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An update on clinical and surgical interventions to reduce sperm DNA fragmentation in infertile men

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Abstract

Background: Sperm chromatin integrity is essential for normal embryo development and pregnancy outcome. Sperm DNA fragmentation (SDF) testing constitutes a diagnostic tool to measure the proportion of spermatozoa with damaged chromatin in the ejaculate. SDF is associated with potentially treatable conditions, including varicocele, male accessory gland infections, inadequate lifestyle, and gonadotoxin exposure, thus prompting their treatment as a means of improving sperm DNA quality and the reproductive outcomes.

Objective: To provide an up-to-date review of the role of clinical and surgical interventions on SDF values in subfertile men.

Materials and methods: An extensive search of studies examining the relationship between male infertility conditions associated with SDF was performed using PubMed and MEDLINE, with a focus on interventional therapy. The start date for the search was not defined, whereas the end date was March 2019. Randomized and non-randomized controlled trials, observational studies, systematic and narrative reviews, and case series were evaluated.

Results: Treating the underlying male infertility factor seems a promising way to alleviate SDF and to increase the likelihood of achieving natural and assisted conception, but data remain limited. The best evidence relates to varicocele repair and hormonal therapy with the follicle-stimulating hormone. Antioxidant therapy and lifestyle changes might alleviate oxidative sperm markers and decrease SDF but their effects on pregnancy outcomes are still unclear. Among men with high SDF undergoing assisted reproductive technology, the use of testicular spermatozoa in preference over ejaculated spermatozoa for intracytoplasmic sperm injection (ICSI) has been shown to improve pregnancy rates possibly owing to the better sperm chromatin quality in testicular spermatozoa than in ejaculated spermatozoa.

Conclusion: Current evidence supports interventional therapy as a means to alleviate sperm DNA damage. Identification of the conditions associated with SDF remains important to enable treatment to potentially improve pregnancy outcomes but given the limited data further research is needed to determine the exact role of specific interventional therapy for subfertile men with impaired sperm chromatin.

KEYWORDS

assisted reproductive technology, male infertility, risk reduction behavior, sperm DNA fragmentation, therapeutics, varicocele repair

1 | INTRODUCTION

Globally, over 180 million people struggle with infertility, and the male factor is partially or entirely accountable for about 50% of cases.[1] While the basis of male infertility remains undefined in up to 50% of patients on routine assessment,[2-4] increasing evidence suggests that sperm DNA fragmentation (SDF) plays an independent and significant role in its etiology.[5-9]

SDF involves multiple non-mutually exclusive causative mechanisms that provoke breaks to DNA.[10-12] Overproduction of reactive oxygen species (ROS) during sperm transport through the seminiferous tubules and epididymis results in oxidative stress (OS), which has been suggested to be the primary causative factor leading to SDF.[13,14] Other mechanisms, including apoptosis during spermatogenesis, deficient chromatin remodeling during spermiogenesis,[15] activation of endogenous caspases and endonucleases,[16] and exogenous factors, such as environmental toxicants, radiotherapy, and chemotherapy,[17-19] might also mediate SDF.

Human spermatozoa are highly vulnerable to OS, an effect which mainly relates to three non-mutually exclusive factors, namely (i) plasma membranes rich in polyunsaturated fatty acid (PUFA), (ii) limited cytosolic content of antioxidant factors, and (iii) truncated DNA damage detection and repair mechanisms.[20] In particular, PUFA is highly susceptible to ROS, and under OS conditions, PUFA amplifies the generation of ROS in a vicious OS circle.[21] Upon reaching the sperm nucleus, ROS can promote harm by modifying bases, creating abasic sites, chromatin protein cross-linking, and DNA strand breaks (both single and double) depending on the magnitude of the oxidative attack.[11] For instance, excessive ROS leads to the formation of oxidized base adducts (eg, 8-oxo-7,8-dihydro-2-deoxyguanosine [8OHdG]. A sperm enzyme named 8-oxoguanine DNA glycosylase 1 (OGG1) cleaves oxidized base adducts out of the DNA, which creates a relatively unstable abasic site more prone to fragmentation.[22-24]

Several male infertility conditions have been associated with SDF, including varicocele, male accessory gland infection (MAGI), endocrine abnormalities of the reproductive system, chronic illness, cancer, advanced paternal age, environmental exposure to toxicants, and lifestyle factors.[25-31] These conditions are also associated with excessive OS, thus suggesting that SDF is probably one of the critical consequences of OS via ROS.

Furthermore, a number of studies suggest that SDF might adversely influence the chances of conception, both natural and assisted,[32-37] albeit the evidence is not unequivocal.[38,39] The reasons explaining the reported impaired pregnancy rates among couples whose male partners have high SDF are not fully understood, but it has been hypothesized that the underlying genetic and epigenetic components associated with the damaged sperm chromatin might be the driving cause of poor reproductive outcomes.[10,40-43]

Spermatozoa with impaired chromatin content can hamper fertilization, early embryo development, implantation, and pregnancy over its effects on the stability of the embryonic genome.[44] The oxidatively induced SDF may dysregulate methylation processes and expression of critical genes for fertilization, embryo development, and implantation.[20] Moreover, 8-OHdG residues might cause transversion mutations (G-C to T-A), thus altering gene expression if not repaired by oocyte enzymes before the zygote's S-phase.[22,23] There is a growing concern that an underlying DNA damage could be transferred to the embryo by defective spermatozoa and thus affect the health of the resulting offspring.[22] At present, little is known about the effectiveness and accuracy of DNA repair at the oocyte level. Apparently, aged oocytes have less effective repair mechanisms, ultimately resulting in persistence of DNA lesions and mutagenic bases might increase the risk of embryo genetic and epigenetic abnormalities.[20-22,45]

Studies have also shown the negative effect of SDF on reproductive outcomes across a diverse range of animal species.[46,47] Increases in chromosome abnormalities at the first cleavage division, whereas reduction in fertilization rates, development to the first cleavage division, and implantation rates was observed when spermatozoa with damaged chromatin from mutant mice were used for intracytoplasmic sperm injection (ICSI).[48] In rabbits, the rate of stillborn pups was significantly higher when artificial insemination was carried out with spermatozoa with high SDF.[49] In another ICSI study, embryo development to the blastocyst stage was only reached from rams whose spermatozoa had low levels of SDF.[50]

In the era of assisted reproductive technology (ART), remarkable energy is invested in improving embryo quality and pregnancy success, but the merit of proper male evaluation and pre-treatment strategies is overlooked as ICSI can provide the couple with a baby without the need to explain the nature of underlying male infertility.[44,51-53] However, the notion that SDF could impair reproductive outcomes has prompted the use of SDF testing in clinical settings,[54] albeit controversies still exist on the utility of SDF as a biomarker for male infertility.[38,55]

Moreover, given the association of SDF and potentially remediable conditions, medical interventions have been explored as a means of improving sperm DNA integrity, with the ultimate goals of enhancing fertility and increasing the likelihood of achieving healthy offspring. In this review, we summarize the current evidence on the role of interventions to reduce SDF, with a primary focus on clinical and surgical treatments. We also provide an overview of SDF effects on male infertility, describe the technical aspects of SDF diagnostic measurement, and consider its indications.

2 | IMPACT OF SPERM DNA FRAGMENTATION ON FERTILITY AND REPRODUCTIVE OUTCOMES

2.1 | Male infertility

A 2018 systematic review—followed by meta-analysis—of twenty-eight studies comparing 2883 infertile to 1294 fertile men demonstrated that infertile men have higher SDF in their ejaculates than fertile counterparts (mean difference: 1.61; 95% confidence interval [CI] 1.21-2.12; $P < .001$).[35] In this study, the authors performed ROC analysis and found that the SDF threshold of 20% best discriminated fertile from infertile men, with an area under the curve

[AUC] of 0.84 ($P < .001$), a sensitivity of 79%, and specificity of 86%. Among the 28 studies included in the meta-analysis by Santi et al, only one study reported SDF values using the Comet assay,[56] which typically reveals higher SDF levels than the sperm chromatin structure assay (SCSA), terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labeling (TUNEL) assay, and sperm chromatin dispersion (SCD) test.[57] The remaining 27 studies, which represented over 95% of the dataset, used TUNEL, SCSA, or SCD. These three assays have shown to be highly correlated, and thresholds ranging from 19% to 23% with AUCs of 0.79 to 0.90 were reported for male infertility diagnosis.[57] Therefore, the SDF threshold of 20% reported by Santi et al is consistent with previous reports, thus suggesting that SDF tests provide distinct and more meaningful information than those of conventional semen analysis.[27,58] Nevertheless, caution should be taken to interpret threshold values when Comet studies are combined with the three assays mentioned above, owing to the increased sensitivity of the former to reveal DNA fragmentation in spermatozoa.

The reasons explaining why SDF values are overall higher in infertile men than fertile counterparts are not fully understood, but current evidence suggests a critical role of OS in the pathophysiology of SDF-associated infertility.[30,59-63] Among men seeking fertility evaluation at one of the authors (SCE) Fertility Center, up to 52% subjects were found to have SDF indices—assessed by the sperm chromatin dispersion (SCD) test—above 20% (Figure 1; unpublished data). Along the same lines and using the commonly reported threshold of 30% for the SCD test,[64] approximately 25% of the men showed high SDF values. Notably, 15% of our patients exhibited high SDF even though conventional semen parameters—according to the 2010 World Health Organization (WHO) criteria—were within normal ranges. Our observations are in line with previously published studies.[8,65] Additionally, data from ART programs using SCSA indicate that SDF affects over 30% of patients undergoing in vitro fertilization (IVF) and ICSI treatment.[66]

2.2 | Natural pregnancy

SDF values assessed by TUNEL and SCSA have been independently associated with the chances of achieving natural pregnancy, with lower SDF thresholds for better outcomes.[47,67-69] Among couples from the general population, SDF by SCSA was associated with failure to achieve natural pregnancy with an odds ratio (OR) of 7.01 (95% CI: 3.68-13.36).[70] High sensitivity of 80-85% and specificity of 85-90% have been reported with the use of SCD and TUNEL in the prediction of natural pregnancy.[71,72] In the SCSA studies, fecundability decreased when SDF values were 20% and over, and for values ≥30%, occurrence of natural pregnancies was quite rare.[73]

Criticism of the studies mentioned above is that they did not utilize live births as an outcome. However, the prospective LIFE (longitudinal investigation of fertility and the environment) study provided level 1 evidence, suggesting that SDF is associated with time to pregnancy (TTP).[74] In this study, carried out in the United States, a total of 473 couples discontinuing contraception whose male partners provided at least one semen sample for evaluation was followed for one year while trying to conceive. Both the DNA fragmentation index (DFI) and the high DNA stainability—assessed by the SCSA assay—were negatively associated with TTP, although the point and interval estimates were close to 1.

2.3 | Intrauterine insemination

Many studies have suggested the adverse role of SDF on pregnancy achievement following intrauterine insemination (IUI). Among couples with unexplained infertility undergoing IUI, pregnancy rates were reduced when SDF values by the SCD test were >20%.[75] Furthermore, the odds of pregnancy were remarkably decreased (by 7.0- to 8.7-fold) in the general population of infertile couples subjected to IUI with sperm specimens from men with SDF levels >30% (by SCSA) in the neat semen.[66,76]

Along the same lines, a 2019 systematic review and meta-analysis compiled the data of ten studies and over 2800 treatment cycles. High SDF, defined in most included studies as values of 25% or higher, using either the SCSA or the SCD test, was associated with decreased pregnancy rates (relative risk [RR]: 0.34, 95% CI 0.22-0.52, $P < .001$). Among the two studies reporting delivery rates after IUI, high SDF values were negatively associated with the likelihood of delivery (RR 0.14, 95% CI: 0.04-0.56, $P < .001$).[77]

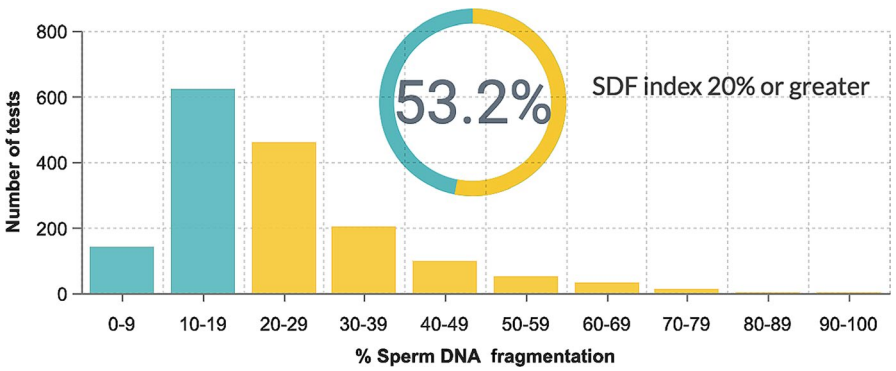

FIGURE 1 Distribution of sperm DNA fragmentation (assessed by the sperm chromatin dispersion test) among men attending a tertiary fertility center. *Source*: Andrology laboratory, ANDROFERT Fertility Center, Campinas, Brazil. Year: 2016-2018

2.4 | In vitro fertilization and intracytoplasmic sperm injection

The most recent meta-analyses of IVF/ICSI studies concur overall that SDF adversely affects pregnancy success. A 2014 study indicated that the likelihood of pregnancy was lower in couples whose male partners had high SDF rates in the ejaculated semen (combined relative risk [RR] = 0.81; 95% CI 0.70-0.95; P = .008).[37] In this report, there was a significant increase in the risk of miscarriage among couples whose male partner had high DNA damage irrespective of the insemination method, IVF or ICSI (OR 2.68, 95% CI 1.40-5.14; P = .003). Furthermore, a 2017 study polling data from 56 studies and over 8,000 treatment cycles showed that SDF adversely affected clinical pregnancy following IVF (OR 1.65, 95% CI 1.34-2.04; P < .0001) and ICSI (OR 1.31, 95% CI 1.08-1.59; P = .0068).[36] In the study mentioned above, the risk of miscarriage also increased among couples undergoing ART with high (versus low) SDF rates (RR 2.16, 95% CI 1.54-3.03; P < .0001). These results were corroborated by a recent meta-analysis of 23 studies comprising 6771 cycles, which showed that among infertile couples whose male partners had high DFI both the clinical pregnancy rates (23 studies; 6771 cycles; RR = 1.57; 95% CI 1.18, 2.09; P < .01) and the miscarriage rates (25 studies; 3992 patients; RR = 0.85, 95% CI 0.75-0.96; P < .01) were negatively impacted; in this study, however, live birth rates were not differentially affected by SDF (10 studies; 1785 couples).[78]

2.5 | Effect of SDF on embryo quality following ART

The role of SDF on human embryo development has yielded mixed results. In a 2011 systematic review of 28 IVF and ICSI studies involving 3226 treatment cycles, SDF was associated with poor embryo quality and/or development in 11 studies, whereas the remaining 17 studies showed no apparent association between SDF and embryo quality and/or development.[79] A limitation of the studies reporting on the effect of SDF on embryo development relates to the lack of adjustment concerning oocyte quality, which was shown to have a role in DNA repair.[21] Notably, a 2017 ICSI study using donor oocytes showed that the blastulation rates were negatively correlated with DFI measured with the TUNEL assay.[80] These findings have been corroborated by recent IVF and ICSI studies using the SCD test,[81-83] although the evidence is not unequivocal.[84]

The subjectivity of embryo assessment using morphological characteristics remains a critical shortcoming of studies evaluating the role of SDF on embryo quality. Non-invasive time-lapse techniques and invasive comprehensive genetic testing might provide more accurate information on overall embryo quality and chromosomal integrity, respectively. ICSI studies using time-lapse technology showed positive correlations between DFI—assessed by the alkaline Comet assay or the SCD test—and the time to reach each stage of embryo development.[85,86] It has been suggested that the type of SDF might differentially affect embryo development, with Comet assay's double-stranded DNA breaks (DS-DB) being more relevant than single-stranded DNA breaks (SS-DB) concerning embryo kinetics and implantation.[85,87] By contrast, recent studies examining blastocyst genetic content using comprehensive 24-chromosome genetic testing suggest that SDF does not seem to affect embryo euploidy status.[88,89]

Collectively, sperm DNA integrity seems essential for healthy human embryo development and successful pregnancy outcome. Current evidence indicates that SDF—measured particularly with the use of TUNEL, Comet, SCD, and SCSA—increases the risk of infertility and impaired reproductive outcomes. Additionally, there seems to be an increased risk of diseases in offspring when natural or artificial inseminations are carried out with specimens from men with high frequencies of fragmented DNA in neat semen, assessed by either the alkaline Comet assay or the SCSA.[22,90,91] The mechanistic effects have not been fully elucidated but seem to involve OS and altered expression of critical genes involved in sperm function, fertilization, and embryo development. However, the data are still limited, and additional prospective studies are necessary to conclusively determine the role of SDF values on natural pregnancy, IUI, and ART. Nonetheless, given the ubiquity of SDF among men facing infertility issues, it is suggested that SDF indices are clinically useful for male infertility diagnosis and management.

3 | CURRENT RECOMMENDATIONS FOR SPERM DNA FRAGMENTATION TESTING

Owing to the essential role of sperm DNA integrity for normal embryo development and successful pregnancy outcome, assessments of sperm DNA damage have been used to obtain information about sperm DNA quality. Despite acknowledging that SDF can be clinically informative for ART outcomes, professional societies, such as the American Society for Reproductive Medicine (ASRM), the American Urological Association, and the European Association of Urology, have not recommended SDF testing in the routine infertility workup.[4,39,55,92] Possible reasons relate to technical issues concerning assays' distinct characteristics and patient population heterogeneity, which have resulted in ambiguous conclusions concerning the clinical utility of SDF testing.[27,58,93] In particular, the ASRM Practice Committee underlies the fact that existing data concerning the association between SDF and reproductive outcomes are too limited to routinely recommend the use of SDF testing for the male partner in an infertile couple. Moreover, it adds that *"…because the prognostic clinical value of DNA integrity testing may not affect the treatment of couples, the routine use of DNA integrity tests in the clinical evaluation of male-factor infertility is controversial."* Nevertheless, in 2015, the ASRM guidelines acknowledged that (i) *"Varicocele repair and antioxidant use may affect sperm DNA integrity,"* and (ii) *"Sperm retrieved from the testis tend to have better sperm DNA quality in men with abnormal ejaculated sperm DNA integrity,"* although a note of caution indicated that "no treatment for abnormal DNA integrity has been proven to have clinical value".[55]

Since the publication of the guidelines mentioned above, new evidence supports a remarkable relationship between SDF and clinical varicocele, unexplained infertility, ART outcomes, and environmental and lifestyle factors, as we will discuss in the following sections. Furthermore, new data emerged concerning the potential benefit of clinical and surgical interventions as a means to reduce SDF, thus emphasizing the need for updates in the existing clinical recommendations.

