy controls (2.0 ± 1.0%) After surgery, a lower percentage of spermatozoa with low MMP was observed compared with baseline (2.0 ± 0.6 vs. 28.0 ± 4.0%, respectively; $P < 0.05$), and results were not different than controls (2.0 ± 0.6%). The percentages of spermatozoa with PS externalization (3.0 ± 3.0 vs. 9.0 ± 4.0%; $P < 0.05$) and decondensed chromatin (6.0 ± 0.5 vs. 22.0 ± 4.0%; $P < 0.05$) were lower than baseline, and results were not difference than controls (4.0 ± 2.0% and 6.0 ± 2.0%, respectively)

Table 3 (continued)

Study	Design	Patients	Controls	SDF assay	Oxidative stress and/or other sperm function markers	Surgical technique	Main results
Ni et al. [93]	Prospective cohort	42 infertile men with clinical left varicocele (grade 1; 15 patients; grade 2: 16 patients; grade 3: 11 patients) and abnormal semen analysis (sperm count < 15 M/mL and/or %motility < 32%) subjected to varicocelectomy	10 normozoospermic fertile controls	SCSA	Sperm protamine-1/2 mRNA ratio	Microsurgical	Mean DFI and protamine-1/2 mRNA ratio were significantly higher in the preoperative group than in the control group (27.4 ± 6.3 vs. 11.5 ± 3.9% and 2.1 ± 1.1 vs. 1.1 ± 0.1 respectively, $P < 0.01$) DFI results in patients who achieved pregnancy after varicocele repair (20.6 ± 3.5%) were not significantly different than controls (11.5 ± 3.9%) but were both lower than preoperative values (27.4 ± 6.3%; $P < 0.01$) and the results of non-pregnant patients (24.7 ± 6.5%; $P < 0.01$) In grade 3 group P1/P2 mRNA ($P < 0.05$) and DFI ($P < 0.01$) were significantly improved, while in grade 2 group only DFI was improved ($P < 0.05$). In grade 1 patients, no differences were noted in P1/P2 mRNA ratio and DFI

Table 3 (continued)

Study	Design	Patients	Controls	SDF assay	Oxidative stress and/or other sperm function markers	Surgical technique	Main results
Mohammed et al. [94]	Prospective cohort	75 infertile men (mean age: 31 years) with clinical varicocele (any grade) and altered semen parameters subjected to varicocelectomy	40 healthy fertile volunteers (mean age: 30.2 years) without varicocele	Acridine orange	Sperm chromatin decondensation by flow cytometry	Subinguinal with loop magnification	Baseline DFI and sperm chromatin decondensation were lower in controls than patients (18.2 ± 4.8 vs. 32.4 ± 7.4, $P = 0.003$; and 12.8 ± 2.2 vs. 25.4 ± 8.8%, $P = 0.005$) DFI reduced after varicocelectomy (pre vs. post: 32.4 ± 7.4 and 20.0 ± 4.1%, $P = 0.05$), but no significant changes were detected regarding DNA chromatin decondensation (25.4 ± 8.8% vs. 22.0 ± 4.1) Positive pregnancy outcome at 1-year follow-up ($n = 15$) had significantly lower DFI (16.4 ± 6.4%) than those who did not (24.2 ± 4.1%, $P = 0.04$), but there no significant difference was observed in sperm DNA decondensation among couples who conceived or did not (20.3 ± 6.8 vs. 23.5 ± 5.4)
Alhatal et al. [95]	Prospective cohort	29 infertile men with clinical varicocele and abnormal semen parameters subjected to varicocelectomy	6 healthy sperm donors with normal sperm parameters	SCSA	Sperm DNA decondensation (aniline blue and iodoacetamide-fluorescein)	Microsurgical subinguinal	Preoperative sperm %DF (20 ± 10.6 vs. 7.4 ± 5%; $P = 0.01$), %positive AB staining (13.5 ± 7.0 vs. 2.5 ± 1%; $P = 0.0009$), and % positive 5-IAF (16.3 ± 6.0 vs. 1.7 ± 1.0%; $P = 0.0001$) of infertile men with varicocele were significantly higher than that of healthy donors The %DFI decreased significantly after surgery (from 20.0 ± 10.6% to 12.0 ± 5.7%; $P = 0.001$). Similarly, the %AB staining (from 13.5 ± 7.0% to 5.4 ± 2.7%; $P = 0.0004$) and %5-IAF (16.3 ± 6.0% to 5.4 ± 2.7%; P0.0004) also decreased after surgery

Table 3 (continued)