Along these lines, two recent clinical practice guidelines (CPG) provide specific recommendations concerning SDF testing. The CPG on recurrent pregnancy loss (RPL) issued by the European Society for Human Reproduction and Embryology stated that "*assessing SDF in couples with RPL*—defined by two or more pregnancy losses from the time of conception until 24 weeks of gestation—*can be considered for explanatory purposes, based on indirect evidence*".[94] A 2019 systematic review and meta-analysis of thirteen prospective studies pooling 579 male partners of women with recurrent pregnancy loss and 434 male partners of fertile control women demonstrated that DFI rates were significantly higher in the former (mean difference [MD] 11.9%, 95% CI 4.9%-18.8%). The pooled MD was higher for the TUNEL assay (14.2%, 95% CI 4.86-23.64) than the SCD test (3.5%, −3.30-10.3%), the Comet assay (5.2%, 95% CI 0.31-10.1), and the SCSA (10.1%, 95% CI 2.1-18.1).[95]

The 2017 CPG on SDF testing based on clinical scenarios issued by the Society for Translational Medicine (STM) also provided evidence-based guidance for recommending SDF testing.[96] This guideline translated the current evidence and provided a framework of standardized care based on clinical scenarios (Table 1). Specifically, the STM guideline recommends testing for couples with unexplained infertility and those suffering from RPL. Male patients with risk factors for oxidative stress, including but not limited to lifestyle conditions (eg, smoking, obesity, metabolic syndrome), varicocele, genital infections, advanced age, and exposure to toxicants (eg, environmental, licit or illicit drugs, radiation, chemotherapy), should also be screened. The guideline mentioned above goes on by recommending testing after failed IUI, IVF, or ICSI provided no other apparent reasons exist to explain that failure. The CPG by the STM was the first collaborative attempt to aggregate the existing evidence and provide clinicians with evidence-based guidance for interventions. The document was reviewed by many experts across the globe, whose authoritative commentaries on its clinical utility can be found elsewhere.[58,97-99]

TABLE 1 Clinical practice guidelines for sperm DNA fragmentation testing by the Society for Translational Medicine

I. Sperm DNA fragmentation testing[a]:
• Neat semen sample should be used for SDF testing • A fixed ejaculatory abstinence before collection of semen sample should be applied • A standardized protocol with stringent quality control is essential for a reliable SDF testing result • SDF threshold reflects the probability on reproductive outcome[b]
II. Recommendations:
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)
Unexplained infertility/IUI failure/recurrent pregnancy loss: • SDF testing should be offered to infertile couples with RPL or prior to initiating IUI (grade C recommendation) • Early IVF or ICSI may be an alternative to infertile couple with RPL or failed IUI (grade C recommendation)
IVF and/or ICSI failure: • SDF testing is indicated in patients with recurrent failure of assisted reproduction (grade C recommendation) • The use of testicular sperm rather than ejaculated sperm may be beneficial in men with oligozoospermia, high SDF, and recurrent IVF failure (grade B–C recommendation)
Borderline abnormal (or normal) semen parameters with risk factor: • SDF testing should be offered to patients who have a modifiable lifestyle risk factor of male infertility (grade C recommendation)

Note: SDF, sperm DNA fragmentation; RPL, recurrent pregnancy loss; IUI, intrauterine insemination; IVF, conventional in vitro fertilization; ICSI, intracytoplasmic sperm injection. Adapted from 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. *Transl Androl Urol* 6(Suppl. 4): 720-733.

[a]Grade B-C recommendation.

[b]SDF levels represent one of the many variables that can affect a couple's reproductive outcome.

4 | DIAGNOSTIC METHODS TO MEASURE SDF

SDF is characterized by the presence of SS-DB and/or DS-DB affecting the DNA. SS-DBs give rise to free 5′–3′ ends affecting only one DNA strand whereas DS-DBs produces blunt 5′–3′ ends affecting both DNA strands. These breaks can be detected in the neat or processed ejaculate, as well in spermatozoa retrieved from the epididymis or testicle, using a variety of tests. Probes or dyes are used to identify DNA breaks with the aid of fluorescence microscopy, optical microscopy, and flow cytometry according to the method type (reviewed by[10]).

The four commonly used tests to measure DNA fragmentation in human spermatozoa are the SCSA,[100] the TUNEL assay,[101] single-cell gel electrophoresis (Comet) assay,[102] and the SCD test.[103] A 2017 survey among forty-nine infertility specialists from nineteen countries indicated that SCSA and SCD test were the most common methods used to evaluate SDF, followed by TUNEL and Comet assays.[97]

Despite often used to refer to any test that assesses the sperm chromatin integrity, the term "fragmentation" should be reserved for the assays mentioned above as not all sperm chromatin damage relates to breaking the DNA into "fragments".[104] Other assays, including aniline blue staining, chromomycin A3 staining, and toluidine blue staining, do not measure SDF,[10,27,58,105] these tests provide a crude measurement of the level of chromatin compaction.[106]

Defects in nucleus condensation predominantly relate to protamine mispackage via defective DNA-protein ionic links at later stages of spermatogenesis, whereas SS-DB and DS-DB are primarily associated with OS.[11,12,22,87,107]

Although deficient chromatin compaction renders the sperm DNA more susceptible to ROS attack, the effect depends on the semen redox properties and OS levels.[27] By contrast, excessive ROS directly affects the sperm membranes and chromatin by inducing DNA breaks.[61,108,109] Thus, tests that measure SDF should be preferred over those that assess chromatin compaction owing to the ubiquity of OS in men with infertility.[11,27,58] In the next sections, we will focus only on the four major SDF tests, namely TUNEL, SCSA, SCD, and Comet.

4.1 | Sperm DNA fragmentation assays

These methods act by incorporating either DNA probes/dyes or modified nucleotides at the site of damage with or without the use of heat, acid, or lysis solution to (open) the DNA strands at the sites of existing single or double DNA strand breaks.[54,110]

The TUNEL assay relies on a terminal deoxynucleotidyl transferase (TdTA) enzyme for the "direct" labeling of 3′ free ends of DNA. The sites of breaks can be identified with the aid of optical fluorescence microscopy or flow cytometry.[101,111,112] The TUNEL test essentially assays the protamine toroid linker DNA as the relatively large TdTA molecule might not penetrate the highly compacted protamine toroid.[105] Protocol modifications have been introduced to increase its sensitivity, including DNA decompaction using dithiothreitol (DTT).[113,114]

SCSA is based on acid denaturation of the chromatin at the sites of existing single- or double-strand breaks.[115] Acridine orange (AO) is used for staining, the dye penetrates the sperm chromatin and intercalates into double-stranded DNA (intact DNA), which fluoresces green when exposed to blue laser light. By contrast, AO attachment to single-strand DNA creates a complex that produces a metachromatic shift to red fluorescence. The red fluorescence represents DNA strands that originate from the sites of single- or double-strand breaks.[116] The fluorescence patterns emitted by spermatozoa are captured using a flow cytometer, and the ratio of red to total (green + red) fluorescence intensity is used to calculate the percentage of spermatozoa with DNA fragmentation (DFI, DNA fragmentation index.[47,117]

The SCD test relies on the principle that spermatozoa with DNA fragmentation fail to produce the characteristic halo of dispersed DNA loops that are observed in spermatozoa with non-fragmented DNA, following acid denaturation and removal of nuclear proteins.[103] Sperm suspensions are embedded in agarose gel on slides and treated with an acid denaturation solution (HCl) to generate restricted single-strand DNA motifs at the sites of existing single- or double-strand breaks. The denaturation is stopped, and spermatozoa are exposed to a lysing solution based on DTT, sodium dodecyl sulfate, and NaCl to remove the sperm membrane and nuclear proteins. Then, the slides are stained with DAPI (4′,6-diamidino-2-phenylindole) or the Diff-Quik stain, and the percentages of spermatozoa with nondispersed and dispersed chromatin loops are manually assessed by fluorescence or bright-field microscopy, respectively.[103,118] The halos correspond to relaxed DNA loops attached to the residual nuclear structure as seen in spermatozoa with low or no SDF. By contrast, spermatozoa with very small or no halos correspond to those exhibiting SDF as confirmed by DNA breakage detection-fluorescence in situ hybridization, a procedure in which the restricted single-stranded DNA motifs generated from DNA breaks can be detected and quantified.[103]

Lastly, the Comet assay relies on DNA decompaction and protein depletion coupled to single-cell electrophoresis in agarose microgel.[102] The removal of protamines and histones creates a nucleoid-like structure containing supercoiled loops of DNA.[119] Alkaline or neutral pH conditions allow the uncoil of double-stranded DNA, which under electrophoresis results in migration of fragments of single- and double-stranded DNA toward the anode, thus forming a comet tail that can be observed under fluorescence microscopy.[10,120] The relative fluorescence in the tail compared with its head reflects the level of SDF; spermatozoa with increased fluorescence intensity in the comet tails have high levels of chromatin damage. Additional quantitative parameters can be used to increase the test's precision, such as nucleus diameter, olive tail movement, and comet length. The alkaline Comet assay detects both single- and double-stranded DNA breaks, as well as alkali-labile sites, whereas the neutral Comet assay detects double-stranded DNA breaks only. Alternatively, the two-tailed Comet assay can assess both types of breaks in the same spermatozoon.[121] The assay firstly applies neutral lysis and electrophoresis to detect double-strand breaks, and then, by turning the slide 90° and applying alkaline lysis and electrophoresis, single-strand breaks are detected.

4.2 | Correlation of test results, biological variation, and standardization

Although the SDF assays discussed above rely on different mechanisms for DNA breaks detection, there is an overall good correlation among TUNEL, SCSA, SCD, and alkaline Comet.[11,57,122] In a study involving both fertile and infertile men, high correlations were found between the SCD test and SCSA (r = 0.71, P < .001), between SCD test and TUNEL assay (r = 0.70; P < .001), and between SCSA and the TUNEL assay (r = 0.79; P < .001), whereas moderate correlations were found between the alkaline Comet assay and the SCD test (r = 0.61; P < .001), between the alkaline Comet and SCSA (r = 0.59; P < .001), and between the alkaline Comet and TUNEL assay (r = 0.72; P < .001).[57] These results have been corroborated by a 2019 study using similar design and methods.[123] Thus, it has been suggested that irrespective of the assay, SDF test results can provide information concerning the quality of the whole semen specimen, not just the damaged spermatozoa detected by the test.[124]

Furthermore, SDF seems to have lower biological intra-individual variation than conventional semen parameters.[100,125,126] In one report, the DFI coefficient of variation (CV)—measured with the aid of SCSA—was significantly lower (9.2%) than that of sperm count (43.0%), progressive motility (28.3%), and sperm morphology (28.3%).[125] Notwithstanding, a study involving 282 infertile men undergoing IUI or ART revealed that the mean CV of DFI for repeated SCSA measurements was 29%, with about 1/3 of the patients crossing the threshold levels in repeated analyses. In this study, however, the ejaculatory abstinence period was quite variable (mean ± SD: 4.6 ± 3.3 days), thus potentially affecting results as there is an inverse relationship between abstinence time and SDF values.[127] Further research is needed as data are still limited concerning the biological variability of SDF using different testing methods.

Proper standardization also remains an issue concerning SDF tests.[58] However, efforts to make protocols standardized and reproducible are increasing steadily, in particular, concerning the flow cytometer TUNEL assay and SCD test, recent reports show low intraobserver variability and high interobserver agreement, thus increasing precision of estimations.[112,128-130]

4.3 | SDF test thresholds

Threshold values beyond which a semen sample can be considered pathological vary according to SDF assay and clinical effect. Although no definite reference values have been agreed upon, cutoff values have been reported for SDF testing with regard to pregnancy prediction and infertility risk.

For instance, threshold values in the range of 16%-22% for TUNEL assay, 18%-20% for SCSA, 20%-26% for the SCD test, and 40%-50% for alkaline Comet assay have been utilized to discriminate fertile from infertile men.[57,72,123] Moreover, a 2018 systematic review of 28 studies including 2883 infertile men and 1294 fertile men indicated that the threshold of 20% was the best value to discriminate fertile from infertile men considering the four most common SDF tests, with a sensitivity of 79%, specificity of 86%, and an area under the curve [AUC] of 0.844. In this report, most studies utilized the TUNEL assay; the ROC built on this subgroup of studies confirmed the overall fit of the analysis (AUC 0.831; $P = .002$).[35]

An early study using the SCSA assay on neat semen indicated that the cutoff value of 30% had high predictive power for the likelihood of pregnancy both in vivo and ART; patients with a SDF < 30% were 7.1 times [95% confidence interval (CI), 3.37-14.91] and ~2.0 times (95% CI, 1.10-2.96) more likely to achieve a pregnancy in vivo and after ART, respectively.[131] However, these results did not hold when the SCSA assay was carried out on specimens processed by the density gradient centrifugation, as no DFI cutoff values could be set for in vivo fertility or clinical pregnancy after IUI and ART.[132]

Furthermore, a DFI of 25.5% by SCD was shown to have a sensitivity of 86.2% and a negative predictive value (NPV) of 72.7% ($P = .02$) in predicting pregnancy achievement in ART treatment.[133] In a recent study, SDF values by SCSA of 27% and over were negatively associated pregnancy outcome with IUI ($\chi^2 = 6.87$, $P < .05$) and ICSI (OR = 5.65; 95% CI: 4.32-7.11; $P < .05$) (.[76]

Collectively, the existing data suggest that the threshold range of 25-30% by SCSA or SCD assessed on neat semen is clinically useful for placing infertile couples into a statistical probability of longer time to achieve natural pregnancy, low odds of pregnancy by IUI and conventional IVF, and a higher risk of miscarriage, both natural and assisted.[134,135] Despite that, the evidence is not equivocal as others have found no robust association between SDF values and pregnancy achievement.[38,137] It is therefore crucial that clinicians be judicious when using this information to predict reproductive outcomes. Consideration of female factors is important as the presence of SS-DB might be repaired by the oocyte repair machinery, thereby alleviating the adverse consequences of SDF. Given the multitude of confounding factors, various cutoff values may be required to ensure optimal performance of the test in distinct clinical scenarios.

In summary, the ideal method to measure SDF and its optimal thresholds are still to be determined, but the four major tests, namely TUNEL, SCSA, Comet, and SCD, seem reliable to provide information about sperm DNA quality and possibly for the identification of a male factor contributing to infertility. Nevertheless, factors such as type (SS-DB or DS-DB), site (intron or exons), and extent of DNA damage on each spermatozoon, along with the ability of the oocyte to repair SDF during fertilization most likely influence the predictive power of SDF tests. Given the different mechanisms for DNA break detection, the type of SDF detected by each assay may be complementary to each other in different clinical settings. Thus, it is critical that clinical decisions are made considering the technical limitations of the assays and the possible benefits of SDF testing for clinical outcomes.

5 | INTERVENTIONS TO DECREASE SPERM DNA FRAGMENTATION

5.1 | Varicocele repair

5.1.1 | Introduction

Varicocele is regarded as the most frequent cause of male infertility.[30,61] The testis responds to varicocele-associated cell stressors, such as heat stress, ischemia, or reflux of adrenal and renal metabolites, by producing excessive amounts of free radicals, which might ultimately lead to SDF.[59] Indeed, the levels of seminal OS markers (ROS, nitric oxide, lipid peroxidation products) are usually higher in both fertile and infertile men with varicocele than in counterparts without varicocele.[108,136,138-140] Moreover, infertile men with varicocele have decreased seminal antioxidant capacity compared to fertile men with varicocele.[141-143] About half of subjects with clinical varicoceles exhibit high SDF values,[144,145] and coincidentally,

OS markers are usually elevated in varicocele men with high SDF indices.[59]

5.1.2 | Hypothesis

It has been hypothesized that varicocele repair might ameliorate OS markers and consequently, SDF indices, with a positive effect on fertility.

5.1.3 | Treatment

The standard treatment for varicocele is surgical repair by an open approach (with or without magnification), laparoscopy, or through percutaneous embolization of the internal spermatic vein. Regardless of the chosen technique, the ultimate goal is the occlusion of the dilated veins that compose the pampiniform plexus. [30,63]

5.1.4 | Summary evidence

Several controlled studies have shown that varicocelectomy mitigates OS and boosts sperm chromatin integrity, thus underscoring the association between varicocele, OS, and SDF[143,146-155] (Table 2). In non-controlled studies, a positive effect on SDF values—as determined by the SCSA—could be observed in 78-90% of the treated patients 3-6 months postoperatively.[144,145]

Reduction in SDF by varicocele repair seems to translate into higher chances of initiating a pregnancy. In one study including 49 infertile men with clinical varicocele and oligozoospermia,[156] couples that become pregnant either naturally or with ART had lower postoperative SDF levels (26.6 ± 13.7%, SCSA) than those who did not (37.3 ± 13.9%; $P = .013$). In another study which enrolled 42 subfertile patients with clinical varicocele and abnormal semen parameters,[152] SDF (by SCSA) decreased significantly from 28.4% to 22.4% ($P = .018$) after varicocele repair, despite that postoperative results were still higher than controls. Nonetheless, SDF values in patients who achieved pregnancy naturally after varicocelectomy (20.6 ± 3.5%) were not significantly different from controls (11.5 ± 3.9%). Moreover, SDF values in pregnant couples were lower than both preoperative values (27.4 ± 6.3%, $P < .01$) and non-pregnant patients (24.7 ± 6.5%; $P < .010$).

By contrast, Baker et al evaluating a cohort of 24 infertile men with clinical varicocele who underwent microsurgical varicocele repair showed that although SDF index was reduced after varicocele repair, results were not different in pregnant and non-pregnant couples (TUNEL DFI 22.2% ± 14.4% vs. 25.7% ± 14.5%, respectively).[148]

A 2018 systematic review compiled the data of 21 studies concerning the effect of varicocele repair on SDF, including 1270 infertile men.[63] Despite using different SDF sperm assays, mixed design, and variable sample size, all studies involving infertile men with palpable varicocele unequivocally reported a significant postoperative decrease in SDF rates in a follow-up period ranging from 3 to 12 months. Of the studies providing pregnancy outcomes,[148,152,156,157] postoperative SDF rates were lower in men from couples who achieved pregnancy success than those who did not in three out of four studies.[152,156,157] In this review, the vast majority of studies that also assessed OS markers reported a concomitant reduction in OS markers and SDF after varicocele repair.

Nineteen of the 21 studies mentioned above provided data to be meta-analyzed.[158] A total of 1153 men with clinical varicocele who had their varicoceles repaired were included. Overall, there was a significant decrease in the SDF levels after varicocele repair (MD −8.3%, 95% CI −10.3%, −6.4%, $P < .0001$). Given the heterogeneity of SDF assays, the results were also analyzed according to the method of SDF measurement, in particular, TUNEL and SCSA, which were the methods used in most studies. The decrease in SDF after varicocele repair remained statistically significant regardless of the SDF method (SCSA: MD −7.1%; 95% CI −9.3%, −4.9%; $P < .0001$, and TUNEL: MD −15.0%; 95% CI −21.3%, −8.7%; $P<.0001$).