Study	Design	Patients	Controls	SDF assay	Oxidative stress and/or other sperm function markers	Surgical technique	Main results
Ni et al. [96]	Not specified	51 men with clinical varicocele and abnormal semen analysis subjected to varicocelectomy	15 men with subclinical varicocele, 22 normozoospermic men with clinical varicocele, and 25 healthy fertile donors	SCSA	Assessment of lipid peroxidation by measurement of seminal MDA concentration	Microsurgical retroperitoneal high ligation	SDF levels were elevated in men with clinical varicocele (all grades, range: 20.6 ± 4.1% to 30.03 ± 8.3%) compared to controls (12.0 ± 7.9%) and subclinical varicocele (14.9 ± 5.1%) ($P < 0.05$) Varicocelectomy reduced SDF in patients with grades 1–3 clinical varicocele and altered semen parameters: Grade 1 ($n = 19$): pre 23.5 ± 7.5%, post 3 months: 20.8 ± 5.6%, post 6 months 19.5 ± 5.5%; $P < 0.01$ Grade 2 ($n = 18$): pre 27.7 ± 9.0%, post 3 months: 22.9 ± 5.2%, post 6 months 22.4 ± 4.5%; $P < 0.01$ Grade 3: pre 30.0 ± 8.2%, post 3 months: 23.3 ± 5.4%, post 6 months 21.8 ± 5.9%; $P < 0.01$ Among men with clinical varicocele, DFI and MDA levels in couples who achieved pregnancy were lower than non-pregnant couples ($P < 0.05$) Seminal MDA levels were higher in men with clinical varicocele (all grades) than subclinical varicocele and fertile controls ($P < 0.01$). After surgery, a significant reduction in seminal MDA was observed at 3 (grade 2, $P < 0.01$; grade 3, $P < 0.05$) and 6 (grade 1, $P < 0.05$; grade 2 and 3, $P < 0.01$) months, which was almost equal to the level of control group A positive correlation was observed between sperm DFI and seminal MDA ($r = 0.504$, $P < 0.01$)

Table 3 (continued)

Study	Design	Patients	Controls	SDF assay	Oxidative stress and/or other sperm function markers	Surgical technique	Main results
Abdelbaki et al. [97]	Prospective controlled cohort	60 infertile men (median age: 31 years) with clinical varicocele (left: 35 patients; bilateral: 25 patients) and abnormal semen parameters subjected to varicocelectomy	20 normozoospermic healthy fertile men with normal standard semen variables according to WHO criteria	SCSA	Measurement of ROS and TAC levels	Inguinal with loop magnification	A higher %DFI (29.9 ± 8.3%) and ROS levels (4.49 ± 0.9 Log(ROS + 1) photons/min, and a lower in TAC (0.97 ± 0.4 mM) was found in varicocele patients than controls (7.56 ± 2.84%, 2.62 ± 0.8 Log(ROS + 1) photons/min, and 1.5 ± 0.5 mM, respectively) The DFI % had a positive correlation ($r = 0.654$; $P < 0.001$) with ROS levels, grade of varicocele and duration of infertility, and a significant negative correlation with TAC ($r = -0.79$; $P < 0.001$); %DFI (18.8 ± 7.2%, $P < 0.001$) and ROS levels (3.3 ± 1.3 Log(ROS + 1) photons/min, $P < 0.001$) decreased after varicocelectomy whereas TAC levels increased (2.0 ± 0.5 mM) at a 3-month follow-up

DFI DNA fragmentation index, *MDA* malondialdehyde, *MMP* mitochondrial membrane potential, *OS* oxidative stress, *ROS* reactive oxygen species, *SCSA* sperm chromatin structure assay, *SCD* sperm chromatin dispersion assay; SDF: sperm DNA fragmentation, *TAC* total antioxidant capacity

(by WHO criteria), it was least ordered for the evaluation of low-grade varicocele in patients with subnormal semen analysis results (46.9%) [60]. In this study, 1/3 participants responded that SDF testing is not currently offered in any of the clinical scenarios listed above in their practices, whereas 1/6 revealed uncertainty about its clinical utility in such situations. The CPG issued by the STM clarify these issues and provide useful guidance to urologists and other healthcare practitioners as to enhance the quality of healthcare deliverable to varicocele patients as well as to discourage potentially harmful or ineffective interventions [105].

While it is important to contemplate that the CPG on SDF testing synthesized their recommendations based on the available evidence, these were based overwhelmingly on non-randomized clinical trials and retrospective studies. Therefore, most recommendations were graded B and C, like those issued by most male infertility guidelines [43, 101, 106, 107]. Concerning varicocelectomy in men with clinical varicocele/normal semen analysis and low-grade varicocele/borderline semen analysis, the evidence is still limited, thereby warranting further research. However, as with all male infertility CPG, the guidelines on SDF testing concerning varicocele is not aimed at dictating an exclusive course of treatment. Other management and treatment strategies might be appropriate, taking into account the available resources, patient needs, and specific practice conditions. The essence of any CPG should be to translate the best evidence into practice and serve as a framework for standardized care while maintaining clinical autonomy and physician judgment [108, 109].

Future research

Despite convincing evidence of a positive effect of varicocele repair on SDF, there exist gaps in knowledge as to the exact prevalence of elevated SDF among varicocele patients and the association between SDF and varicocele grade (reviewed by Esteves et al.) [39]. Although reduction in SDF levels after surgery is shown to be more common in men who have a concomitant improvement in conventional semen parameters [70], further research is needed to clarify whether improvements in SDF alone after varicocele repair in men with clinical varicocele and semen analysis within normal ranges can increase pregnancy success. Additionally, investigations are warranted to ascertain the proportion of patients with high SDF levels that resolve to normal levels after varicocelectomy. Lastly, there is a need for further evidence that SDF is reduced in patients with low-grade varicocele and borderline routine semen analysis and that such decline in SDF levels translates into better pregnancy outcomes.

Conclusions

Current evidence supports oxidative stress as a primary factor in the pathophysiology of varicocele-related infertility. The mechanisms by which varicocele increases oxidative stress are not fully elucidated, but reactive oxygen species generation in response to scrotal hyperthermia, testicular hypoxia, reflux of adrenal/renal metabolites, and cadmium accumulation is the leading theory. The testis and epididymis react to oxidative stress via several mechanisms—including the generation of antioxidants that may maintain fertility potential in men with varicocele. Failure of these mechanisms might explain testicular/epididymal dysfunction and infertility observed in a subset of men with varicocele. In this scenario, increased sperm DNA fragmentation—as often seen in men with clinical varicocele—is likely the final result of this oxidative-induced damage. Many assays are available to identify abnormal sperm DNA fragmentation levels in semen of men with varicocele. Surgical varicocele repair seems beneficial not only for decreasing sperm DNA fragmentation but also for increasing the likelihood of pregnancy, both natural and assisted, in men with palpable varicocele and damaged sperm chromatin. While gaps in knowledge exist, particularly concerning the understanding of varicocele grade on sperm DNA fragmentation and the utility of varicocelectomy in men with palpable varicocele and normal/borderline semen analysis, recent guidelines have provided evidence-based indications for SDF testing and guidance for management of the infertile man with varicocele.

Review criteria

An extensive search of studies examining the relationship between varicocele and sperm DNA fragmentation was performed using PubMed and MEDLINE. The start date for the search was not specified, and the end date was December 2017. The overall strategy for study identification and data extraction was based on the following keywords: "varicocele," "male infertility," "sperm DNA fragmentation," "sperm DNA damage," "varicocele repair," "varicocelectomy," "varicocele treatment," "varicocele embolization," and "antioxidants," with the filters "humans" and "English language." Data that were solely published in conference or

Table 4 Excerpt of the evidence-based clinical practice guidelines issued by the Society for Translational Medicine on indications for sperm DNA testing in varicocele. Adapted from Agarwal et al. [105] with permission

Indications for SDF testing
Clinical varicocele
SDF testing is recommended in patients with grade 2/3 varicocele with normal conventional semen parameters (grade C recommendation)
SDF testing is recommended in patients with grade 1 varicocele with borderline/abnormal conventional semen parameter results (grade C recommendation)

SDF sperm DNA fragmentation; grades of recommendations according to quality of evidence: Grade A, based on clinical studies of good quality and consistency with at least one randomized trial; Grade B, based on well-designed studies (prospective, cohort) but without good randomised clinical trials; Grade C, based on poorer quality studies (retrospective, case series, expert opinion). Modified from Oxford Centre for Evidence-Based Medicine (http://www.cebm.net/oxford-centre-evidence-based-medicine-levels-evidence-march-2009/)

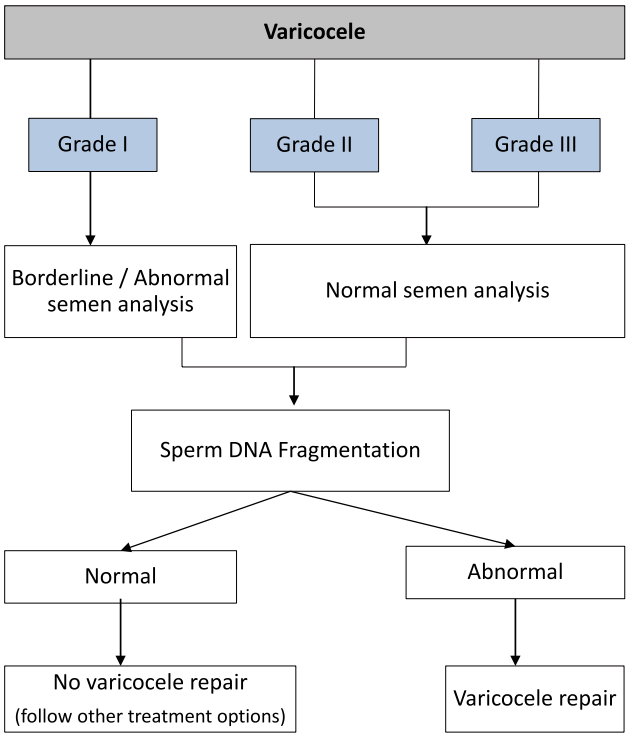

Fig. 2 Algorithm for sperm DNA fragmentation testing in patients with clinical varicocele. Reprinted with permission from Esteves et al., A Strengths–Weaknesses–Opportunities–Threats (SWOT) analysis on the clinical utility of sperm DNA fragmentation testing in specific male infertility scenarios. Transl Androl Urol. 2017 Sep; 6(Suppl 4):S734–S760

meeting proceedings, websites or books were not included. Citations dated outside the search dates were only included if provided conceptual content.