5.1.5 | Mechanism of action

It has been speculated that in men with varicocele SDF is probably a critical OS endpoint (Figure 2). The concurrent impairment in sperm DNA integrity and elevated OS markers supports this assumption. ROS and nitrogen species are released in the endothelium of varicose veins, testicular cells, and principal cells of the epididymis.[59,159] The imbalance between excessive ROS production and antioxidant protection harms the sperm membrane via lipid peroxidation, and the chromatin through nuclear and mitochondrial DNA breaks.[61,108,109] As a result, the overall sperm DNA quality is reduced, thus making a subset of varicocele men less fertile.[7,59,61,160] Varicocele repair might ameliorate OS markers and consequently, SDF indices, with a positive effect on fertility.[63]

Nevertheless, it is still unclear how infertility is obviated in fertile men with varicocele. Individual factors, including antioxidant enzymes such as catalase, superoxide dismutase, vitamin C, and glutathione peroxidase, could protect fertile men from the adverse effect of varicocele.[59] Additionally, other defensive mechanisms might act synergistically, including a slower rate of germ cell apoptosis, increased turnover machinery for the oxidized proteins impeding their aggregation, and reduced cellular signal-transducing effects of ROS.[30,161] By contrast, disruption of these protective mechanisms could exacerbate the harmful effects of oxidation in infertile varicocele men.

5.1.6 | Conclusion

Collectively, current evidence underpins OS as a central factor in the pathophysiology of infertility caused by varicocele. The testis and epididymis respond to OS via distinct mechanisms, including the production of antioxidants that might sustain the fertility potential in men with varicocele. Failure of these mechanisms could explain

TABLE 2 Controlled studies evaluating the effect of varicocelectomy on sperm DNA fragmentation

Author and Year	Design	Patients	Controls	SDF testing method	Oxidative stress and/or other sperm function markers	Varicocele repair method	Main Results
Sakamoto et al[143]	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 varicocele repair	SDF values 6 mo after surgery were lower than preoperative values (post-op: 27.5 ± 19.4%; pre-op: 79.6 ± 13.6%; $P < .001$). Oxidative stress markers were significantly higher in varicocele patients than controls ($P < .05$). There was a significant reduction in the level of NO, HEL, 8-OHdG, and SOD activity after surgery.
Lacerda et al[150]	Prospective cohort	21 adolescents between 15 and 19 y old with grades 2 or 3 varicocele subjected to varicocelectomy	NA	Comet	Mitochondrial activity and thiobarbituric acid-reactive substances (TBARS) levels	Microsurgical subinguinal varicocele repair	Comet class I cells (undamaged DNA) increased after varicocelectomy (49.6 ± 23.1% to 64.5 ± 13.6%; $P = .011$); Percentage of spermatozoa with mostly inactive mitochondria (diaminobenzidine [DAB] class III) decreased after varicocelectomy (20.2 ± 4.9 to 17.1 ± 3.2; $P = .013$). The TBARS levels remained unaltered.
La Vignera et al[149]	Not specified	30 men (mean age: 26.5 y) 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 varicocele repair	SDF values decreased after surgery (4 mo) from 5.0 ± 3.0% to 2.1 ± 0.4% ($P < .05$); the postoperative SDF results were similar to that of healthy controls (2.0 ± 1.0%); After surgery, the % of spermatozoa with low MMP was reduced (post-op.: 2.0 ± 0.6%; pre-op.: 28.0 ± 4.0%, $P < .05$), and results were not different than controls (2.0 ± 0.6%). Similarly, the % of spermatozoa with PS externalization (3.0 ± 3.0% vs. 9.0 ± 4.0%; $P < .05$) and decondensed chromatin (6.0 ± 0.5% vs. 22.0 ± 4.0%; $P < .05$) was lower than pre-op. values, and the postoperative results were not difference than controls (4.0 ± 2.0% and 6.0 ± 2.0%, respectively)
Li et al[151]	Not specified	19 infertile men (mean age: 33.1 y) with clinical varicocele (left: 19 patients; bilateral: two patients) subjected to varicocelectomy	19 normozoospermic controls	SCSA	Not assessed	Microsurgical subinguinal varicocele repair	DFI decreased from 28.4 ± 15.6% before surgery to 22.4 ± 12.9% 3 months after surgery ($P = .018$); the postoperative DFI in varicocele group was similar to controls
Baker et al[148]	Retrospective cohort	22 men with clinical varicocele subjected to varicocelectomy	NA	TUNEL	ROS and TAC levels	Microsurgical subinguinal varicocele repair	DFI decreased from a preoperative mean of 40.8% to a postoperative mean of 24.5% ($P = .001$. A higher preoperative DFI was associated with a larger decrease in postoperative DFI (r2 = 0.53; $P = .01$); DFI results in pregnant and non-pregnant couples did not differ (22.2 ± 14.4 vs. 25.7 ± 14.5%, respectively); Mean TAC decreased from 2292 μM preoperatively to 1885 μM postoperatively ($P = .03$) 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

(Continues)

TABLE 2 (Continued)

Author and Year	Design	Patients	Controls	SDF testing method	Oxidative stress and/or other sperm function markers	Varicocele repair method	Main Results
Ni et al[152]	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 varicocele repair	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%; $P < .01$) and non-pregnant patients (24.7 ± 6.5%; $P < .01$); In grade 3 group, P1/P2 mRNA ($P < .05$) and DFI ($P < .01$) were significantly improved, while in grade 2 group, only DFI was improved ($P < .05$). In grade 1 patients, no differences were noted in P1/P2 mRNA ratio and DFI
Pourmand et al[154]	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	DFI decreased after surgery in both groups (Group 1:14.0% vs. 9.5%; $P = .02$; Group 2:13.9% vs. 8.5%, $P < .001$); results were not different between groups; Improvement in protamine damage from before to after surgery (6 mo) in group 2 only (44.9% vs. 33.7%, $P < .001$)
Tavalaee et al[155]	Not specified	23 infertile men (mean age: 31.3 y) 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% pre-op. vs. 10.8 ± 1.1% post-op., $P < .001$), % spermatozoa with protamine deficiency (46.7 ± 2.6% pre-op. vs. 39.4 ± 2.6% post-op.; $P = .02$), and % spermatozoa with OS (47.6 ± 6.6% pre-op. vs. 36.6 ± 3.8% post-op.; $P = .03$) improved 3 months after surgery; Percentage of spermatozoa 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 ($P = .03$)
Alhathal et al[147]	Prospective cohort	29 infertile men with clinical varicocele and abnormal semen parameters subjected to varicocelectomy	Six healthy sperm donors with normal sperm parameters	SCSA	Sperm DNA decondensation (aniline blue and iodoacetamide-fluorescein)	Microsurgical subinguinal varicocele repair	SDF values decreased after surgery (from 20.0 ± 10.6% to 12.0 ± 5.7%; $P = .001$). Similarly, the %AB staining (from 13.5 ± 7.0% to 5.4 ± 2.7%; $P = .0004$) and %5-IAF (16.3 ± 6.0% to 5.4 ± 2.7%; P0.0004) also decreased after surgery

(Continues)

TABLE 2 (Continued)

Author and Year	Design	Patients	Controls	SDF testing method	Oxidative stress and/or other sperm function markers	Varicocele repair method	Main Results
Ni et al[153]	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 of varicose veins	Varicocelectomy reduced SDF in patients with abnormal semen parameters and grades 1–3 clinical varicocele: Grade 1 (n = 19): pre-op. 23.5 ± 7.5%, post-op. 3 months: 20.8 ± 5.6%, post-op. 6 months 19.5 ± 5.5%; $P < .01$; Grade 2 (n = 18): pre-op. 27.7 ± 9.0%, post-op. 3 months: 22.9 ± 5.2%, post-op. 6 months 22.4 ± 4.5%; $P < .01$; Grade 3: pre-op. 30.0 ± 8.2%, post-op. 3 months: 23.3 ± 5.4%, post-op. 6 months 21.8 ± 5.9%; $P < .01$; Among couples in whom the partner had the varicocele treated, DFI and MDA levels were lower in those who achieved pregnancy than non-pregnant couples ($P < .05$); After surgery, a significant reduction in seminal MDA was observed at 3 months (grade 2, $P < .01$; grade 3, $P < .05$) and 6 months (grade 1, $P < .05$; grade 2 and 3, $P < .01$); these values were similar to that of controls
Abdelbaki et al[146]	Prospective controlled cohort	60 infertile men (median age: 31 y) 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 varicocele repair with loop magnification	SDF values had a positive correlation ($r = 0.654$; $P < .001$) with ROS levels, grade of varicocele and duration of infertility, and a significant negative correlation with TAC ($r = -0.79$; $P < .001$); SDF values (18.8 ± 7.2%, $P < .001$) and ROS levels (3.3 ± 1.3 Log(ROS + 1) photons/min, $P < .001$) decreased after varicocelectomy whereas TAC levels increased (2.0 ± 0.5 mM) at a 3-month postoperative follow-up.

Abbreviations: DCFH-DA: 2′, 7′-dichlorodihydrofluorescein diacetate; DFI, DNA fragmentation index; MDA, malondialdehyde; MMP, mitochondrial membrane potential; OS, oxidative stress; NA, not applicable; NR, not reported; ROS, Reactive oxygen species; SCSA, sperm chromatin structure assay; SCD, sperm chromatin dispersion assay; SDF, sperm DNA fragmentation; TAC, total antioxidant capacity; TUNEL, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling.

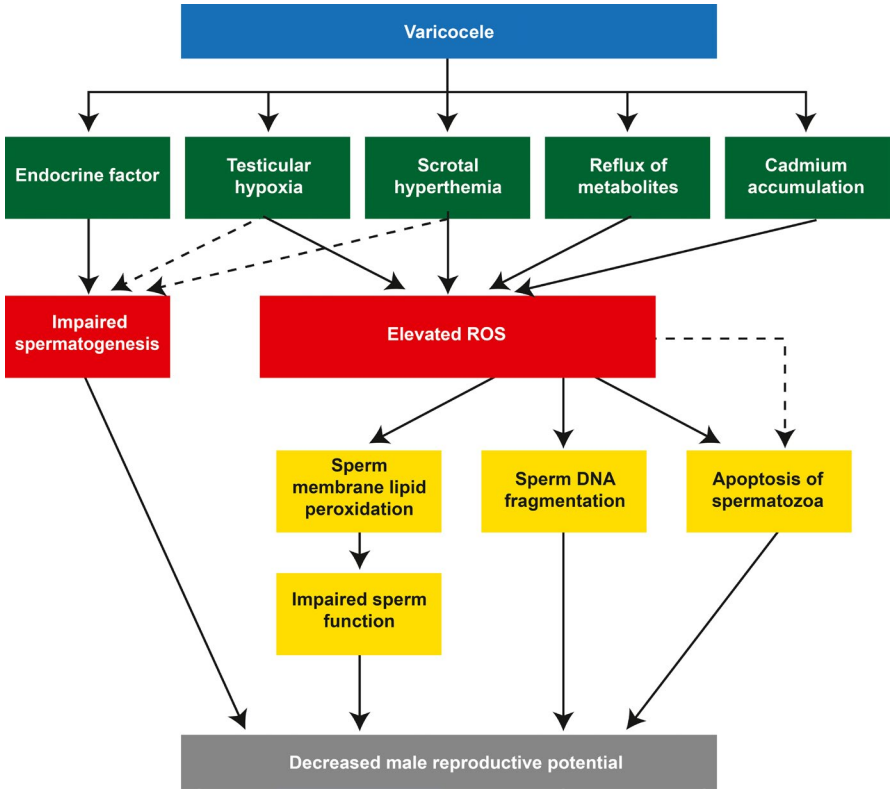

FIGURE 2 Pathophysiology of varicocele and its association with oxidative stress and sperm DNA fragmentation

testicular and epididymal dysfunctions, and consequently infertility, in some men with varicocele. Increased SDF levels, commonly seen in men with clinical varicocele, are plausibly the endpoint of the oxidative-induced damage. Current data reassure the clinical utility of varicocele repair to both alleviating oxidatively induced SDF and increasing the likelihood of both natural and assisted pregnancy (reviewed by[63]). Therefore, it has been suggested that practitioners providing care to infertile couples should advise those men with palpable varicoceles of the connection between sperm DNA damage and oxidative stress and discuss varicocele repair as a possible way to reduce SDF and improve fertility[96] (Figure 3).

5.2 | Treatment of male genital tract infection

5.2.1 | Introduction

Specific microbial pathogens activate seminal leukocytes. The latter generates a significant amount of ROS, thereby causing OS and eventually affecting sperm DNA integrity.[162] Among asymptomatic patients with ultrasound signs of male genital tract infection (MAGI), aerobes (enterobacteria and Gram-positive bacteria), anaerobes, *Chlamydia trachomatis*, and *Ureaplasma urealyticum* are detected in 58%, 11%, 20%, and 11% of patients, respectively.[163] Oxidative stress markers and SDF indices are higher in men with bacteriospermia or leukocytospermia.[164,165] It has been suggested that ART outcomes could be affected in infected patients due to impaired sperm chromatin integrity.[166]

5.2.2 | Hypothesis

It has been hypothesized that among infertile men with MAGI, SDF is caused by excessive OS generated by leukocytes. Leukocytes are the primary source of ROS in the male reproductive tract. Treatment of the underlying infection decreases the number of leukocytes, thus normalizing OS levels and consequently, SDF indices, with a positive effect on fertility.

5.2.3 | Treatment

MAGI is usually treated with antibiotics, in particular, broad antibacterial spectrum agents against Gram-negative and Gram-positive pathogens, as well as *Chlamydia trachomatis* and *Ureaplasma urealyticum*. The preferred remedies are quinolones (eg, ofloxacin), tetracyclines (eg, doxycycline), or macrolides (eg, azithromycin), which are excreted primarily by the kidneys with minimal metabolism and therefore have adequate penetration into the inflamed sexual glands, low adverse effect on spermatozoa, and high specific in vitro susceptibility.[167-169]

5.2.4 | Summary evidence

Among 122 asymptomatic men with MAGI (>10^5 colony-forming units (CFU)/ml], seminal leukocytes and ROS levels were significantly reduced after antibiotic treatment.[163] In this study, the female

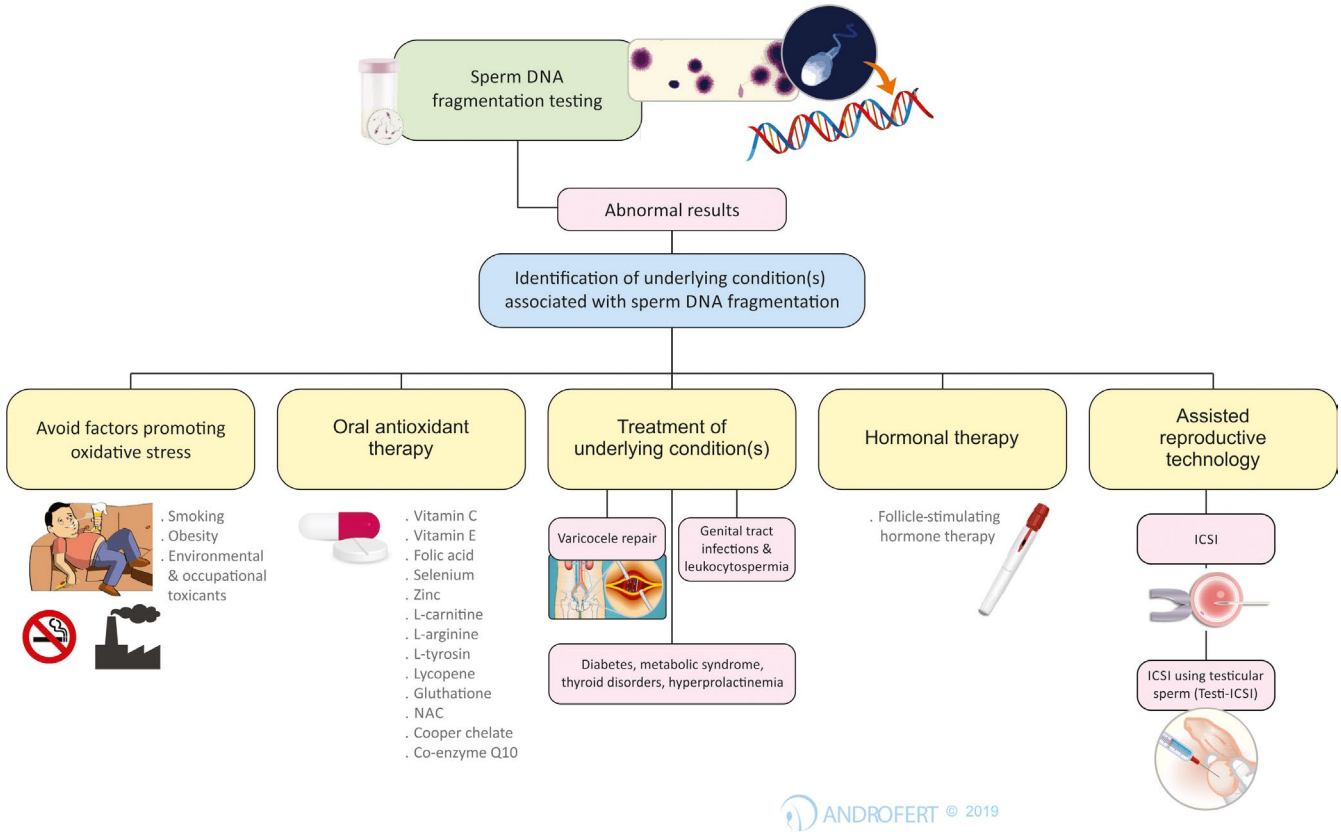

FIGURE 3 Medical and surgical strategies to potentially alleviate sperm DNA fragmentation and improve reproductive outcomes

partners were also treated, and there was a significant difference in the likelihood of achieving natural pregnancy between treated (28.5%) and untreated couples (5.4%, $P = .0097$). Notably, ROS production was reduced in parallel with the reduction in microbial and/or white blood cells, in particular, among couples achieving pregnancy success. SDF, however, was not assessed.

Another study evaluated fourteen infertile patients with bacteriospermia and SDF values over 30%—as measured by SCSA—who completed a two-week course of antibiotics.[144] *Enterococcus* and *Enterobacteria* (*Enterobacter cloacae* and *Escherichia coli*) were treated with ciprofloxacin (500 mg twice a day or extended-release (XL) 1000 mg daily) or amoxicillin (500 mg three times a day), whereas *Ureaplasma urealyticum* was treated with azithromycin 250 mg for five days. In this study, SDF rates were significantly lower after antibiotic treatment, even in patients with a coexistent palpable varicocele (pre-treatment: 53.4% ± 24.3 vs. post-treatment: 43.5% ± 20.1; $P < .01$).

In a study involving men with MAGI caused by chlamydia or mycoplasma, SDF rates (by SCD test) were 3.2 times higher (35.2% ± 13.5%) than in controls without infection (10.8% ± 5.6%).[170] In this study, seminal leukocytes were 5.2 times higher in patients than in controls. Following specific antibiotic therapy using a macrolide, a tetracycline, or a quinolone, combined with a course of anti-inflammatory agents, SDF rates decreased in 91% of patients, from 37.7% ± 13.6% to 24.2% ± 11.2% ($P < .0001$), with a 35.7% median SDF relative improvement after treatment. In the above study, a total of 86% of couples that attempted pregnancy succeeded 3-6 months after therapy. Lastly, when comparing patients with and without pregnancy success, the only differences found were a lower SDF index in the former (32.2% ± 7.6% vs. 43.3% ± 14.1%, $P = .047$), as well as better sperm morphology.