Authors' contributions MR participated in the acquisition of data, helped in data interpretation, and drafted the manuscript. SCE designed the study, helped in data interpretation and coordination, and drafted the manuscript. Both authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest MR has nothing to disclose. SCE is a member of the advisory panel that developed the clinical practice guidelines for sperm DNA fragmentation testing based on clinical scenarios issued by the Society for Translational Medicine (http://www.thestm.org/about/internationAdvisoryCommitee).

References

1. Esteves SC, Miyaoka R, Agarwal A (2011) An update on the clinical assessment of the infertile male. [corrected]. Clinics (Sao Paulo) 66:691–700
2. Shiraishi K, Matsuyama H, Takihara H (2012) Pathophysiology of varicocele in male infertility in the era of assisted reproductive technology. Int J Urol 19:538–550
3. Masson P, Brannigan RE (2014) The varicocele. Urol Clin North Am 41:129–144
4. Jungwirth A, Giwercman A, Tournaye H, Diemer T, Kopa Z, Dohle G et al (2012) European Association of Urology guidelines on Male Infertility: the 2012 update. Eur Urol 62:324–332
5. Practice Committee of the American Society for Reproductive Medicine; Society for Male Reproduction and Urology (2014) Report on varicocele and infertility: a committee opinion. Fertil Steril 102:1556–1560
6. National Collaborating Centre for Women's and Children's Health (2013) Fertility: assessment and treatment for people with fertility problems. National Institute for Health and Clinical Excellence (NICE), London, p. 63 (clinical guideline; no. 156). http://www.nice.org.uk/guidance/CG156. Accessed 8 Feb 2018
7. Miyaoka R, Esteves SC (2012) A critical appraisal on the role of varicocele in male infertility. Adv Urol 2012:597495
8. Tiseo BC, Esteves SC, Cocuzza MS (2016) Summary evidence on the effects of varicocele treatment to improve natural fertility in subfertile men. Asian J Androl 18:239–245
9. Agarwal A, Hamada A, Esteves SC (2012) Insight into oxidative stress in varicocele-associated male infertility: part 1. Nat Rev Urol 9:678–690
10. Hamada A, Esteves SC, Agarwal A (2012) Insight into oxidative stress in varicocele-associated male infertility: part 2. Nat Rev Urol 10:26–37
11. Agarwal A, Sharma RK, Desai NR, Prabakaran S, Tavares A, Sabanegh E (2009) Role of oxidative stress in pathogenesis of varicocele and infertility. Urology 73:461–469
12. Zini A, Dohle G (2011) Are varicoceles associated with increased deoxyribonucleic acid fragmentation? Fertil Steril 96:1283–1287
13. Cho CL, Esteves SC, Agarwal A (2016) Novel insights into the pathophysiology of varicocele and its association with reactive oxygen species and sperm DNA fragmentation. Asian J Androl 18:186–193
14. Esteves SC, Hamada A, Kondray V, Pitchika A, Agarwal A (2012) What every gynecologist should know about male infertility: an update. Arch Gynecol Obstet 286:217–229
15. Tremellen K (2008) Oxidative stress and male infertility—a clinical perspective. Hum Reprod Update 14:243–258
16. Blumer CG, Restelli AE, Giudice PT, Soler TB, Fraietta R, Nichi M, Bertolla RP, Cedenho AP (2012) Effect of varicocele on sperm function and semen oxidative stress. BJU Int 109:259–265

17. Aitken RJ, Krausz C (2001) Oxidative stress, DNA damage and the Y chromosome. Reproduction 122:497–506
18. Seli E, Gardner DK, Schoolcraft WB, Moffatt O, Sakkas D (2004) Extent of nuclear DNA damage in ejaculated spermatozoa impacts on blastocyst development after in vitro fertilization. Fertil Steril 82:378–383
19. Zini A, Boman JM, Belzile E, Ciampi A (2008) Sperm DNa damage is associated with an increased risk of pregnancy loss after IVF and ICSI: systematic review and meta-analysis. Hum Reprod 23:2663–2668
20. Robinson L, Gallos ID, Conner SJ, Rajkhowa M, Miller D, Lewis S, Kirkman-Brown J, Coomarasamy A (2012) The effect of sperm DNA fragmentation on miscarriage rates: a systematic review and meta-analysis. Hum Reprod 27:2908–2917
21. Eisenberg ML, Sapra KJ, Kim SD, Chen Z, Buck Louis GM (2017) Semen quality and pregnancy loss in a contemporary cohort of couples recruited before conception: data from Longitudinal Investigation on Fertility and the Environment (LIFE) Study. Fertil Steril 108:613–619
22. Esteves SC, Gosálvez J, López-Fernández C, Núñez-Calonge R, Caballero P, Agarwal A, Fernández J (2015) Diagnostic accuracy of sperm DNA degradation index (DDSi) as a potential noninvasive biomarker to identify men with varicocele-associated infertility. Int Urol Nephrol 47:1471–1477
23. Esteves SC, Agarwal A (2016) Afterword to varicocele and male infertility: current concepts and future perspectives. Asian J Androl 18:319–322
24. Griveau JF, Le Lannou D (1997) Reactive oxygen species and human spermatozoa: physiology and pathology. Int J Androl 20:61–69
25. Mostafa T, As T, Imam H, El-Nashar AR, Osman IA (2009) Seminal reactive oxygen species—antioxidant relationship in fertile males with and without varicocele. Andrologia 41:125–129
26. Zylbersztejn DS, Andreoni C, Del Giudice PT, Spaine DM, Borsari L, Souza GH, Bertolla RP, Fraietta R (2013) Proteomic analysis of seminal plasma in adolescents with and without varicocele. Fertil Steril 99:92–98
27. Agarwal A, Cho C-L, Esteves SC, Majzoub A (2017) Reactive oxygen species and sperm DNA fragmentation. Transl Androl Urol 6(Suppl 4):S695–S696. https://doi.org/10.21037/tau.2017.05.40
28. Mehraban D, Ansari M, Keyhan H, Sedighi Gilani M, Naderi G, Esfehani F (2005) Comparison of nitric oxide concentration in seminal fluid between infertile patients with and without varicocele and normal fertile men. J Urol 2:106–110
29. Sakamoto Y, Ishikawa T, Kondo Y, Yamaguchi K, Fujisawa M (2008) The assessment of oxidative stress in infertile patients with and without varicocele. BJU Int 101:1547–1552
30. Pasqualotto FF, Sundaram A, Sharma RK, Borges E Jr, Pasqualotto EB, Agarwal A (2008) Semen quality and oxidative stress scores in fertile and infertile patients with varicocele. Fertil Steril 2008(89):602–607
31. Mostafa T, Anis TH, El-Nashar A, Imam H, Othman IA (2001) Varicocelectomy reduces reactive oxygen species levels and increases antioxidant activity of seminal plasma from infertile men with varicocele. Int J Androl 24:261–265
32. Köksal IT, Tefekli A, Usta M, Erol H, Abbasoglu S, Kadioglu A (2000) The role of reactive oxygen species in testicular dysfunction associated with varicocele. BJU Int 86:549–552
33. Allamaneni SS, Naughton CK, Sharma RK, Thomas AJ Jr, Agarwal A (2004) Increased seminal reactive oxygen species levels in patients with varicoceles correlate with varicocele grade but not with testicular size. Fertil Steril 82:1684–1686
34. Ishikawa T, Fujioka H, Ishimura T, Takenaka A, Fujisawa M (2007) Increased testicular 8-hydroxy-2′-deoxyguanosine in patients with varicocele. BJU Int 100:863–866