5.2.5 | Mechanism of action

Antibiotic treatment reduces microbial and therefore the number of activated ROS-producing leukocytes. Consequently, a reduction in OS markers might be achieved, with a positive effect on oxidatively induced SDF.

5.2.6 | Conclusion

Collectively, the current evidence suggests that MAGI increases inflammatory response and ROS production, ultimately affecting sperm chromatin integrity. Antibiotic treatment can reduce seminal leukocytes and ROS levels, thus lowering the frequency of spermatozoa with fragmented DNA (Figure 3). However, data on pregnancy outcomes in treated and untreated men with MAGI remain scanty. Minimal evidence suggests that antibiotic therapy might be relevant to increase the likelihood of pregnancy in infertile men with MAGI.

5.3 | Treatment of endocrine disorders

5.3.1 | Introduction

Diabetes and metabolic syndrome are associated with impaired spermatogenesis, poor sperm chromatin quality, and sexual dysfunction.[171-174] The subjacent mechanisms are not fully understood but seem to include dysfunction of the hypothalamic–pituitary–gonadal axis, OS, disrupted sympathetic innervation and increased inflammatory response by interleukins (such as IL-17 and IL-18).[175] Furthermore, thyroid dysfunction seems to adversely affect the testicular function and redox status.[176] Men with hyperthyroidism or hypothyroidism can have abnormal serum levels of sex hormone-binding globulin as well as free and bioavailable testosterone concentrations. Hyperprolactinemia might also lead to infertility via direct and indirect effects on spermatogenesis and steroidogenesis.[177]

5.3.2 | Hypothesis

It has been speculated that treatment of endocrine disorders helps in regulating testicular function, and could improve steroidogenesis, proliferation and differentiation of non-germ cells, semen characteristics, and testicular redox status.

5.3.3 | Treatment

Management of metabolic syndrome and type 2 diabetes includes weight loss, healthy diet, regular exercise, and possibly, diabetes medication (eg, metformin, sulfonylureas, meglitinides, thiazolidinediones, dipeptidyl peptidase 4 inhibitors, long-acting glucagon-like peptide 1 receptor agonists, and sodium-glucose cotransporter-2 inhibitors) or insulin therapy. Thyroxine replacement therapy or antithyroid drugs are used in cases of hypothyroidism and hyperthyroidism, respectively, whereas bromocriptine and cabergoline are commonly prescribed to treat idiopathic hyperprolactinemia.

5.3.4 | Summary evidence

Studies focusing on the role of endocrine disorders in SDF are scarce, and to date, no studies have examined the possible benefit of treatment of endocrine disorders on sperm DNA integrity. However, a few reports suggest that SDF rates are higher in men with type II diabetes and in those with metabolic syndrome (MetS), which are summarized below.

In one cohort study involving a group of infertile men undergoing ART, SDF values (by SCD) were higher among non-insulin-dependent diabetic men than non-diabetic men (37.0 ± 12.7 vs. 21.0 ± 10.1; $P < .001$).[178] Notably, increased SDF in the diabetic cohort was positively correlated with adverse embryo development and pregnancy outcome. In a case–control study involving 150 men attending a fertility center, diabetic men (both obese and non-obese) were shown to have higher SDF rates (by SCSA) than non-obese and non-diabetic counterparts. (non-obese: 47.8 ± 1.2, obese: 51.7 ± 2.1 (obese); controls: 23.4 ± 1.6; $P = .003$).[179]

Furthermore, a cohort study involving 120 men attending an andrology unit showed that SDF rates (using the Comet assay) were significantly higher among those with MetS (both fertile and infertile) than fertile controls.[180] Interestingly, sperm insulin and CIDEA (cell death-inducing DNA fragmentation factor-α-like effector A) gene expression were also significantly increased in infertile men with MetS compared with both fertile counterparts as well as fertile controls, and the values in both groups of men with MetS were significantly higher than in the control group. CIDEA, which belongs to the CIDE family of proapoptotic proteins, has a role in lipid metabolism, body weight regulation, and the development of metabolic disorders.[181] Since obesity, hyperglycemia, and dyslipidemia were reported to induce an OS state in the testicular microenvironment by increasing the production of ROS,[182-184] it has been conjectured that both DNA fragmentation and CIDEA gene expression represent OS endpoints.

5.3.5 | Mechanism of action

Restoration of normal gonadal function and redox status.

5.3.6 | Conclusion

Despite the plausible association between endocrine disorders and SDF, the effect of endocrine treatment on SDF and reproductive outcomes remains unknown.

5.4 | Lifestyle changes

5.4.1 | Introduction

Exposure to environmental, occupational, and therapeutic toxicants can cause SDF. A positive relationship between environmental exposure to air pollutants (particulate matter 2.5 and 10, nitrogen oxides, sulfur oxides, and ozone) and SDF has been documented.[185,186] Likewise, environmental and occupational exposure to polycyclic aromatic hydrocarbons, ionizing radiation, organophosphate, and carbamate pesticides has been shown to increase SDF rates.[187-191] Chromic exposure to bisphenol A, an environmental endocrine disruptor utilized in the production chain of plastics and resins, has also been associated with increased SDF values.[192] Moreover, environmental and occupational exposure to lead might result in OS and defective sperm function, including impaired sperm DNA integrity.[193] Lastly, therapeutic exposure to chemotherapy and radiotherapy can also induce single- and double-strand DNA breaks. In one prospective study, patients

with lymphoma treated with chemotherapy had SDF values (by SCSA and TUNEL) higher than controls for up to two years after treatment.[194] Similarly, testicular cancer patients who received radiotherapy had persistently higher levels of SDF than controls for up to two years after exposure.[195] In patients with testicular germ cell tumors, SDF values by SCSA seem to be significantly higher among those treated with radiotherapy than those treated with chemotherapy.[196]

Many studies have consistently reported the adverse influence of smoking on semen quality and sperm DNA integrity.[197-202] Cigarette smoke contains over 7000 chemicals, including free radicals, several of which have been shown to harm sperm function through alterations in the plasma membrane, DNA integrity, and proteomic profile.[203] At present, convincing evidence indicates that tobacco users tend to have increased levels of ROS, 8-OHdG, and higher SDF rates than non-smokers.[204]

Obesity has been suggested to be a contributing factor to impaired sperm chromatin integrity. The mechanisms by which obesity influences sperm DNA quality are not fully understood, but a multifactorial nature is postulated.[205] Secondary hypogonadism due to excess peripheral conversion of testosterone to estrogen, increased levels of ROS, and increased testicular temperatures owing to excessive suprapubic fat are the candidate mechanisms. Despite that, a 2017 systematic review and meta-analysis including a total of seven studies and 3250 subjects from both IVF clinics and the general population found no obvious association between body mass index and SDF.[206] Overall, SDF values using all assays combined were slightly increased in both overweight (mean difference [MD] =0.62%; 95% CI −2.20, 3.44] I^2 = 93%) and obese men (MD = 0.64%; 95% CI −3.79, 5.07, I^2 = 94%), when compared with normal body mass index (BMI) men, but the results were not statistically different. In this study, the pooled effect estimates were neither affected by the method to measure SDF nor the study population (general population vs. infertile men). Nonetheless, statistical heterogeneity was high across all comparisons, thus suggesting a marked variation across the included studies. Lastly, the association between BMI and pregnancy outcomes remains equivocal. In another study evaluating infertile couples undergoing ART, being overweight or obese had no apparent effect on live birth rates (LBR) with either IVF (OR 0.91, 95% CI 0.78-1.06) or ICSI (OR 1.00, 95% CI 0.50-1.99).[207]

5.4.2 | Hypothesis

Changes in lifestyle and avoid exposure to toxicants may help to alleviate related testicular dysfunctions. As a result, the genetic and epigenetic constitution of spermatozoa might improve.

5.4.3 | Treatment

Avert exposure to environmental and occupational toxicants, smoking cessation, dietary and lifestyle changes, and weight loss.

5.4.4 | Summary evidence

Clinical data on the effects of averting exposure to environmental and occupational chemicals on fertility, and SDF in particular, are lacking. Likewise, the effect of smoking cessation as a means to reduce SDF remains unclear as no study has yet evaluated its impact on this population.

By contrast, recent studies suggest that dietary patterns could influence sperm DNA integrity. In a 2018 study from Poland, 336 infertile men with normal sperm count (as per the 2010 World Health Organization criteria) were interviewed as regards their diet.[208] Patients were classified into three groups based on their dietary pattern, namely Western, Mixed, or Prudent. The Prudent diet consisted of high intakes of fish, chicken, fruit, vegetables, and whole grains, whereas the Western diet included high intakes of red and processed meat, butter, high-fat dairy, refined grains, fast food, high energy drinks, and sweets. After controlling for ejaculatory abstinence, age, smoking, previous diseases, and alcohol consumption, the authors noted that the Prudent diet was associated with increased sperm counts, higher testosterone serum levels, and decreased SDF rates compared with the Western diet (SCSA: 15.2% ± 10.4% vs. 17.9% ± 8.1, $P < .05$).

Along the same lines, a 2016 study from India suggested that improvement in SDF could be obtained by adopting meditation and yoga-based lifestyle.[90] The authors assessed the levels of ROS, SDF (by SCSA), 8-OHdG, and telomere length in 56 fathers of children with childhood cancer (retinoblastoma) and 50 controls (fathers of healthy children) according to yoga, meditation practice, and smoking status at day 0, and after 3 and 6 months of the intervention. The intervention program consisted of two hours of theory and practice sessions each day and lasted for six months. The seminal mean levels of ROS (RLU/s/million: 36.1 ± 1.8 vs. 20.5 ± 2.7, $P < .01$), SDF by SCSA (31.5% ± 6.7% vs. 21.9% ± 9.4; $P < .01$), and 8-OHdG (66.0 pg/mL ± 2.9 vs. 23.1 pg/mL ± 2.7; $P < .01$) were higher in fathers of children with retinoblastoma than in controls, whereas the relative mean sperm telomere length was shorter in the former (telomere to single-copy gene ratio: 0.35 ± 0.02 vs. 0.38 ± 0.02; $P < .05$). Levels of ROS were reduced in tobacco users ($P < .05$) as well as in alcohol users ($P < .05$) after the adoption of meditation and yoga-based lifestyle modification. SDF values decreased ($P < .05$) after six months of yoga and meditation practice in both groups, whereas a decrease in the levels of 8-OHdG was evident from the third month of practice.

Studies evaluating the impact of weight loss on SDF values are rare. To our knowledge, only a small cohort study involving six obese men was published to date.[209] In this study, a nutritionist-led individualized dietary program coupled with exercise was used to reduce intra-abdominal fat over a 3- to 8-month period. All men had unexplained infertility and SDF values—by TUNEL—of 25% or higher. The DNA quality improved ($P = .01$) in all patients who had their semen specimens analyzed both before and after the intervention, and their partners achieved pregnancy and full-term deliveries, either naturally or assisted.

5.4.5 | Mechanism of action

Lifestyle modifications could influence the endocrine regulation of the male reproductive system and testicular redox status.

5.4.6 | Conclusion

Collectively, minimal data are available concerning the clinical utility of lifestyle changes as a means to decrease SDF values. Some evidence suggests that dietary changes and yoga might benefit men with high SDF values, but further research is needed both to confirm these preliminary data and to determine how these changes could translate into better reproductive results (Figure 3). Nonetheless, the information provided by SDF tests might help to implement lifestyle modifications as well as to monitor patient compliance in health improvement programs. Moreover, knowledge about the SDF status can be used to reinforce patients' counseling concerning the overall reproductive health and treatment prognosis.

5.5 | Oral antioxidants

5.5.1 | Introduction

Antioxidants constitute the primary defense mechanism against OS induced by free radicals. These substances are readily available compounds that can be consumed through diet or as oral supplements.[98] Oral antioxidant supplementation is among the most common therapies used for the treatment of male infertility.[210] The reasons relate to the common belief that excessive free radicals cause SDF and contribute to male infertility, and therefore, scavenging free radicals by antioxidants could add clinical benefit. Moreover, antioxidants are listed as dietary supplements rather than medical drugs, thus making them easily available, and its continued use has been associated with few side effects and relatively low cost. However, antioxidant therapy to men who have infertility remains empirical, since the literature is scarce in studies investigating the association between real deficiencies of specific antioxidants and the effect of oral supplementation.

5.5.2 | Hypothesis

Oral antioxidants scavenge excess free radicals and enhance seminal antioxidant capacity. As a result, the oxidatively induced SDF is minimized.

5.5.3 | Treatment

Oral antioxidant therapy is usually prescribed as a combination of non-enzymatic (eg, vitamins E and C, carotenoids, flavonoids, carnitine, coenzyme Q10, zinc, and selenium) and enzymatic antioxidants (eg, N-acetyl cysteine).

5.5.4 | Summary evidence

Improvements in conventional semen parameters, OS markers, and SDF values have been observed with the use of oral antioxidant therapy in men with infertility (reviewed by[98]). However, the evidence is not unequivocal, and the effect might be age-dependent as the magnitude of improvement in SDF values—measured by SCSA—was shown to be lower in men older than 40 years of age.[144] Nonetheless, most published studies included small patient cohorts and used a mixture of antioxidants in short treatment protocols, thus limiting clinical recommendations concerning the ideal regimen and duration[211-220] (Table 3).

A 2019 Cochrane meta-analysis of 61 randomized controlled trials on the use of antioxidants in 6,264 infertile men found that these regimens may improve pregnancy rates in couples attending fertility clinics.[221] Although a total of 18 different oral antioxidants were analyzed, only seven trials reported on live birth. The pooled results of these trials indicated that antioxidant therapy was associated with increased live birth rates compared with placebo or no treatment (OR: 1.79, 95% CI 1.20-2.67; $P = .005$). Nonetheless, no significant effect on live birth was observed when the studies with a high risk of bias were removed from the analysis. The study mentioned above is an update of a 2014 meta-analysis,[222] in which only two trials involving 100 patients[223,224] evaluated the impact of antioxidant therapy on SDF values; the pooled effect estimates revealed a significant reduction in SDF values—assessed by the TUNEL assay—after oral antioxidants (MD: −13.8%; 95% CI: −17.3%, −10.4%; $P < .000001$). In the 2019 updated review, additional RCTs were included,[225,226] totaling four studies and 254 men. The updated pooled estimates indicated that antioxidant use was associated with a tendency to lower SDF than placebo (MD −5.0%, 95% CI −12.6% to 2.6%, six intervention arms, $P = .20$, $I^2 = 89\%$). This association became more evident when the study of Barekat *et al* was removed from the analysis (MD −10.0%, 95% CI −12.9% to −7.2%, 219 men, 3 RCTs, $P < .001$, $I^2 = 74\%$).[221] Although this study reported decreased SDF rates (by TUNEL) with the use of enzymatic antioxidants, therapy was given as an adjuvant to varicocele repair, which could have biased the overall effect estimates due to the positive association of varicocele and SDF.[225] Noteworthy, the study of Raigani and colleagues, which reported a weak association between the use of oral antioxidant therapy and SDF, applied other assays for SDF assessment than the four major tests included in this review.[226]

By contrast, some data indicate that the indiscriminate use of antioxidants might induce a state of "reductive stress," which enhances ROS generation by mitochondria.[227] In one study, male partners of couples with failed IVF or ICSI received antioxidant treatment consisting of daily oral vitamins C and E (400 mg each), β-carotene (18 mg), zinc (500 μmol), and selenium (1 μmol) for 90 days. The

TABLE 3 Studies examining the effect of antioxidant therapy on sperm DNA fragmentation

Author and Year	Antioxidants (daily intake)	Study design	Number of subjects	Treatment duration	SDF testing method	Main results
Greco et al[213]	Vitamin C (1000 mg) + Vitamin E (1000 mg)	Prospective observational study	38 (patients with high SDF and prior failed ICSI)	2 mo	TUNEL	Decrease in SDF values (−15.8 ± 3.6%, $P < .001$); Improvement in ICSI clinical pregnancy rates (48.2% vs. 6.9%, $P < .05$) and implantation rates (19.6% versus 2.2%, $P < .01$) compared with the pre-treatment ICSI outcomes
Greco et al[212]	Vitamin C (1000 mg) + Vitamin E (1000 mg)	Randomized placebo-controlled study	64 men with unexplained infertility and high SDF levels	2 mo	TUNEL	Decrease in SDF levels (−13.0% ± 0.5%, $P < .01$)
Menezo et al[107]	b-carotene (18 mg), vitamin C (400 mg), vitamin E (400 mg), zinc (500 µmol), and Selenium(1 µmol)	Prospective observational study	58	3 mo	SCSA	Decrease in SDF (−19.1%, $P < .01$)
Omu et al[217]	Group 1: Zinc (400 mg) Group 2: Zinc (400 mg) + vitamin E (20 mg): Group 3: Zinc (400 mg) + vitamins E (20 mg) + vitamin C (10 mg)	Randomized placebo-controlled study	45 treatment (group 1: n = 11, group 2: n = 12; group 3: n = 14) 8 placebo group	3 mo	SCSA	Improvement in SDF measures with treatment[a]
Tunc et al[219]	Vitamin C (100 mg), E (400 IU), Selenium (26 µg), Zinc (25 mg), Folic acid (0.5 mg) and garlic (1 mg)	Prospective observational study	50	3 mo	TUNEL	Decrease in SDF values (−4% (interquartile range [IR]: −3.1%; −3.5%, $P < .01$)
Vani et al[220]	Vitamin C (1000 mg) 5 consecutive days in a week	Prospective comparative study	240:120 men exposed to lead, and 120 healthy human subjects	3 mo	Comet	Decrease in alkaline-labile sites and mean tail length of the Comet when compared to the control group ($P < .01$)
Abad et al[211]	L-Carnitine (1500 mg); vitamin C (60 mg); coenzyme Q10 (20 mg); vitamin E (10 mg); zinc (10 mg); vitamin (200 µg) B9 selenium (50 µg); vitamin B12 (1 µg)	Prospective observational study	20 infertile patients diagnosed with asthenoteratozoospermia	3 mo	SCD measurement done following various periods of sperm storage (0, 2, 6, 8 and 24 h) at 37°C	Decrease in SDF values (at 0 h: −8.4% ± 6.7%, $P < .01$; at 2 h: −8.1% ± 5.0%, $P < .01$; at 6 h: −8.6% ± 0.8%, $P < .01$; at 8 h: −9.0% ± 2.8%, $P < .01$; at 24 h: −20.9% ± 8.6%, $P < .01$)
Gual-Frau et al[214]	L-Carnitine (1500mg); vitamin C (60mg); coenzyme Q10 (20mg); vitamin E (10mg); zinc (10mg); vitamin B9 (200 µg), selenium (50µg), vitamin B12 (1µg)	Prospective observational study	20 infertile men with grade 1 varicocele	3 mo	SCD	Decrease in SDF values (−22.1%; $P = .02$); 31.3% fewer highly degraded sperm cells ($P = .07$)
Martínez-Soto et al[215]	Docosahexaenoic acid (DHA) (1500 mg)	Randomized, double-blind, placebo-controlled, parallel-group study	57; 32 treatment group and 25 placebo group	10 wk	TUNEL	Decrease in SDF values (−17.2 ± 2.8%, $P < .001$) in treatment group vs (+11.2 ± 1.9%, $P > .05$) in the placebo group