35. Abd-Elmoaty MA, Saleh R, Sharma R, Agarwal A (2010) Increased levels of oxidants and reduced antioxidants in semen of infertile men with varicocele. Fertil Steril 94:1531–1534
36. Pasqualotto FF, Sharma RK, Nelson DR, Thomas AJ Jr, Agarwal A (2000) Relationship between oxidative stress, semen characteristics, and clinical diagnosis in men undergoing infertility investigation. Fertil Steril 73:459–464
37. Sharma RK, Pasqualotto FF, Nelson DR, Thomas AJ Jr, Agarwal A (1999) The reactive oxygen species-total antioxidant capacity score is a new measure of oxidative stress to predict male infertility. Hum Reprod 14:2801–2807
38. Hendin BN, Kolettis PN, Sharma RK, Thomas AJ Jr, Agarwal A (1999) Varicocele is associated with elevated spermatozoal reactive oxygen species production and diminished seminal plasma antioxidant capacity. J Urol 161:1831–1834
39. Esteves SC, Agarwal A, Cho CL, Majzoub A (2017) A strengths-weaknesses-opportunities-threats (SWOT) analysis on the clinical utility of sperm DNA fragmentation testing in specific male infertility scenarios. Tranl Androl Urol 6:S734–S760
40. Hurtado de Catalfo GE, Ranieri-Casilla A, Marra FA, de Alaniz MJ, Marra CA (2007) Oxidative stress biomarkers and hormonal profile in human patients undergoing varicocelectomy. Int J Androl 30:519–530
41. Chen SS, Huang WJ, Chang LS, Wei YH (2008) Attenuation of oxidative stress after varicocelectomy in subfertile patients with varicocele. J Urol 179:639–642
42. Esteves SC, Sharma RK, Gosálvez J, Agarwal A (2014) A translational medicine appraisal of specialized andrology testing in unexplained male infertility. Int Urol Nephrol 2014(46):1037–1052
43. Esteves SC, Chan P (2015) A systematic review of clinical practice guidelines and best practice statements for the evaluation of the infertile male. Int Urol Nephrol 47:1441–1456
44. Clavijo RI, Carrasquillo R, Ramasamy R (2017) Varicoceles: prevalence and pathogenesis in adult men. Fertil Steril 108:364–369
45. Gat Y, Zukerman Z, Chakraborty J, Gornish M (2005) Varicocele, hypoxia and male infertility. Fluid mechanics analysis of the impaired testicular venous drainage system. Hum Reprod 20:2614–2619
46. Gat Y, Gornish M, Navon U, Chakraborty J, Bachar GN, Ben-Shlomo I (2006) Right varicocele and hypoxia, crucial factors in male infertility: fluid mechanics analysis in male infertility: fluid mechanics analysis of the impaired testicular drainage system. Reprod Biomed Online 13:510–515
47. Ambrosini G, Nath AK, Sierra-Honigmann MR, Flores-Riveros J (2002) Transcriptional activation of the human leptin gene in response to hypoxia. Involvement of hypoxia inducible factor 1. J Biol Chem 277:34601–34609
48. Nallella KP, Allamaneni SS, Pasqualotto FF, Sharma RK, Thomas AJ Jr, Agarwal A (2004) Relationship of interleukin-6 with semen characteristics and oxidative stress in patients with varicocele. Urology 64:1010–1013
49. Sahin Z, Celik-Ozenci C, Akkoyunlu G, Korgun ET, Acar N, Erdogru T, Demir R, Ustunel I (2006) Increased expression of interleukin-1α and interleukin-1β is associated with experimental varicocele. Fertil Steril 85(Suppl 1):1265–1275
50. Ito H, Fuse H, Minagawa H, Kawamura K, Murakami M, Shimazaki J (1982) Internal spermatic vein prostaglandins in varicocele patients. Fertil Steril 37:218–222
51. Benoff S, Hurley IR, Barcia M, Mandel FS, Cooper GW, Hershlag A (1997) A potential role for cadmium in the etiology of varicocele-associated infertility. Fertil Steril 67:336–347
52. Benoff SH, Millan C, Hurley IR, Napolitano B, Marmar JL (2004) Bilateral increased apoptosis and bilateral accumulation

of cadmium in infertile men with left varicocele. Hum Reprod 19:616–627
53. Jeng SY, Wu SM, Lee JD (2009) Cadmium accumulation and metallothionein overexpression in internal spermatic vein of patients with varicocele. Urology 73:1231–1235
54. Lewis SE, John Aitken R, Conner SJ, Iuliis GD, Evenson DP, Henkel R, Giwercman A, Charagozloo O (2013) The impact of sperm DNA damage in assisted conception and beyond: recent advances in diagnosis and treatment. Reprod Biomed Online 27:325–337
55. Gosálvez J, Caballero P, López-Fernández C, Ortega L, Guijarro JA, Fernández JL, Johnston SD, Nuñez-Calonge R (2013) Can DNA fragmentation of neat or swim-up spermatozoa be used to predict pregnancy following ICSI of fertile oocyte donors? Asian J Androl 15:812–818
56. Feijó CM, Esteves SC (2014) Diagnostic accuracy of sperm chromatin dispersion test to evaluate sperm deoxyribonucleic acid damage in men with unexplained infertility. Fertil Steril 101:58–63
57. Majzoub A, Esteves SC, Gonsálvez J, Agarwal A (2016) Specialized sperm function tests in varicocele and the future of andrology laboratory. Asian J Androl 18:205
58. Gosálvez J, Lopez-Fernandez C, Fernandez JL et al (2015) Unpacking the mysteries of sperm DNA fragmentation: ten frequently asked questions. J Reprod Biotechnol Fertil 4:1–16
59. Esteves SC (2016) Novel concepts in male factor infertility: clinical and laboratory perspectives. J Assist Reprod Genet 33:1319–1335
60. Majzoub A, Agarwal A, Cho CL, Esteves SC (2017) Sperm DNA fragmentation testing: a cross sectional survey on current practices of fertility specialists. Transl Androl Urol 6:S710–S719
61. Sharma R, Ahmad G, Esteves SC, Agarwal A (2016) Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay using bench top flow cytometer for evaluation of sperm DNA fragmentation in fertility laboratories: protocol, reference values, and quality control. J Assist Reprod Genet 33:291–300
62. Avendaño C, Franchi A, Duran H, Oehninger S (2010) DNA fragmentation of normal spermatozoa negatively impacts embryo quality and intracytoplasmic sperm injection outcome. Fertil Steril 94:549–557
63. Buck Louis GM, Sundaram R, Schisterman EF, Sweeney A, Lynch CD, Kim S, Maisog JM, Gore-Langton R, Eisenberg ML, Chen Z (2014) Semen quality and time to pregnancy: the Longitudinal Investigation of Fertility and the Environment Study. Fertil Steril 101:453–462
64. Agarwal A, Cho CL, Esteves SC (2016) Should we evaluate and treat sperm DNA fragmentation? Curr Opin Obstet Gynecol 2016(28):164–171
65. Sakkas D, Alvarez JG (2010) Sperm DNA fragmentation: mechanisms of origin, impact on reproductive outcome, and analysis. Fertil Steril 93:1027–1036
66. Esteves SC, Sánchez-Martín F, Sánchez-Martín P, Schneider DT, Gosálvez J (2015) Comparison of reproductive outcome in oligozoospermic men with high sperm DNA fragmentation undergoing intracytoplasmic sperm injection with ejaculated and testicular sperm. Fertil Steril 104:1398–1405
67. Tesarik J, Greco E, Mendoza C (2004) Late, but not early, paternal effect on human embryo development is related to sperm DNA fragmentation. Hum Reprod 19:611–615
68. Bungum M, Humaidan P, Axmon A, Spano M, Bungum L, Erenpreiss J, Giwercman A (2007) Sperm DNA integrity assessment in prediction of assisted reproduction technology outcome. Hum Reprod 22:174–179
69. Agarwal A, Sharma R, Harlev A, Esteves SC (2016) Effect of varicocele on semen characteristics according to the new 2010 World Health Organization criteria: a systematic review and meta-analysis. Asian J Androl 18:163–170
70. Smith R, Kaune H, Parodi D, Madariaga M, Rios R, Morales I, Castro A (2006) Increased sperm DNA damage in patients with varicocele: relationship with seminal oxidative stress. Hum Reprod 21:986–993
71. Wang YJ, Zhang RQ, Lin YJ, Zhang RG, Zhang WL (2012) Relationship between varicocele and sperm DNA damage and the effect of varicocele repair: a meta-analysis. Reprod Biomed Online 25:307–314
72. Janghorban-Laricheh E, Ghazavi-Khorasgani N, Tavalaee M, Zohrabi D, Abbasi H, Nasr-Esfahani MH (2012) An association between sperm PLCζ levels and varicocele? J Assist Reprod Genet 33:1649–1655
73. Vivas-Acevedo G, Lozano-Hernández R, Camejo MI (2014) Varicocele decreases epididymal neutral α-glucosidase and is associated with alteration of nuclear DNA and plasma membrane in spermatozoa. BJU Int 113:642–649
74. Marmar JL (2016) The evolution and refinements of varicocele surgery. Asian J Androl 18:171–178
75. Camargo M, Intasqui P, Bertolla RP (2016) Proteomic profile of seminal plasma in adolescents and adults with treated and untreated varicocele. Asian J Androl 18:194–201
76. Kruger T (2016) Critical appraisal of conventional semen analysis in the context of varicocele. Asian J Androl 18:202–204
77. Esteves SC, Miyaoka R, Roque M, Agarwal A (2016) Outcome of varicocele repair in men with nonobstructive azoospermia: systematic review and meta-analysis. Asian J Androl 18:246–253
78. Esteves SC, Roque M, Agarwal A (2016) Outcome of assisted reproductive technology in men with treated and untreated varicocele: systematic review and meta-analysis. Asian J Androl 18:254–258
79. Zini A, Blumenfeld A, Libman J et al (2005) Beneficial effect of microsurgical subinguinal varicocelectomy on human sperm DNA integrity. Hum Reprod 20:1018–1021
80. Werthman P, Wixon R, Kasperson K, Evenson DP (2008) Significant decrease in sperm deoxyribonucleic acid fragmentation after varicocelectomy. Fertil Steril 90:1800–1804
81. Moskovtsev SI, Lecker I, Mullen JB, Jarvi K, Willis J, White J, Lo KC (2009) Cause-specific treatment in patients with high sperm DNA damage resulted in significant DNA improvement. Syst Biol Reprod Med 55:109–115
82. Smit M, Romijn JC, Wildhagen MF, Veldhoven JL, Weber RF, Dohle GR (2010) Decreased sperm DNA fragmentation after surgical varicocelectomy is associated with increased pregnancy rate. J Urol 183:270–274
83. Kadioglu TC, Aliyev E, Celtik M (2014) Microscopic varicocelectomy significantly decreases the sperm DNA fragmentation index in patients with infertility. Biomed Res Int 2014:695713
84. Telli O, Sarici H, Kabar M, Ozgur BC, Resorlu B, Bozkurt S (2015) Does varicocelectomy affect DNA fragmentation in infertile patients? Indian J Urol 31:116–119
85. Sun XL, Wang JL, Peng YP, Gao QQ, Song T, Yu W, Xu ZP, Chen Y, Dai YT (2017) Bilateral is superior to unilateral varicocelectomy in infertile males with left clinical and right subclinical varicocele: a prospective randomized controlled study. Int Urol Nephrol. https://doi.org/10.1007/s11255-017-1749-x
86. Zaazaa A, Adel A, Fahmy I, Elkhiat Y, Awaad AA, Mostafa T (2018) Effect of varicocelectomy and/or mast cells stabilizer on sperm DNA fragmentation in infertile patients with varicocele. Andrology 6:146–150
87. Lacerda JI, Del Giudice PT, da Silva BF, Nichi M, Fariello RM, Fraietta R, Restelli AE, Blumer CG, Bertolla RP, Cedenho AP (2011) Adolescent varicocele: improved sperm function after varicocelectomy. Fertil Steril 95:994–999