(Continues)

TABLE 3 (Continued)

Author and Year	Antioxidants (daily intake)	Study design	Number of subjects	Treatment duration	SDF testing method	Main results
Barekat et al[225]	N-acetyl-L-cysteine (NAC) 200 mg three times daily	Randomized controlled trial	35 infertile men with varicocele, subjected to varicocele repair; 20 control group; 15 treatment group	3 mo	TUNEL	Improvement in sperm chromatin integrity among men subjected to varicocelectomy who received NAC post-surgery compared to those who did not (11.8% ± 2.01% vs. 4.7% ± 1.3, $P < .01$)
Stenqvist et al[218]	Commercial fertility supplement containing vitamins (vitamin C 30 mg, vitamin E 5 mg and vitamin B12 0.5 μg), antioxidants (l-carnitine 750 mg, coenzyme Q10 10 mg and folic acid 100 μg) and oligoelements (zinc 5 mg and selenium 25 μg)	Randomized, double-blind, Placebo-controlled study	77; 37 treatment group 40 placebo group	6 mo	SCSA	No significant decreased in SDF values

Abbreviations: DDS, DNA degraded sperm; SCSA, sperm chromatin structure assay; SDF, sperm DNA fragmentation; TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labeling.
[a]No values were provided.

authors observed that while SDF values (by SCSA) significantly decreased as a result of treatment, sperm decondensation increased by 25% overall.[216] The authors speculated that owing to its high redox potential, vitamin C reduced cystine to two cysteine moieties and opened the interchain disulfide bridges in the protamines. Notably, high rates of decondensed spermatozoa might offset the positive effect of antioxidants on SDF as it can result in asynchronous chromosome condensation.[60]

At present, the best candidates for antioxidant therapy, the optimal regimen, dosage, and duration are yet to be determined. Ideally, antioxidant therapy should be offered to infertile men with high OS markers. Nonetheless, most techniques used to measure ROS are complex, which limit their widespread utilization as a routine procedure in the andrology clinic. SDF values could be used as a surrogate measure of the OS status, but as already mentioned, SDF is not always associated with OS. Moreover, studies using transgenic animal models indicate that even moderate sperm DNA oxidation, not resulting in SDF, might lead to reproductive failures.[228] Thus, screening the infertile patient for OS using low-cost assays developed for rapid assessment of total OS in the human ejaculate has been recommended before initiating antioxidant therapy.[10,229-232]

5.5.5 | Mechanism of action

Oral antioxidant therapy scavenges ROS thus boosting seminal antioxidant capacity. A drop in ROS levels results in alleviation of OS, which could decrease the oxidatively induced SDF.[98]

5.5.6 | Conclusion

Oral antioxidant therapy might be used to decrease the oxidatively induced SDF. Despite the overall beneficial effect, the absolute reduction on SDF values is small. There are limited data concerning oral antioxidant therapy in infertile men with high SDF, and therefore, further research is needed to determine its clinical utility in terms of pregnancy success as well as the ideal candidates, antioxidant regimen, and treatment duration.

5.6 | Hormonal therapy

5.6.1 | Introduction

In adult men, FSH stimulates DNA synthesis in spermatogonia and preleptotene spermatocytes and acts on Sertoli cells to enhance survival of premeiotic germ cells.[233] The FSH receptor (FSHR) mediates FSH action on Sertoli cells. FSHR is prone to single nucleotide polymorphisms (SNP) that might affect receptor sensitivity.[234] Sperm DNA fragmentation predominantly originates either in the testis resulting from an abortive apoptotic mechanism or OS during transit in

the male genital tract.[12] FSH administration seems to reduce sperm apoptosis and improve the qualitative properties of the acrosome, axoneme, and chromatin.[235]

5.6.2 | Hypothesis

Follicle-stimulating hormone (FSH) therapy could be used to reduce apoptosis-associated SDF.

5.6.3 | Treatment

Exogenous FSH administration (recombinant or urinary FSH), subcutaneously, 75-150 IU, twice or thrice a week, for approximately twelve weeks.

5.6.4 | Summary evidence

A 2012 RCT compared the effects of recombinant human FSH (rec-hFSH) treatment (150 IU on alternate days for 90 days) with non-antioxidant vitamins on SDF rates in 65 men with idiopathic oligoasthenoteratozoospermia.[236] After treatment, SDF rates assessed by TUNEL were significantly lower in the treatment group than in the control group (12.6% vs. 23.9%, respectively, $P < .05$). Subsequently, in 2018, the same group investigated the role of rec-hFSH on SDF on a prospective study enrolling 115 men with unexplained infertility.[237] In this study, FSH (150 IU) administered subcutaneously every other day for three months reduced SDF values in approximately 70% of patients, with a 35% average relative decrease compared with baseline. The authors noted that the effect of treatment was more pronounced in men with basal SDF values lower than 17% (as assessed by TUNEL) and in those with FSH basal levels between 2.16 and 4.27 IU/L. However, pregnancy outcomes were not assessed in either study.

A prospective study involving 89 men with idiopathic infertility and high SDF rates (DFI > 15% by TUNEL) explored the effect of 150 IU of rec-hFSH given every other day for 12 weeks.[238] The authors found a significant decrease ($P = .008$) in SDF values in patients who were carriers of the homozygous N polymorphism of FSH receptor (*FSHR p*.N680S) compared to those with the S allele (*p*.N680S). Apparently, the SNP *FSHB* −211G>T genotype modulated the observed effect as patients with this genotype were the most responsive to therapy. The authors suggested that *FSHR* genotype could be a pharmacogenetic marker of response to FSH therapy. In their study, however, the natural pregnancy rates (21.4% vs. 15.8%) achieved during and after the conclusion of the trial were not significantly different between homozygous carriers of N and S *p*.N680 allele. Lastly, a cohort study involving 166 patients treated with purified human FSH (150 IU 3x/wk) for three months resulted in a 10% relative reduction in SDF values (assessed by TUNEL), which was followed by improved pregnancy outcomes in the population who responded to the therapy.[239]

A recent meta-analysis, combining clinical trials that included men with idiopathic infertility, evaluated FSH treatment efficacy on SDF.[35] Six prospective trials were identified, including 383 men treated with either recombinant or urinary FSH,[236,238,239,240,241,242] (Table 4). FSH therapy reduced SDF values (using the four major assays combined) by 4.24% (95% CI: 0.24-8.25%; $P = .04$) after a treatment period of three months, although a significant increase in the total sperm count was not detected. These results support the clinical utility of SDF testing in the management and follow-up of infertile men during hormonal treatment.

5.6.5 | Mechanism of action

It has been speculated that FSH administration decreases SDF by reducing apoptosis in the testis via its cellular pro-survival properties that counteract the intratesticular intrinsic apoptotic pathway.[243]

5.6.6 | Conclusion

Collectively, hormone therapy with exogenous FSH administration might reduce SDF in men with idiopathic infertility (Figure 3). In particular, FSH treatment seems to improve SDF mainly in idiopathic infertile men with the *p*.N680S homozygous N *FSHR*. Higher FSH doses and/or duration of therapy might be beneficial to the other genotypes. Further studies are warranted to determine how the effect of this intervention translates into better reproductive outcomes.

5.7 | Testicular sperm for ICSI

5.7.1 | Introduction

The integrity of the sperm genome and epigenome is vital for the birth of healthy infants.[244] As the spermatozoon loses most cytosolic antioxidants during spermiogenesis, the male gamete is highly susceptible to oxidative-induced DNA damage. Abnormal levels of critical DNA repair enzymes could explain the persistence of SDF in ejaculated spermatozoa from infertile men (reviewed by[29,59]) The fertilization of oocytes by spermatozoa with damaged chromatin through ICSI might lead to an increased risk of fertilization failure, embryo development arrest, implantation failure, miscarriage, congenital malformations, as well as perinatal and postnatal morbidity.[32,54,245]

5.7.2 | Hypothesis

The use of testicular spermatozoa in preference over ejaculated spermatozoa might be a useful strategy to overcome the oxidative-induced SDF in non-azoospermic ICSI candidates.

TABLE 4 Studies examining the effect of follicle-stimulating hormone (FSH) therapy on sperm DNA fragmentation

Author and Year	Antioxidants (daily intake)	Study design	Number of subjects	Treatment duration	SDF testing method	Main results
Palomba et al [242]	Urinary FSH	Prospective observational study	36 men with idiopathic infertility	3 mo	SCD	SDF reduction after FSH administration, from 24.6 ± 12.7 to 16.7 ± 7.8%
Colacurci et al[236]	Recombinant FSH	Case–control prospective study	65 men with oligoasthenoteratozoospermia 64 controls	3 mo	TUNEL	SDF reduction after FSH administration, from 23.7 ± 9.4 to 12.6 ± 7.0%
Ruvolo et al [240]	Recombinant FSH	Prospective observational study	53 men with oligoasthenoteratozoospermia	3 mo	TUNEL	No SDF changes after FSH administration (from 10.5 ± 4.2 to 11.4 ± 4.5%)
Garolla et al [241]	Urinary FSH	Case–control prospective study	92 men with oligozoospermia 82 controls	3 mo	TUNEL	No SDF changes after FSH administration (from 24.4 ± 9.5 to 24.1 ± 8.2%)
Simoni et al[238]	Recombinant FSH	Prospective observational study	55 men with idiopathic infertility	3 mo	TUNEL	SDF reduction after FSH administration, from 57.8 ± 17.3 to 52.9 ± 18.2%
Garolla et al[239]	Urinary FSH	Case–control prospective study	84 men with oligozoospermia 82 controls	3 mo	TUNEL	SDF reduction after FSH administration, from 26.7 ± 7.9 to 23.4 ± 7.4%

Abbreviations: FSH, follicle-stimulating hormone; SCD, sperm chromatin dispersion; SDF, sperm DNA fragmentation; TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labeling.

5.7.3 | Treatment

Both percutaneous and open sperm retrieval procedures have been used to harvest spermatozoa from the seminiferous tubules in non-azoospermic men with high SDF in the ejaculate.[32] These methods are commonly carried out on an outpatient basis and the same day of oocyte retrieval.

5.7.4 | Summary evidence

Evidence from both animal and human studies shows that SDF is lower in testicular spermatozoa than in ejaculated or epididymal spermatozoa.[48,62,223,246-251] Studies examining paired testicular and ejaculated specimens from the same men confirmed that SDF values are threefold to fivefold lower in the former.[62,247,249] In a 2017 meta-analysis of five studies, SDF levels were compared between ejaculated and testicular spermatozoa for 143 patients who served as their controls.[32] The pooled estimates showed that the mean difference in SDF rates (using TUNEL and SCD combined between testicular spermatozoa and ejaculated spermatozoa was −24.6% (95% CI −32.53% to −16.64%, I^2 = 92%; P < .001). Among the studies, all but one used the TUNEL assay for the assessment of SDF in paired specimens. When only TUNEL studies were combined, the MD in SDF values was −19.78% (95% CI −22.35% to −17.21%; I^2 = 15%; P < .001). In the single study using the SCD method, the MD was −32.4% (95% CI −34.85% to −29.95%; P < .001) favoring testicular sperm.

The meta-analysis mentioned above also compiled the data of four ICSI studies (Table 5).[62,223,252,253] A total of 507 cycles was carried out in infertile couples with high SDF values on the neat semen, in which 3840 oocytes were injected with either ejaculated sperm or testicular sperm. The OR for clinical pregnancy (2.42, 95% CI 1.57-3.73; I^2 = 34%; P < .0001) and live birth (OR: 2.58, 95% CI 1.54-4.35, I^2 = 0%; P = .0003) favored the use of testicular sperm (Testi-ICSI) in preference over ejaculated sperm.[32] Likewise, the OR for miscarriage (0.28, 95% CI 0.11-0.68, I^2 = 11%; P = .005) was also in favor of the Testi-ICSI group. Recent studies using the SCD, SCSA, and TUNEL assay corroborate the superiority of testicular sperm over ejaculated sperm for ICSI in men with high SDF values (Table 5).[254-256]

Furthermore, data from a 2019 study using whole-exome sequencing molecular karyotype to assess sperm aneuploidy rates in ejaculated and testicular spermatozoa suggested that aneuploidy rates were lower in testicular spermatozoa.[257] The authors studied fertile donors and infertile patients (both men with non-obstructive azoospermia and non-azoospermic men with high SDF assessed by TUNEL). Aneuploidy rates in testicular specimens were as low as those of ejaculated samples from fertile donors (1.9% vs. 1.2%), whereas aneuploidy rates were 11.1% for ejaculated specimens of patients (P < .001). More importantly, paired assessments in ejaculated and testicular specimens of non-azoospermic men with high SDF showed that both SDF rates and aneuploidy rates were significantly lower in testicular spermatozoa (8% and 1.2%) than in ejaculated spermatozoa (20% and 8.4%). This report was corroborated by a recent ICSI study that evaluated blastocyst

TABLE 5 Characteristics and main outcome measures of studies reporting ICSI outcomes with testicular versus ejaculated spermatozoa in non-azoospermic men with high sperm DNA fragmentation in the semen

Author and Year	Design	Subjects and cohort size (N)	SDF testing method	SDF cutoff values	Paired SDF results in testicular and ejaculated spermatozoa (%)	Sperm retrieval method	Fertilization rate (%)	Clinical pregnancy rate (%)	Ongoing pregnancy or live birth rates[f] (%)
Greco et al[223]	Case series; intervention applied in consecutive patients	Predominantly normozoospermic infertile men (18); Couples with history of ICSI failure	TUNEL	15%	23.6 ± 5.1% (E) and 4.8 ± 3.6% (T) ($P < .001$)	TESE and TESA	74.9[b]	44.4 (T)[c]	NR
Esteves et al[62]	Prospective cohort	Oligozoospermic infertile men (172); Couples with no history of ICSI failure	SCD	30%	40.9% ± 10.2% (E) and 8.3% ± 5.3% (T) ($P < .001$)	TESE and TESA	69.4 (E) vs. 56.1 (T) ($P = .0001$)	40.2 (E) vs. 51.9 (T) (NS)	LBR: 26.4 (E) vs. 46.7 (T) ($P = .007$)
Bradley et al[252]	Retrospective cohort	Predominantly oligozoospermic men infertile men (228)[a]	SCIT	29%	NR	TESE and TESA	66.0 (E) vs. 57.0 (T) ($P < .001$)	27.5 (E) vs. 49.5 (T) ($P < .01$)	24.2 (E) vs. 49.8 (T) ($P < .05$)
Pabuccu et al[253]	Retrospective cohort	Normozoospermic infertile men (71); Couples with history of ICSI failure	TUNEL	30%	41.7 ± 8.2 (E)	TESA	74.1 ± 20.7 (T) and 71.1 ± 26.9 (E) (NS)	41.9 (T) and 20.0 (E) ($P = .04$)	OPR: 38.7 (T) vs. 15.0 (E) (0.02)
Arafa et al[254]	Prospective cohort; intervention applied in consecutive patients	Oligozoospermic and normozoospermic infertile men (36); Couples with history of ICSI failure	SCD	30%	56.3 ± 15.3 (E)	TESA	46.4 (T) and 47.8 (E) (NS)	38.9 (T) and 13.8 (E) ($P < .0001$)	LBR: 38.9 (T) vs. 8.0 (E) ($P < .0001$)
Zhang et al[256]	Prospective cohort[e]	Oligozoospermic and normozoospermic infertile men (102); Couples with no history of ICSI failure	SCSA	30%	NR	TESA	70.4 (T) vs. 75.0 (E) (NS)	36.0 (T) vs. 14.6 (E) ($P = .01$)	LBR: 36.0 (T) vs. 9.8 (E) ($P = .001$)
Herrero et al[255]	Retrospective cohort	Couples with no previous live births and a history of at least two previous failed ICSI cycles with ejaculated spermatozoa (145)	SCSA and TUNEL	SCSA (≥25%); TUNEL (≥36%)	NR	TESE	DFI ≥ 25% (SCSA): 66.3% (T); 62.9% (E) (NS) DFI ≥ 36% (TUNEL): 61.2% (T); 57.6% (E) (NS)	DFI ≥ 25% (SCSA): 18.2% (T); 9.1% (E) ($P < .02$) DFI ≥ 36% (TUNEL): 23.1% (T); 0.0% (E) ($P < .02$)	[d]DFI ≥ 25% (SCSA): 21.7% (T); 9.1% (E) ($P < .01$) DFI ≥ 36% (TUNEL): 20.0% (T); 0% (E) ($P < .02$)

[a]Number of ICSI cycles; LBR, live birth rate; NS, not significantly different; OPR, ongoing pregnancy rate; SDF, sperm DNA fragmentation; TESE, testicular sperm extraction, TESA, testicular sperm aspiration; micro-TESE, microdissection testicular sperm extraction; NR, not reported; SCD, sperm chromatin dispersion test; SCIT, sperm chromatin integrity test, a variation of sperm chromatin structure assay (SCSA); TUNEL, terminal deoxyribonucleotide transferase-mediated dUTP nick-end labeling assay; E, ejaculated sperm group; T, testicular sperm group.

[b]2PN fertilization rate with use of testicular spermatozoa; data from previous cycles with use of ejaculated spermatozoa not provided;

[c]The authors reported only one pregnancy with ejaculated spermatozoa which miscarried;

[d]Cumulative live birth rates reported;

[e]Inferred from the study reported data; authors not contacted for providing clarification;

[f]Herrero et al reported the cumulative live birth rate.

euploidy probability—by comprehensive 24-chromosome genetic testing with next-generation sequencing—after sperm injections with either ejaculated or testicular spermatozoa from non-azoospermic men with high SDF values (by the SCD test) on neat semen.[88] The authors showed that the probability of a metaphase II oocyte turn into a euploid blastocyst was not adversely affected by the use of testicular spermatozoa, thus supporting the safe utilization of testicular spermatozoa.

Notably, infertile men showing high SDF values on the neat semen usually have an overall preserved sperm count; therefore, sperm retrieval success is high regardless of the acquisition method.[258] Nonetheless, there are risks involved with sperm retrieval, including infection, hematoma, and testicular atrophy. Although the risks are low (<5%),[259] sperm retrieval should be performed by reproductive urologists who are familiar with testicular anatomy. Identification of the male factor associated with SDF might ease treatment to mitigate SDF, thus potentially making possible natural conception or ART with ejaculated spermatozoa. Currently, Testi-ICSI is reserved for men with persistently high SDF after the use of other measures to alleviate SDF.[260,261]

5.7.5 | Mechanism of action

ICSI using spermatozoa with a lower risk of having SDF might explain, at least in part, the improved reproductive outcome with testicular spermatozoa as consistently reported in various studies. However, other mechanisms involving epigenetic factors might play a role and warrant further investigation.

5.7.6 | Conclusion

Collectively, contemporary evidence indicates that SDF rates are lower in testicular spermatozoa than in ejaculated spermatozoa of infertile men undergoing ART. Testi-ICSI seems to be advantageous over ICSI with ejaculated spermatozoa to infertile men with high levels of SDF in the semen, with improvements reported in rates of clinical pregnancy, miscarriage, and live birth (Figure 3). Possible reasons explaining the reported better pregnancy outcomes by Testi-ICSI over Ejac-ICSI in this patient subset seem to involve the injection of spermatozoa with a lower risk of DNA damage rather than the transfer of embryos with better embryo ploidy status. Nonetheless, more research is needed to confirm the clinical efficacy of Testi-ICSI in prospective trials and its safe utilization concerning the health of offspring.

6 | CONCLUSIONS

At present, limited data exist concerning the clinical utility of medical and surgical interventions to alleviate SDF. The best available evidence concerns clinical varicocele, in which surgical repair has been shown to decrease SDF values with a potentially positive effect on pregnancy outcomes, and the use of FSH therapy in men with idiopathic infertility. Additionally, some evidence suggests that lifestyle changes and oral antioxidant therapy might alleviate SDF, but the effects on pregnancy are unclear. Among ICSI candidates with high SDF, the use of testicular spermatozoa in preference over ejaculated spermatozoa is associated with lower SDF values and better reproductive outcomes. Given the notable association between underlying male infertility conditions and SDF, a comprehensive andrological evaluation remains essential to identify the causes of infertility and enable treatment to improve the chances of achieving natural or assisted conception potentially. However, further research is needed to determine the exact role of medical and surgical interventions for subfertile men with abnormal seminal levels of sperm DNA fragmentation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

SCE designed the manuscript, helped in data interpretation and coordination, and drafted the manuscript. DS and MS participated in the acquisition of data, data interpretation, and drafted the manuscript. All authors read and approved the final manuscript.

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REFERENCES

1. Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. *Reprod Biol Endocrinol*. 2015;13:37.
2. Esteves SC, Chan P. A systematic review of recent clinical practice guidelines and best practice statements for the evaluation of the infertile male. *Int Urol Nephrol*. 2015;47:1441-1456.
3. Hamada A, Esteves SC, Nizza M, Agarwal A. Unexplained male infertility: diagnosis and management. *Int Braz J Urol*. 2012;38:576-594.
4. Jungwirth A, Giwercman A, Tournaye H, et al. European association of urology guidelines on male infertility: the 2012 update. *Eur Urol*. 2012;62:324-332.
5. Aitken RJ. Oxidative stress and the etiology of male infertility. *J Assist Reprod Genet*. 2016;33:1691-1692.
6. Bui AD, Sharma R, Henkel R, Agarwal A. Reactive oxygen species impact on sperm DNA and its role in male infertility. *Andrologia*. 2018;50:e13012.
7. Esteves SC, Gosálvez J, López-Fernández C, et al. Diagnostic accuracy of sperm DNA degradation index (DDSi) as a potential noninvasive biomarker to identify men with varicocele-associated infertility. *Int Urol Nephrol*. 2015;47:1471-1477.
8. Saleh RA, Agarwal A, Nelson DR, et al. Increased sperm nuclear DNA damage in normozoospermic infertile men: a prospective study. *Fertil Steril*. 2002;78:313-318.

9. Sergerie M, Laforest G, Boulanger K, Bissonnette F, Bleau G. Longitudinal study of sperm DNA fragmentation as measured by terminal uridine nick end-labelling assay. *Hum Reprod*. 2005;20:1921-1927.
10. Esteves SC, Sharma RK, Gosálvez J, Agarwal A. A translational medicine appraisal of specialized andrology testing in unexplained male infertility. *Int Urol Nephrol*. 2014;46:1037-1052.
11. Gosálvez J, Lopez-Fernandez C, Fernandez JL, Esteves SC, Johnston SD. Unpacking the mysteries of sperm DNA fragmentation: ten frequently asked questions. *J Reprod Biotechnol Fertil*. 2015;4:1-16.
12. Muratori M, Tamburrino L, Marchiani S, et al. Investigation on the origin of sperm DNA fragmentation: role of apoptosis, immaturity and oxidative stress. *Mol Med*. 2015;21:109-122.
13. Ollero M, Gil-Guzman E, Lopez MC, et al. Characterization of subsets of human spermatozoa at different stages of maturation: implications in the diagnosis and treatment of male infertility. *Hum Reprod*. 2001;16:1912-1921.
14. Sakkas D, Alvarez JG. Sperm DNA fragmentation: mechanisms of origin, impact on reproductive outcome, and analysis. *Fertil Steril*. 2010;93:1027-1036.
15. McPherson S, Longo FJ. Chromatin structure-function alterations during mammalian spermatogenesis: DNA nicking and repair in elongating spermatids. *Eur J Histochem*. 1993;37:109-128.
16. Sotolongo B, Huang TT, Isenberger E, Ward WS. An endogenous nuclease in hamster, mouse, and human spermatozoa cleaves DNA into loop-sized fragments. *J Androl*. 2005;26:272-280.
17. O'Flaherty C, Vaisheva F, Hales BF, Chan P, Robaire B. Characterization of sperm chromatin quality in testicular cancer and Hodgkin's lymphoma patients prior to chemotherapy. *Hum Reprod*. 2008;23:1044-1052.
18. Rubes J, Selevan SG, Sram RJ, Evenson DP, Perreault SD. GSTM1 genotype influences the susceptibility of men to sperm DNA damage associated with exposure to air pollution. *Mutat Res*. 2007;625:20-28.
19. Sakkas D, Moffatt O, Manicardi GC, Mariethoz E, Tarozzi N, Bizzaro D. Nature of DNA damage in ejaculated human spermatozoa and the possible involvement of apoptosis. *Biol Reprod*. 2002;66:1061-1067.
20. Dada R. Sperm DNA damage diagnostics: when and why. *Transl Androl Urol*. 2017;6(Suppl 4):691-694.
21. Champroux A, Torres-Carreira J, Gharagozloo P, Drevet JR, Kocer A. Mammalian sperm nuclear organization: resiliencies and vulnerabilities. *Basic Clin Androl*. 2016;26:17.
22. Aitken RJ. DNA damage in human spermatozoa; important contributor to mutagenesis in the offspring. *Transl Androl Urol*. 2017;6(Suppl 4):761-764.
23. Feng Z, Hu W, Amin S, Tang MS. Mutational spectrum and genotoxicity of the major lipid peroxidation product, trans-4-hydroxy-2-nonenal, induced DNA adducts in nucleotide excision repair-proficient and -deficient human cells. *Biochemistry*. 2003;42:7848-7854.
24. Lopes S, Jurisicova A, Sun JG, Casper RF. Reactive oxygen species: potential cause for DNA fragmentation in human spermatozoa. *Hum Reprod*. 1998;13:896-900.
25. Bertoncelli Tanaka M, Agarwal A, Esteves SC. Paternal age and assisted reproductive technologies: problem solver or trouble maker? *Panminerva Med*. 2018;61:138-151.
26. Bisht S, Faiq M, Tolahunase M, Dada R. Oxidative stress and male infertility. *Nat Rev Urol*. 2017;14:470-485.
27. Esteves SC, Agarwal A, Majzoub A. The complex nature of the sperm DNA damage process. *Transl Androl Urol*. 2017;6(Suppl 4):S557-559.
28. Esteves SC, Miyaoka R, Agarwal A. An update on the clinical assessment of the infertile male. *Clinics (Sao Paulo)*. 2011;66:691-700.
29. Esteves SC. Novel concepts in male factor infertility: clinical and laboratory perspectives. *J Assist Reprod Genet*. 2016;33:1319-1335.
30. Hamada A, Esteves SC, Agarwal A. Insight into oxidative stress in varicocele associated male infertility: part 2. *Nat Rev Urol*. 2013;10:26-37.
31. Sharma R, Biedenharn KR, Fedor JM, Agarwal A. Lifestyle factors and reproductive health: taking control of your fertility. *Reprod Biol Endocrinol*. 2013;11:66.
32. Esteves SC, Roque M, Bradley CK, Garrido N. Reproductive outcomes of testicular versus ejaculated sperm for intracytoplasmic sperm injection among men with high levels of DNA fragmentation in semen: systematic review and meta-analysis. *Fertil Steril*. 2017;108:456-467.
33. Larson KL, DeJonge CJ, Barnes AM, Jost LK, Evenson DP. Sperm chromatin structure assay parameters as predictors of failed pregnancy following assisted reproductive techniques. *Hum Reprod*. 2000;15:1717-1722.
34. Robinson L, Gallos ID, Conner SJ, et al. The effect of sperm DNA fragmentation on miscarriage rates: a systematic review and meta-analysis. *Hum Reprod*. 2012;27:2908-2917.
35. Santi D, Spaggiari G, Simoni M. Sperm DNA fragmentation index as a promising predictive tool for male infertility diagnosis and treatment management – meta-analyses. *Reprod Biomed Online*. 2018;37:315-326.
36. Simon L, Emery BR, Carrell DT. Review: Diagnosis and impact of sperm DNA alterations in assisted reproduction. *Best Pract Res Clin Obstet Gynaecol*. 2017;44:38-56.
37. Zhao J, Zhang Q, Wang Y, Li Y. Whether sperm deoxyribonucleic acid fragmentation has an effect on pregnant and miscarriage after in vitro fertilization/intracytoplasmic sperm injection: a systematic review and meta-analysis. *Fertil Steril*. 2014;102:998-1005.
38. Cissen M, Wely MV, Scholten I, et al. Measuring sperm DNA fragmentation and clinical outcomes of medically assisted reproduction: a systematic review and meta-analysis. *PLoS ONE*. 2016;11:e0165125.
39. Practice Committee of the American Society for Reproductive Medicine. The clinical utility of sperm DNA integrity testing: a guideline. *Fertil Steril*. 2013;99:673-677.
40. Esteves SC, Agarwal A. Reproductive outcomes, including neonatal data, following sperm injection in men with obstructive and nonobstructive azoospermia: case series and systematic review. *Clinics (Sao Paulo)*. 2013;68:141-150.
41. Esteves SC, Prudencio C, Seol B, Verza S, Knoedler C, Agarwal A. Comparison of sperm retrieval and reproductive outcome in azoospermic men with testicular failure and obstructive azoospermia treated for infertility. *Asian J Androl*. 2014;16:602-606.
42. Mitchell V, Rives N, Albert M, et al. Outcome of ICSI with ejaculated spermatozoa in a series of men with distinct ultrastructural flagellar abnormalities. *Hum Reprod*. 2006;21:2065-2074.
43. Strassburger D, Friedler S, Raziel A, Schachter M, Kasterstein E, Ron-el R. Very low sperm count affects the result of intracytoplasmic sperm injection. *J Assist Reprod Genet*. 2000;17:431-436.
44. Esteves SC, Roque M, Bedoschi G, Haahr T, Humaidan P. Intracytoplasmic sperm injection for male infertility and consequences for offspring. *Nat Rev Urol*. 2018;15:535-562.
45. Jin J, Pan C, Fei Q, et al. Effect of sperm DNA fragmentation on the clinical outcomes for in vitro fertilization and intracytoplasmic sperm injection in women with different ovarian reserves. *Fertil Steril*. 2015;103:910-916.
46. Cortés-Gutiérrez EI, López-Fernández C, Fernández JL, Dávila-Rodríguez MI, Johnston SD, Gosálvez J. Interpreting sperm DNA damage in a diverse range of mammalian sperm by means of the two-tailed comet assay. *Front Genet*. 2014;5:404.
47. Evenson DP. The Sperm Chromatin Structure Assay (SCSA®) and other sperm DNA fragmentation tests for evaluation of sperm nuclear DNA integrity as related to fertility. *Anim Reprod Sci*. 2016;169:56-75.

48. Suganuma R, Yanagimachi R, Meistrich ML. Decline in fertility of mouse sperm with abnormal chromatin during epididymal passage as revealed by ICSI. *Hum Reprod*. 2005;20:3101-3108.
49. Johnston SD, López-Fernández C, Arroyo F, et al. Reduced sperm DNA longevity is associated with an increased incidence of still born; evidence from a multi-ovulating sequential artificial insemination animal model. *J Assist Reprod Genet*. 2016;33:1231-1238.
50. Palazzese L, Gosálvez J, Anzalone DA, Loi P, Saragusty J. DNA fragmentation in epididymal freeze-dried ram spermatozoa impairs embryo development. *J Reprod Dev*. 2018;64:393-400.
51. Barratt CLR, Björndahl L, De Jonge CJ, et al. The diagnosis of male infertility: an analysis of the evidence to support the development of global WHO guidance-challenges and future research opportunities. *Hum Reprod Update*. 2017;23:660-680.
52. Niederberger C, Pellicer A, Cohen J, et al. Forty years of IVF. *Fertil Steril*. 2018;110:185-324.
53. Wilkinson J, Bhattacharya S, Duffy J, et al. Reproductive medicine: still more ART than science? *BJOG*. 2019;126:138-141.
54. Agarwal A, Majzoub A, Esteves SC, Ko E, Ramasamy R, Zini A. Clinical utility of sperm DNA fragmentation testing: practice recommendations based on clinical scenarios. *Transl Androl Urol*. 2016a;5:935-950.
55. Practice Committee of the American Society for Reproductive Medicine. Diagnostic evaluation of the infertile male: a committee opinion. *Fertil Steril*. 2015;103:e18-25.
56. Dorostghoal M, Kazeminejad SR, Shahbazian N, Pourmehdi M, Jabbari A. Oxidative stress status and sperm DNA fragmentation in fertile and infertile men. *Andrologia*. 2017;49(10):e12762.
57. Ribas-Maynou J, García-Peiró A, Fernández-Encinas A, et al. Comprehensive analysis of sperm DNA fragmentation by five different assays: TUNEL assay, SCSA, SCD test and alkaline and neutral Comet assay. *Andrology*. 2013;1:715-722.
58. Esteves SC, Agarwal A, Cho CL, Majzoub A. 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;6(Suppl 4):S734-S760.
59. Agarwal A, Hamada A, Esteves SC. Insight into oxidative stress in varicocele-associated male infertility: part 1. *Nat Rev Urol*. 2012;9:678-690.
60. Aitken RJ, Smith TB, Jobling MS, Baker MA, De Iuliis GN. Oxidative stress and male reproductive health. *Asian J Androl*. 2014;16:31-38.
61. Cho CL, Esteves SC, Agarwal A. Novel insights into the pathophysiology of varicocele and its association with reactive oxygen species and sperm DNA fragmentation. *Asian J Androl*. 2016;18:186-193.
62. Esteves SC, Sanchez-Martin F, Sanchez-Martin P, Schneider DT, Gosalvez J. Comparison of reproductive outcome in oligozoospermic men with high sperm DNA fragmentation undergoing intracytoplasmic sperm injection with ejaculated and testicular sperm. *Fertil Steril*. 2015;10:1398-1405.
63. Roque M, Esteves SC. Effect of varicocele repair on sperm DNA fragmentation: a review. *Int Urol Nephrol*. 2018;50:583-603.
64. Cho CL, Agarwal A, Majzoub A, Esteves SC. The correct interpretation of sperm DNA fragmentation test. *Transl Androl Urol*. 2017;6(Suppl 4):S621-S623.
65. Erenpreiss J, Elzanaty S, Giwercman A. Sperm DNA damage in men from infertile couples. *Asian J Androl*. 2008;10:786-790.
66. Bungum M, Humaidan P, Axmon A, et al. Sperm DNA integrity assessment in prediction of assisted reproduction technology outcome. *Hum Reprod*. 2007;22:174-179.
67. Evenson DP, Jost LK, Marshall D, et al. Utility of the sperm chromatin structure assay as a diagnostic and prognostic tool in the human fertility clinic. *Hum Reprod*. 1999;14:1039-1049.
68. Malić Vončina S, Golob B, Ihan A, Kopitar AN, Kolbezen M, Zorn B. Sperm DNA fragmentation and mitochondrial membrane potential combined are better for predicting natural conception than standard sperm parameters. *Fertil Steril*. 2016;105:637-644.
69. Spanò M, Kolstad AH, Larsen SB, et al. The applicability of the flow cytometric sperm chromatin structure assay in epidemiological studies. *Asclepios. Hum Reprod*. 1998;13:2495-2505.
70. Zini A. Are sperm chromatin and DNA defects relevant in the clinic? *Syst Biol Reprod Med*. 2011;57:78-85.
71. Chenlo PH, Curi SM, Pugliese MN, et al. Fragmentation of sperm DNA using the TUNEL method. *Actas Urol Esp*. 2014;38:608-612.
72. Wiweko B, Utami P. Predictive value of sperm deoxyribonucleic acid (DNA) fragmentation index in male infertility. *Basic Clin Androl*. 2017;27:1.
73. Evenson DP, Wixon R. Data analysis of two in vivo fertility studies using Sperm Chromatin Structure Assay-derived DNA fragmentation index vs. pregnancy outcome. *Fertil Steril*. 2008;90:1229-1231.
74. Buck Louis GM, Sundaram R, Schisterman EF, et al. Semen quality and time to pregnancy: the Longitudinal Investigation of Fertility and the Environment Study. *Fertil Steril*. 2014;101(2):453–62. https://doi.org/10.1016/j.fertnstert.2013.10.022.
75. Vandekerckhove FW, De Croo I, Gerris J, Vanden Abbeel E, De Sutter P. Sperm chromatin dispersion test before sperm preparation is predictive of clinical pregnancy in cases of unexplained infertility treated with intrauterine insemination and induction with clomiphene citrate. *Front Med (Lausanne)*. 2016;3:63.
76. Rilcheva VS, Ayvazova NP, Ilieva LO, Ivanova SP, Konova EI. Sperm DNA integrity test and assisted reproductive technology (Art) outcome. *J Biomed Clin Res*. 2016;9:21-29.
77. Chen Q, Zhao JY, Xue X, Zhu GX. The association between sperm DNA fragmentation and reproductive outcomes following intrauterine insemination, a meta analysis. *Reprod Toxicol*. 2019;86:50-55.
78. Deng C, Li T, Xie Y, et al. Sperm DNA fragmentation index influences assisted reproductive technology outcome: A systematic review and meta-analysis combined with a retrospective cohort study. *Andrologia*. 2019;51:e13263.
79. Zini A, Jamal W, Cowan L, Al-Hathal N. Is sperm DNA damage associated with IVF embryo quality? A systematic review. *J Assist Reprod Genet*. 2011;28:391-397.
80. Alvarez Sedó C, Bilinski M, Lorenzi D, et al. Effect of sperm DNA fragmentation on embryo development: clinical and biological aspects. *JBRA Assist Reprod*. 2017;21:343-350.
81. Borges E Jr, Zanetti BF, Setti AS, Braga DPAF, Provenza RR, Iaconelli A Jr. Sperm DNA fragmentation is correlated with poor embryo development, lower implantation rate, and higher miscarriage rate in reproductive cycles of non-male factor infertility. *Fertil Steril*. 2019;112:483-490.
82. Kim SM, Kim SK, Jee BC, Kim SH. Effect of sperm DNA fragmentation on embryo quality in normal responder women in *in vitro* fertilization and intracytoplasmic sperm injection. *Yonsei Med J*. 2019;60:461-466.
83. Zheng WW, Song G, Wang QL, et al. Sperm DNA damage has a negative effect on early embryonic development following in vitro fertilization. *Asian J Androl*. 2018;20:75-79.
84. Antonouli S, Papatheodorou A, Panagiotidis Y, et al. The impact of sperm DNA fragmentation on ICSI outcome in cases of donated oocytes. *Arch Gynecol Obstet*. 2019;300:207-215.
85. Casanovas A, Ribas-Maynou J, Lara-Cerrillo S, et al. Double-stranded sperm DNA damage is a cause of delay in embryo development and can impair implantation rates. *Fertil Steril*. 2019;111:699-707.
86. Wdowiak A, Bakalczuk S, Bakalczuk G. The effect of sperm DNA fragmentation on the dynamics of the embryonic development in intracytoplasmatic sperm injection. *Reprod Biol*. 2015;15:94-100.
87. Ribas-Maynou J, Benet J. Single and double strand sperm DNA damage: different reproductive effects on male fertility. *Genes (Basel)*. 2019;1:10.