88. Li F, Yamaguchi K, Okada K, Matsushita K, Ando M, Chiba K, Yue H, Fujisawa M (2012) Significant improvement of sperm DNA quality after microsurgical repair of varicocele. Syst Biol Reprod Med 58:274–277
89. Baker K, McGill J, Sharma R, Agarwal A, Sabanegh E Jr (2013) Pregnancy after varicocelectomy: impact of postoperative motility and DFI. Urology 81:760–766
90. Pourmand G, Movahedin M, Dehghani S, Mehrsai A, Ahmadi A, Pourhosein M, Hoseini M, Ziloochi M, Heidari F, Beladi L, Noori M (2014) Does L-carnitine therapy add any extra benefit to standard inguinal varicocelectomy in terms of deoxyribonucleic acid damage or sperm quality factor indices: a randomized study. Urology 84:821–825
91. Tavalaee M, Bahreinian M, Barekat F, Abbasi H, Nasr-Esfahani MH (2015) Effect of varicocelectomy on sperm functional characteristics and DNA methylation. Andrologia 47:904–909
92. Vignera La, Condorelli R, Vicari E, D'Agata R, Calogero AE (2012) Effects of varicocelectomy on sperm DNA fragmentation, mitochondrial function, chromatin condensation, and apoptosis. J Androl 2012(33):389–396
93. Ni K, Steger K, Yang H, Wang H, Hu K, Chen B (2014) Sperm protamine mRNA ratio and DNA fragmentation index represent reliable clinical biomarkers for men with varicocele after microsurgical varicocele ligation. J Urol 192:170–176
94. Mohammed EE, Mosad E, Zahran AM, Hameed DA, Taha EA, Mohamed MA (2015) Acridine orange and flow cytometry: which is better to measure the effect of varicocele on sperm DNA integrity? Adv Urol 2015:814150
95. Alhathal N, San Gabriel M, Zini A (2016) Beneficial effects of microsurgical varicocoelectomy on sperm maturation, DNA fragmentation, and nuclear sulfhydryl groups: a prospective trial. Andrology 4:1204–1208
96. Ni K, Steger K, Yang H, Wang H, Hu K, Zhang T, Chen B (2016) A Comprehensive investigation of sperm DNA damage and oxidative stress injury in infertile patients with subclinical, normozoospermic and astheno/oligozoospermic clinical varicocele. Andrology 4:816–824
97. Abdelbaki SA, Sabry JH, Al-Adl AM, Sabry HH (2017) The impact of coexisting sperm DNA fragmentation and seminal oxidative stress on the outcome of varicocelectomy in infertile patients: a prospective controlled study. Arab J Urol 15:131–139
98. García-Peiró A, Ribas-Maynou J, Oliver-Bonet M, Navarro J, Checa MA, Nikolaou A, Amengual MJ, Abad C, Benet J (2014) Multiple determinations of sperm DNA fragmentation show that varicocelectomy is not indicated for infertile patients with subclinical varicocele. Biomed Res Int 2014:181396
99. Gual-Frau J, Abad C, Amengual MJ, Hannaoui N, Checa MA, Ribas-Maynou J, Lozano I, Nikolaou A, Benet J, García-Peiró A, Prats J (2015) Oral antioxidant treatment partly improves integrity of human sperm DNA in infertile grade I varicocele patients. Hum Fertil (Camb) 18:225–229
100. American Urological Association and American Society for Reproductive Medicine (2001) Report on varicocele and infertility: an AUA best practice policy and ASRM practice committee report. https://www.auanet.org/Documents/education/clinical/Varicocele-Archive.pdf. Accessed 6 June 2017
101. Shridharani A, Owen RC, Elkelany OO et al (2016) The significance of clinical practice guidelines on adult varicocele detection and management. Asian J Androl 18:269–275
102. Esteves SC (2014) Clinical relevance of routine semen analysis and controversies surrounding the 2010 World Health Organization criteria for semen examination. Int Braz J Urol 40:443–453
103. Esteves SC, Zini A, Aziz N, Alvarez JG, Sabanegh ES Jr, Agarwal A (2012) Critical appraisal of World Health Organization's new reference values for human semen characteristics and effect on diagnosis and treatment of subfertile men. Urology 79:16–22
104. Sharma R, Harlev A, Agarwal A, Esteves SC (2016) Cigarette smoking and semen quality: a new meta-analysis examining the effect of the 2010 World Health Organization Laboratory methods for the examination of human semen. Eur Urol 70:635–645
105. Agarwal A, Cho CL, Majzoub A, Esteves SC (2017) The Society for Translational Medicine: clinical practice guidelines for sperm DNA fragmentation testing in male infertility. Trans Androl Urol 6:S720–S733
106. Roque M, Esteves SC (2016) A systematic review of clinical practice guidelines and best practice statements for the diagnosis and management of varicocele in children and adolescents. Asian J Androl 18:262–268
107. Esteves SC, Agarwal A, Majzoub A (2017) Unraveling the utility and limitations of clinical practice guidelines. Transl Androl Urol 6:S506–S508
108. Esteves SC, Agarwal A, Majzoub A (2017) Best practice statements are not intended to dictate an exclusive course of management. Trans Androl Urol 6:S683–S684
109. Greenhalgh T, Howick J, Maskrey N, Evidence Based Medicine Renaissance Group (2014) Evidence based medicine: a movement in crisis? BMJ 348:g3725