88. Figueira R, Carvalho JF, Bento FC, Melo AA, Martinhago CD, Esteves SC. ICSI using surgically retrieved testicular sperm of non-azoospermic men with high sperm DNA fragmentation index and blastocyst ploidy: a safe approach. Abstracts of the 35th Annual Meeting of the European Society of Human Reproduction and Embryology. *Hum Reprod* 2019; 34(Supp 1): i1-i543.
89. Gat I, Tang K, Quach K, et al. Sperm DNA fragmentation index does not correlate with blastocyst aneuploidy or morphological grading. *PLoS ONE*. 2017;12:e0179002.
90. Rima D, Shiv BK, Bhavna CH, Shilpa B, Saima KH. Oxidative stress induced damage to paternal genome and impact of meditation and yoga – can it reduce incidence of childhood cancer? *Asian Pac J Cancer Prev*. 2016;17:4517-4525.
91. Vande Loock K, Ciardelli R, Decordier I, Plas G, Haumont D, Kirsch-Volders M. Preterm newborns show slower repair of oxidative damage and paternal smoking associated DNA damage. *Mutagenesis*. 2012;27:573-580.
92. Jarow J, Sigman M, Kolettis PN, Lipshultz LR, McClure D, Nangia AJ, Naughton CK, Prins GS, Sandlow JI, Schlegel PN. The optimal evaluation of the infertile male: best practice statement reviewed and validity confirmed; 2011. https://www.auanet.org/education/guidelines/male-infertility-d.cfm
93. Bach PV, Schlegel PN. Sperm DNA damage and its role in IVF and ICSI. *Basic Clin Androl*. 2016;26:15.
94. Atik RB, Christiansen OB, Elson J, et al. ESHRE guideline: recurrent pregnancy loss. *Human Reprod Open*. 2018;2: hoy004.
95. McQueen DB, Zhang J, Robins JC. Sperm DNA fragmentation and recurrent pregnancy loss: a systematic review and meta-analysis. *Fertil Steril*. 2019;112:54-60.
96. Agarwal A, Cho CL, Majzoub A, Esteves SC. The Society for Translational Medicine: clinical practice guidelines for sperm DNA fragmentation testing in male infertility. *Transl Androl Urol*. 2017a;6(Suppl. 4):720-733.
97. Majzoub A, Agarwal A, Cho CL, Esteves SC. Sperm DNA fragmentation testing: across sectional survey on current practices of fertility specialists. *Transl Androl Urol*. 2017;6(Suppl 4):S710-S719.
98. Majzoub A, Agarwal A, Esteves SC. Antioxidants for elevated sperm DNA fragmentation: a mini review. *Transl Androl Urol*. 2017c;6(Suppl 4):S649-653.
99. Translational Andrology and Urology (Sperm DNA Fragmentation 2017), 6(Suppl. 4). http://tau.amegroups.com/issue/view/612.
100. Evenson DP, Jost LK, Baer RK, Turner TW, Schrader SM. Individuality of DNA denaturation patterns in human sperm as measured by the sperm chromatin structure assay. *Reprod Toxicol*. 1991;5:115-125.
101. Gorczyca W, Traganos F, Jesionowska H, Darzynkiewicz Z. Presence of DNA strand breaks and increased sensitivity of DNA in situ to denaturation in abnormal human sperm cells: analogy to apoptosis of somatic cells. *Exp Cell Res*. 1993;207:202-205.
102. Singh NP, Stephens RE. X-ray induced DNA double-strand breaks in human sperm. *Mutagenesis*. 1998;13:75-79.
103. Fernández JL, Muriel L, Rivero MT, Goyanes V, Vazquez R, Alvarez JG. The sperm chromatin dispersion test: a simple method for the determination of sperm DNA fragmentation. *J Androl*. 2003;24:59-66.
104. Christensen P, Humaidan P. Testing of sperm DNA damage and clinical recommendations. *Transl Androl Urol*. 2017;6(Suppl 4):S607-S609.
105. Ward WS. Eight tests for sperm DNA fragmentation and their roles in the clinic. *Transl Androl Urol*. 2017;6(Suppl 4):S468-S470.
106. Carrell DT, Hotaling J. Using sperm testing to improve patient and offspring health: rational, evidence-based care of the infertile male in the ART clinic. *Transl Androl Urol*. 2017;6(Suppl 4):S443-S445.
107. Menezo Y, Clement P, Amar E. Evaluation of sperm DNA structure, fragmentation and decondensation: an essential tool in the assessment of male infertility. *Transl Androl Urol*. 2017;6(Suppl 4):S553-S556.
108. Blumer CG, Restelli AE, Giudice PT, et al. Effect of varicocele on sperm function and semen oxidative stress. *BJU Int*. 2012;109:259-265.
109. Chen SS, Huang WJ, Chang LS, Wei YH. Attenuation of oxidative stress after varicocelectomy in subfertile patients with varicocele. *J Urol*. 2008;179:639-642.
110. Evenson DP. Evaluation of sperm chromatin structure and DNA strand breaks is an important part of clinical male fertility assessment. *Transl Androl Urol*. 2017;6(Suppl 4):S495-S500.
111. Feijó CM, Esteves SC. Diagnostic accuracy of sperm chromatin dispersion test to evaluate sperm deoxyribonucleic acid damage in men with unexplained infertility. *Fertil Steril*. 2014;101:58-63.
112. Sharma R, Ahmad G, Esteves SC, Agarwal A. 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*. 2016;33:291-300.
113. Sharma R, Masaki J, Agarwal A. Sperm DNA fragmentation analysis using the TUNEL assay. *Methods Mol Biol*. 2013;927:121-136.
114. Mitchell LA, De Iuliis GN, Aitken RJ. The TUNEL assay consistently underestimates DNA damage in human spermatozoa and is influenced by DNA compaction and cell vitality: Development of an improved methodology. *Int J Androl*. 2011;34:2-13.
115. Evenson DP, Darzynkiewicz Z, Melamed MR. Relation of mammalian sperm chromatin heterogeneity to fertility. *Science*. 1980;210:1131-1133.
116. Darzynkiewicz Z, Traganos F, Sharpless T, Melamed MR. Thermal denaturation of DNA in situ as studied by acridine orange staining and automated cytofluorometry. *Exp Cell Res*. 1975;90:411-428.
117. Evenson D, Jost L. Sperm chromatin structure assay is useful for fertility assessment. *Methods Cell Sci*. 2000;22:169-189.
118. Fernández JL, Muriel L, Goyanes V, et al. Simple determination of human sperm DNA fragmentation with an improved sperm chromatin dispersion test. *Fertil Steril*. 2005;84:833-842.
119. Simon L, Carrell DT. Sperm DNA damage measured by comet assay. *Methods Mol Biol*. 2013;927:137-146.
120. Ribas-Maynou J, García-Peiró A, Abad C, Amengual MJ, Navarro J, Benet J. Alkaline and neutral Comet assay profiles of sperm DNA damage in clinical groups. *Hum Reprod*. 2012;27:652-658.
121. Enciso M, Sarasa J, Agarwal A, Fernández JL, Gosálvez J. A two-tailed Comet assay for assessing DNA damage in spermatozoa. *Reprod Biomed Online*. 2009;18:609-616.
122. Simon L, Liu L, Murphy K, et al. Comparative analysis of three sperm DNA damage assays and sperm nuclear protein content in couples undergoing assisted reproduction treatment. *Hum Reprod*. 2014;29:904-917.
123. Javed A, Talkad MS, Ramaiah MK. Evaluation of sperm DNA fragmentation using multiple methods: a comparison of their predictive power for male infertility. *Clin Exp Reprod Med*. 2019;46:14-21.
124. Zini A, Bach PV, Al-Malki AH, Schlegel PN. Use of testicular sperm for ICSI in oligozoospermic couples: how far should we go? *Hum Reprod*. 2017;32:7-13.
125. Smit M, Dohle GR, Hop WC, Wildhagen MF, Weber RF, Romijn JC. Clinical correlates of the biological variation of sperm DNA fragmentation in infertile men attending an andrology outpatient clinic. *Int J Androl*. 2007;30:48-55.
126. Zini A, Kamal K, Phang D, Willis J, Jarvi K. Biologic variability of sperm DNA denaturation in infertile men. *Urology*. 2001;58:258-261.
127. Agarwal A, Gupta S, Du Plessis S, et al. Abstinence time and its impact on basic and advanced semen parameters. *Urology*. 2016b;94:102-110.

128. McEvoy A, Roberts P, Yap K, Matson P. Development of a simplified method of human semen storage for the testing of sperm DNA fragmentation using the Halosperm G2 test kit. *Fertil Steril*. 2014;102:981-988.
129. Ribeiro S, Sharma R, Gupta S, Cakar Z, De Geyter C, Agarwal A. Inter- and intra-laboratory standardization of TUNEL assay for assessment of sperm DNA fragmentation. *Andrology*. 2017;5:477-485.
130. Sharma R, Gupta S, Henkel R, Agarwal A. Critical evaluation of two models of flow cytometers for the assessment of sperm DNA fragmentation: an appeal for performance verification. *Asian J Androl*. 2019;21:438-444.
131. Bungum M, Humaidan P, Spano M, Jepson K, Bungum L, Giwercman A. The predictive value of sperm chromatin structure assay (SCSA) parameters for the outcome of intrauterine insemination, IVF and ICSI. *Hum Reprod*. 2004;19:1401-1408.
132. Bungum M, Spanò M, Humaidan P, Eleuteri P, Rescia M, Giwercman A. Sperm chromatin structure assay parameters measured after density gradient centrifugation are not predictive for the outcome of ART. *Hum Reprod*. 2008;23:4-10.
133. López G, Lafuente R, Checa MA, Carreras R, Brassesco M. Diagnostic value of sperm DNA fragmentation and sperm high-magnification for predicting outcome of assisted reproduction treatment. *Asian J Androl*. 2013;15:790-794.
134. Majzoub A, Agarwal A, Esteves SC. Insights on the predictive accuracy of the sperm DNA fragmentation tests on male infertility. *Transl Androl Urol*. 2017b;6:S644-S646.
135. Evenson DP. Sperm chromatin structure assay (SCSA®). *Methods Mol Biol*. 2013;927:147-164.
136. Agarwal A, Cho C-L, Esteves SC, Majzoub A. Reactive oxygen species and sperm DNA fragmentation. *Transl Androl Urol*. 2017b;6(-Suppl 4):695-696.
137. Lin MH, Kuo-Kuang Lee R, Li SH, Lu CH, Sun FJ, Hwu YM. Sperm chromatin structure assay parameters are not related to fertilization rates, embryo quality, and pregnancy rates in in vitro fertilization and intracytoplasmic sperm injection, but might be related to spontaneous abortion rates. *Fertil Steril*. 2008;90:352-359.
138. Mehraban D, Ansari M, Keyhan H, Sedighi Gilani M, Naderi G, Esfehani F. Comparison of nitric oxide concentration in seminal fluid between infertile patients with and without varicocele and normal fertile men. *J Urol*. 2005;2:106-110.
139. Mostafa T, As T, Imam H, El-Nashar AR, Osman IA. Seminal reactive oxygen species—antioxidant relationship in fertile males with and without varicocele. *Andrologia*. 2009;41:125-129.
140. Zylbersztejn DS, Andreoni C, Del Giudice PT, et al. Proteomic analysis of seminal plasma in adolescents with and without varicocele. *Fertil Steril*. 2013;99:92-98.
141. Mostafa T, Anis TH, El-Nashar A, Imam H, Othman IA. Varicocelectomy reduces reactive oxygen species levels and increases antioxidant activity of seminal plasma from infertile men with varicocele. *Int J Androl*. 2001;24:261-265.
142. Pasqualotto FF, Sundaram A, Sharma RK, Borges E Jr, Pasqualotto EB, Agarwal A. Semen quality and oxidative stress scores in fertile and infertile patients with varicocele. *Fertil Steril*. 2008;89:602-607.
143. Sakamoto Y, Ishikawa T, Kondo Y, Yamaguchi K, Fujisawa M. The assessment of oxidative stress in infertile patients with and without varicocele. *BJU Int*. 2008;101:1547-1552.
144. Moskovtsev SI, Lecker I, Mullen JB, et al. Cause-specific treatment in patients with high sperm DNA damage resulted in significant DNA improvement. *Syst Biol Reprod Med*. 2009;55:109-115.
145. Werthman P, Wixon R, Kasperson K, Evenson DP. Significant decrease in sperm deoxyribonucleic acid fragmentation after varicocelectomy. *Fertil Steril*. 2008;90:1800-1804.
146. Abdelbaki SA, Sabry JH, Al-Adl AM, Sabry HH. 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*. 2017;15:131-139.
147. Alhathal N, San Gabriel M, Zini A. Beneficial effects of microsurgical varicocoelectomy on sperm maturation, DNA fragmentation, and nuclear sulfhydryl groups: a prospective trial. *Andrology*. 2016;4:1204-1208.
148. Baker K, McGill J, Sharma R, Agarwal A, Sabanegh E Jr. Pregnancy after varicocelectomy: impact of postoperative motility and DFI. *Urology*. 2013;81:760-766.
149. La Vignera S, Condorelli R, Vicari E, D'Agata R, Calogero AE. Effects of varicocelectomy on sperm DNA fragmentation, mitochondrial function, chromatin condensation, and apoptosis. *J Androl*. 2012;33:389-396.
150. Lacerda JI, Del Giudice PT, da Silva BF, et al. Adolescent varicocele: improved sperm function after varicocelectomy. *Fertil Steril*. 2011;95:994-999.
151. Li F, Yamaguchi K, Okada K, et al. Significant improvement of sperm DNA quality after microsurgical repair of varicocele. *Syst Biol Reprod Med*. 2012;58:274-247.
152. Ni K, Steger K, Yang H, Wang H, Hu K, Chen B. Sperm protamine mRNA ratio and DNA fragmentation index represent reliable clinical biomarkers for men with varicocele after microsurgical varicocele ligation. *J Urol*. 2014;192:170-176.
153. Ni K, Steger K, Yang H, et al. A Comprehensive investigation of sperm DNA damage and oxidative stress injury in infertile patients with subclinical, normozoospermic and astheno/oligozoospermic clinical varicocele. *Andrology*. 2016;4:816-824.
154. Pourmand G, Movahedin M, Dehghani S, et al. 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*. 2014;84:821-825.
155. Tavalaee M, Bahreinian M, Barekat F, Abbasi H, Nasr-Esfahani MH. Effect of varicocelectomy on sperm functional characteristics and DNA methylation. *Andrologia*. 2015;47:904-909.
156. Smit M, Romijn JC, Wildhagen MF, Veldhoven JL, Weber RF, Dohle GR. Decreased sperm DNA fragmentation after surgical varicocelectomy is associated with increased pregnancy rate. *J Urol*. 2010;183(1):270–4. https://doi.org/10.1016/j.juro.2009.08.161.
157. Mohammed EE, Mosad E, Zahran AM, Hameed DA, Taha EA, Mohamed MA. Acridine orange and flow cytometry: which is better to measure the effect of varicocele on sperm DNA integrity? *Adv Urol*. 2015;2015:1-6.
158. Roque M, Bedoschi G, Esteves SC. Effect of varicocele repair on sperm DNA fragmentation: a systematic review and meta-analysis. *Fertil Steril*. 2018;110:e162.
159. Hurtado de Catalfo GE, Ranieri-Casilla A, Marra FA, de Alaniz MJ, Marra CA. Oxidative stress biomarkers and hormonal profile in human patients undergoing varicocelectomy. *Int J Androl*. 2007;30:519-530.
160. Esteves SC, Agarwal A. Afterword to varicocele and male infertility: current concepts and future perspectives. *Asian J Androl*. 2017;18:319-322.
161. Hamada AJ, Esteves SC, Agarwal A. A comprehensive review of genetics and genetic testing in azoospermia. *Clinics (Sao Paulo)*. 2013;68(Suppl 1):39-60.
162. Gosálvez J, Rodríguez-Predreira M, Mosquera A, et al. Characterisation of a subpopulation of sperm with massive nuclear damage, as recognised with the sperm chromatin dispersion test. *Andrologia*. 2014;46:602-609.
163. Vicari E. Effectiveness and limits of antimicrobial treatment on seminal leukocyte concentration and related reactive oxygen species production in patients with male accessory gland infection. *Hum Reprod*. 2000;15:2536-2544.

164. Fraczek M, Hryhorowicz M, Gill K, et al. The effect of bacteriospermia and leukocytospermia on conventional and nonconventional semen parameters in healthy young normozoospermic males. *J Reprod Immunol*. 2016;118:18-27.
165. Ochsendorf FR. Infections in the male genital tract and reactive oxygen species. *Hum Reprod Update*. 1999;5:399-420.
166. Zeyad A, Hamad M, Amor H, Hammadeh ME. Relationships between bacteriospermia, DNA integrity, nuclear protamine alteration, sperm quality and ICSI outcome. *Reprod Biol*. 2018;18:115-121.
167. Cunha BA, Garabedian-Ruffalo SM. Tetracyclines in urology: current concepts. *Urology*. 1980;36:54856.
168. Naber KG, Kinzig M, Sorgel F, Weigel D. Penetration of ofloxacin into prostatic fluid, ejaculate and seminal fluid. *Infection*. 1993;21:98-100.
169. Schramm P. Ofloxacin: concentration in human ejaculate and influence on sperm motility. *Infection*. 1986;14(Suppl. 4):274-275.
170. Gallegos G, Ramos B, Santiso R, Goyanes V, Gosalvez J, Fernandez JL. Sperm DNA fragmentation in infertile men with genitourinary infection by Chlamydia trachomatis and Mycoplasma. *Fertil Steril*. 2008;90:328-334.
171. Condorelli RA, La Vignera S, Mongioì LM, Alamo A, Calogero AE. Diabetes mellitus and infertility: different pathophysiological effects in type 1 and type 2 on sperm function. *Front Endocrinol (Lausanne)*. 2018;9:268.
172. Leisegang K, Bouic PJ, Henkel RR. Metabolic syndrome is associated with increased seminal inflammatory cytokines and reproductive dysfunction in a case-controlled male cohort. *Am J Reprod Immunol*. 2016;76:155-163.
173. Lu X, Huang Y, Zhang H, Zhao J. Effect of diabetes mellitus on the quality and cytokine content of human semen. *J Reprod Immunol*. 2017;123:1-2.
174. Maresch CC, Stute DC, Alves MG, Oliveira PF, de Kretser DM, Linn T. Diabetes-induced hyperglycemia impairs male reproductive function: a systematic review. *Hum Reprod Update*. 2018;24:86-105.
175. La Vignera S, Vita R, Condorelli RA, et al. Impact of thyroid disease on testicular function. *Endocrine*. 2017;58:397-407.
176. La Vignera S, Vita R. Thyroid dysfunction and semen quality. *Int J Immunopathol Pharmacol*. 2018;32:2058738418775241.
177. Singh P, Singh M, Cugati G, Singh AK. Hyperprolactinemia: An often missed cause of male infertility. *J Hum Reprod Sci*. 2011;4:102-103.
178. Rama Raju GA, Jaya Prakash G, Murali Krishna K, Madan K, Siva Narayana T, Ravi Krishna CH. Noninsulin-dependent diabetes mellitus: effects on sperm morphological and functional characteristics, nuclear DNA integrity and outcome of assisted reproductive technique. *Andrologia*. 2012;44(Suppl 1):490-498.
179. Abbasihormozi SH, Babapour V, Kouhkan A, et al. Stress hormone and oxidative stress biomarkers link obesity and diabetes with reduced fertility potential. *Cell J*. 2019;21:307-313.
180. Elsamanoudy AZ, Abdalla HA, Hassanien M, Gaballah MA. Spermatozoal cell death-inducing DNA fragmentation factor-α-like effector A (CIDEA) gene expression and DNA fragmentation in infertile men with metabolic syndrome and normal seminogram. *Diabetol Metab Syndr*. 2016;8:76.
181. Nordstrom EA, Ryden M, Backlund EC, et al. A human-specific role of cell death-inducing DFFA (DNA fragmentation factor-α)-like effector A (CIDEA) in adipocyte lipolysis and obesity. *Diabetes*. 2005;54:1726-1734.
182. Dupont C, Faure C, Sermondade N, et al. Obesity leads to higher risk of sperm DNA damage in infertile patients. *Asian J Androl*. 2013;15:622-625.
183. Fariello RM, Pariz JR, Spaine DM, Cedenho AP, Bertolla RP, Fraietta R. Association between obesity and alteration of sperm DNA integrity and mitochondrial activity. *BJU Int*. 2012;110:863-867.
184. Kasturi SS, Tannir J, Brannigan RE. The metabolic syndrome and male infertility. *J Androl*. 2008;29:251-259.
185. Lafuente R, García-Blàquez N, Jacquemin B, Checa MA. Outdoor air pollution and sperm quality. *Fertil Steril*. 2016;106:880-896.
186. Radwan M, Jurewicz J, Polańska K, et al. Exposure to ambient air pollution–does it affect semen quality and the level of reproductive hormones? *Ann Hum Biol*. 2016;43:50-56.
187. Jamal F, Haque QS, Singh S, Rastogi SK. The influence of organophosphate and carbamate on sperm chromatin and reproductive hormones among pesticide sprayers. *Toxicol Ind Health*. 2016;32:1527-1536.
188. Jeng HA, Pan CH, Chao MR, et al. Sperm quality and DNA integrity of coke oven workers exposed to polycyclic aromatic hydrocarbons. *Int J Occup Med Environ Health*. 2016;29:915-926.
189. Miranda-Contreras L, Cruz I, Osuna JA, et al. Effects of occupational exposure to pesticides on semen quality of workers in an agricultural community of Merida state, Venezuela. *Invest Clin*. 2015;56:123-136.
190. Sánchez-Peña LC, Reyes BE, López-Carrillo L, et al. Organophosphorous pesticide exposure alters sperm chromatin structure in Mexican agricultural workers. *Toxicol Appl Pharmacol*. 2004;196:108-113.
191. Zhou DD, Hao JL, Guo KM, Lu CW, Liu XD. Sperm quality and DNA damage in men from Jilin Province, China, who are occupationally exposed to ionizing radiation. *Genet Mol Res*. 2016;15(1).
192. Zhu WJ, Qiao J. Male reproductive toxicity of bisphenol A. *Zhonghua Nan Ke Xue*. 2015;21:1026-1030.
193. Gandhi J, Hernandez RJ, Chen A, et al. Impaired hypothalamic-pituitary-testicular axis activity, spermatogenesis, and sperm function promote infertility in males with lead poisoning. *Zygote*. 2017;25:103-110.
194. Bujan L, Walschaerts M, Brugnon F, et al. Impact of lymphoma treatments on spermatogenesis and sperm deoxyribonucleic acid: a multicenter prospective study from the CECOS network. *Fertil Steril*. 2014;102:667-674.
195. Ståhl O, Eberhard J, Jepson K, et al. Sperm DNA integrity in testicular cancer patients. *Hum Reprod*. 2006;21:3199-3205.
196. Smit M, van Casteren NJ, Wildhagen MF, Romijn JC, Dohle GR. Sperm DNA integrity in cancer patients before and after cytotoxic treatment. *Hum Reprod*. 2010;25:1877–1883.
197. Sharma R, Harlev A, Agarwal A, Esteves SC. 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*. 2016;70:635-645.
198. Aboulmaouahib S, Madkour A, Kaarouch I, et al. Impact of alcohol and cigarette smoking consumption in male fertility potential: Looks at lipid peroxidation, enzymatic antioxidant activities and sperm DNA damage. *Andrologia*. 2018;50(3):e12926.
199. Boeri L, Capogrosso P, Ventimiglia E, et al. Heavy cigarette smoking and alcohol consumption are associated with impaired sperm parameters in primary infertile men. *Asian J Androl*. 2019;21:478-485.
200. Cui X, Jing X, Wu X, Wang Z, Li Q. Potential effect of smoking on semen quality through DNA damage and the downregulation of Chk1 in sperm. *Mol Med Rep*. 2016;14:753-761.
201. Mostafa RM, Nasrallah YS, Hassan MM, Farrag AF, Majzoub A, Agarwal A. The effect of cigarette smoking on human seminal parameters, sperm chromatin structure and condensation. *Andrologia*. 2018;50(3):e12910.
202. Ranganathan P, Rao KA, Thalaivarasai Balasundaram S. Deterioration of semen quality and sperm-DNA integrity as influenced by cigarette smoking in fertile and infertile human male smokers—A prospective study. *J Cellular Biochem*. 2019;120(7):11784-11793.
203. Gunes S, Metin Mahmutoglu A, Arslan MA, Henkel R. Smoking-induced genetic and epigenetic alterations in infertile men. *Andrologia*. 2018;50:e13124.

204. Kumar SB, Chawla B, Bisht S, Yadav RK, Dada R. Tobacco use increases oxidative DNA damage in sperm – possible etiology of childhood cancer. *Asian Pac J Cancer Prev*. 2015;16:6967-6972.
205. Morrison CD, Brannigan RE. Metabolic syndrome and infertility in men. *Best Pract Res Clin Obstet Gynaecol*. 2015;29:507-515.
206. Sharma R, Agarwal A, Harlev A, Esteves SC. A meta-analysis to study the effects of body mass index on sperm DNA fragmentation index in reproductive age men. *Fertil Steril*. 2017;108: e138-139.
207. Le W, Su SH, Shi LH, Zhang JF, Wu DL. Effect of male body mass index on clinical outcomes following assisted reproductive technology: a meta-analysis. *Andrologia*. 2016;48:406-424.
208. Jurewicz J, Radwan M, Sobala W, Radwan P, Bochenek M, Hanke W. Dietary patterns and their relationship with semen quality. *Am J Mens Health*. 2018;12:575-583.
209. Faure C, Dupont C, Baraibar MA, et al. In subfertile couple, abdominal fat loss in men is associated with improvement of sperm quality and pregnancy: a case-series. *PLoS ONE*. 2014;2:e86300.
210. Agarwal A, Majzoub A. Role of antioxidants in assisted reproductive techniques. *World J Mens Health*. 2017c;35:77-93.
211. Abad C, Amengual MJ, Gosálvez J, et al. Effects of oral antioxidant treatment upon the dynamics of human sperm DNA fragmentation and subpopulations of sperm with highly degraded DNA. *Andrologia*. 2013;45:211-216.
212. Greco E, Iacobelli M, Rienzi L, Ubaldi F, Ferrero S, Tesarik J. Reduction of the incidence of sperm DNA fragmentation by oral antioxidant treatment. *J Androl*. 2005a;26:349-353.
213. Greco E, Romano S, Iacobelli M, et al. ICSI in cases of sperm DNA damage: beneficial effect of oral antioxidant treatment. *Hum Reprod*. 2005b;20:2590-2594.
214. Gual-Frau J, Abad C, Amengual MJ, et al. Oral antioxidant treatment partly improves integrity of human sperm DNA in infertile grade I varicocele patients. *Hum Fertil (Camb)*. 2015;18:225-229.
215. Martínez-Soto JC, Domingo JC, Cordobilla B, et al. Dietary supplementation with docosahexaenoic acid (DHA) improves seminal antioxidant status and decreases sperm DNA fragmentation. *Syst Biol Reprod Med*. 2016;62:387-395.
216. Ménézo YJ, Hazout A, Panteix G, et al. Antioxidants to reduce sperm DNA fragmentation: an unexpected adverse effect. *Reprod Biomed Online*. 2007;14:418-421.
217. Omu AE, Al-Azemi MK, Kehinde EO, Anim JT, Oriowo MA, Mathew TC. Indications of the mechanisms involved in improved sperm parameters by zinc therapy. *Med Princ Pract*. 2008;17:108-116.
218. Stenqvist A, Oleszczuk K, Leijonhufvud I, Giwercman A. Impact of antioxidant treatment on DNA fragmentation index: a double-blind placebo-controlled randomized trial. *Andrology*. 2018;6:811-816.
219. Tunc O, Thompson J, Tremellen K. Improvement in sperm DNA quality using an oral antioxidant therapy. *Reprod Biomed Online*. 2009;18:761-768.
220. Vani K, Kurakula M, Syed R, Alharbi K. Clinical relevance of vitamin C among lead-exposed infertile men. *Genet Test Mol Biomarkers*. 2012;16:1001-1006.
221. Smits RM, Mackenzie-Proctor R, Yazdani A, Stankiewicz MT, Jordan V, Showell MG. Antioxidants for male subfertility. *Cochrane Database Syst Rev*. 2019;3:CD007411.
222. Showell MG, Mackenzie-Proctor R, Brown J, Yazdani A, Stankiewicz MT, Hart RJ. Antioxidants for male subfertility. *Cochrane Database Syst Rev*. 2014;CD007411.
223. Greco E, Scarselli F, Iacobelli M, et al. Efficient treatment of infertility due to sperm DNA damage by ICSI with testicular spermatozoa. *Hum Reprod*. 2005;20:226-230.
224. Martinez-Soto JC, Domingo JC, Cardobilla LP, Palbero L, Pellicer A, Landeras J. Effect of dietary DHA supplementation on sperm DNA integrity. *Fertil Steril*. 2010;94:S235-S236.
225. Barekat F, Tavalaee M, Deemeh MR, et al. A Preliminary Study: N-acetyl-L-cysteine Improves Semen Quality following Varicocelectomy. *Int J Fertil Steril*. 2016;10:120-126.
226. Raigani M, Yaghmaei B, Amirjannti N, et al. The micronutrient supplements, zinc sulphate and folic acid, did not ameliorate sperm functional parameters in oligoasthenoteratozoospermic men. *Andrologia*. 2014;46:956-962.
227. Bauersachs J, Widder JD. Reductive stress: linking heat shock protein 27, glutathione, and cardiomyopathy? *Hypertension*. 2010;55:1299-1300.
228. Chabory E, Damon C, Lenoir A, et al. Epididymis seleno-independent glutathione peroxidase 5 maintains sperm DNA integrity in mice. *J Clin Invest*. 2009;119:2074-2085.
229. Agarwal A, Sharma R, Roychoudhury S, Du Plessis S, Sabanegh E. MiOXSYS: a novel method of measuring oxidation reduction potential in semen and seminal plasma. *Fertil Steril*. 2016c;106: 566–573.
230. Agarwal A, Arafa M, Chandrakumar R, Majzoub A, AlSaid S, Elbardisi H. A multicenter study to evaluate oxidative stress by oxidation-reduction potential, a reliable and reproducible method. *Andrology*. 2017d;5:939-945.
231. Gosálvez J, Coppola L, Fernández JL, et al. Multi-centre assessment of nitroblue tetrazolium reactivity in human semen as a potential marker of oxidative stress. *Reprod Biomed Online*. 2017;34:513-521.
232. Gosálvez J, Fernández JL, Esteves SC. Response: Nitroblue tetrazolium (NBT) assay. *Reprod BioMed Online*. 2018;36(1): 92-93.
233. Shiraishi K, Matsuyama H. Gonadotoropin actions on spermatogenesis and hormonal therapies for spermatogenic disorders. *Endocr J*. 2017;64:123-131.
234. Casarini L, Moriondo V, Marino M, et al. FSHR polymorphism p. N680S mediates different responses to FSH in vitro. *Mol Cell Endocrinol*. 2014;393:83-91.
235. Kamischke A, Behre HM, Bergmann M, Simoni M, Schäfer T, Nieschlag E. Recombinant human follicle stimulating hormone for treatment of male idiopathic infertility: a randomized, double-blind, placebo-controlled, clinical trial. *Hum Reprod*. 1998;13: 596-603.
236. Colacurci N, Monti MG, Fornaro F, et al. Recombinant human FSH reduces sperm DNA fragmentation in men with idiopathic oligoasthenoteratozoospermia. *J Androl* 2012;33:588-593.
237. Colacurci N, De Leo V, Ruvolo G, et al. Recombinant FSH improves sperm DNA damage in male infertility: a phase II clinical trial. *Front Endocrinol (Lausanne)*. 2018;9:383.
238. Simoni M, Santi D, Negri L, et al. Treatment with human, recombinant FSH improves sperm DNA fragmentation in idiopathic infertile men depending on the FSH receptor polymorphism p. N680S: a pharmacogenetic study. *Hum Reprod*. 2016;31:1960-1969.
239. Garolla A, Ghezzi M, Cosci I, et al. FSH treatment in infertile males candidate to assisted reproduction improved sperm DNA fragmentation and pregnancy rate. *Endocrine*. 2017;56:416-425.
240. Ruvolo G, Roccheri MC, Brucculeri AM, Longobardi S, Cittadini E, Bosco L. Lower sperm DNA fragmentation after r-FSH administration in functional hypogonadotropic hypogonadism. *J Assist Reprod Genet*. 2013;30(4):497–503. https://doi.org/10.1007/s10815-013-9951-y.
241. Garolla A, Selice R, Engl B, et al. Spermatid count as a predictor of response to FSH therapy. *Reprod Biomed Online*. 2014;29(1):102–12. https://doi.org/10.1016/j.rbmo.2014.02.014.
242. Palomba S, Falbo A, Espinola S, Rocca M, Capasso S, Cappiello F, Zullo F. Effects of highly purified folliclestimulating hormone on sperm DNA damage in men with male idiopathic subfertility: a pilot study. *J Endocrinol Invest*. 2011;34(10):747–52. https://doi.org/10.3275/7745.

243. Ruwanpura SM, McLachlan RI, Matthiesson KL, Meachem SJ. Gonadotrophins regulate germ cell survival, not proliferation, in normal adult men. *Hum Reprod*. 2008;23:403-411.
244. Krawetz SA. Paternal contribution: new insights and future challenges. *Nat Rev Genet*. 2005;6:633-642.
245. Lewis SE, Aitken RJ. DNA damage to spermatozoa has impacts on fertilization and pregnancy. *Cell Tissue Res*. 2005;322:33-41.
246. Hammoud I, Bailly M, Bergere M, et al. Testicular spermatozoa are of better quality than epididymal spermatozoa in patients with obstructive azoospermia. *Urology*. 2017;103:106-111.
247. Mehta A, Bolyakov A, Schlegel PN, Paduch DA. Higher pregnancy rates using testicular sperm in men with severe oligospermia. *Fertil Steril*. 2015;104:1382-1387.
248. Moskovtsev SI, Alladin N, Lo KC, Jarvi K, Mullen JB, Librach CL. A comparison of ejaculated and testicular spermatozoa aneuploidy rates in patients with high sperm DNA damage. *Syst Biol Reprod Med*. 2012;58:142-148.
249. Moskovtsev SI, Jarvi K, Mullen JB, Cadesky KI, Hannam T, Lo KC. Testicular spermatozoa have statistically significantly lower DNA damage compared with ejaculated spermatozoa in patients with unsuccessful oral antioxidant treatment. *Fertil Steril*. 2010;93:1142-1146.
250. O'Connell M, McClure N, Lewis SE. Mitochondrial DNA deletions and nuclear DNA fragmentation in testicular and epididymal human sperm. *Hum Reprod*. 2002;17:1565-1570.
251. Steele EK, McClure N, Maxwell RJ, Lewis SE. A comparison of DNA damage in testicular and proximal epididymal spermatozoa in obstructive azoospermia. *Mol Hum Reprod*. 1999;5:831-835.
252. Bradley CK, McArthur SJ, Gee AJ, Weiss KA, Schmidt U, Toogood L. Intervention improves assisted conception intracytoplasmic sperm injection outcomes for patients with high levels of sperm DNA fragmentation: a retrospective analysis. *Andrology*. 2016;4:903-910.
253. Pabuccu EG, Caglar GS, Tangal S, Haliloglu AH, Pabuccu R. Testicular versus ejaculated spermatozoa in ICSI cycles of normozoospermic men with high sperm DNA fragmentation and previous ART failures. *Andrologia*. 2017;49(2):e12609.
254. Arafa M, AlMalki A, AlBadr M, et al. ICSI outcome in patients with high DNA fragmentation: Testicular versus ejaculated spermatozoa. *Andrologia*. 2018;50(1):e12835.
255. Herrero MB, Lusignan MF, Son WY, Sabbah M, Buckett W, Chan P. ICSI outcomes using testicular spermatozoa in non-azoospermic couples with recurrent ICSI failure and no previous live births. *Andrology*. 2019. https://doi.org/10.1111/andr.12591.
256. Zhang J, Xue H, Qiu F, Zhong J, Su J. Testicular spermatozoon is superior to ejaculated spermatozoon for intracytoplasmic sperm injection to achieve pregnancy in infertile males with high sperm DNA damage. *Andrologia*. 2019;51:e13175.
257. Cheung S, Schlegel PN, Rosenwaks Z, Palermo GD. Revisiting aneuploidy profile of surgically retrieved spermatozoa by whole exome sequencing molecular karyotype. *PLoS ONE*. 2019;14:e0210079.
258. Miyaoka R, Orosz JE, Achermann AP, Esteves SC. Methods of surgical sperm extraction and implications for assisted reproductive technology success. *Panminerva Med*. 2019;61:164-177.
259. Ramasamy R, Yagan N, Schlegel PN. Structural and functional changes to the testis after conventional versus microdissection testicular sperm extraction. *Urology*. 2005;65:1190-1194.
260. Esteves SC. Should a couple with failed in vitro fertilization or intracytoplasmic sperm injection and elevated sperm DNA fragmentation use testicular sperm for the next cycle? *Eur Urol Focus*. 2018;4:296-298.
261. Esteves SC. Testicular versus ejaculated sperm should be used for intracytoplasmic sperm injection (ICSI) in cases of infertility associated with sperm DNA fragmentation | Opinion: Yes. *Int Braz J Urol*. 2018b;44:667-675.

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