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Clinical Consultation Guide – Andrology

European Association of Urology Guidelines Panel on Male Sexual and Reproductive Health: A Clinical Consultation Guide on the Indications for Performing Sperm DNA Fragmentation Testing in Men with Infertility and Testicular Sperm Extraction in Nonazoospermic Men

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Abstract

Accumulating evidence has highlighted the contribution of oxidative stress and sperm DNA fragmentation (SDF) in the pathophysiology of male infertility. SDF has emerged as a novel biomarker of risk stratification for patients undergoing assisted reproductive technologies. Studies have also supported the use of testicular over ejaculated sperm at the time of intracytoplasmic sperm injection, as testicular sperm may have lower SDF than ejaculated samples. The European Association of Urology Working Panel on Male Sexual and Reproductive Health provides an evidence-based consultation guide on the indications for SDF testing in male infertility and also for testicular sperm extraction (TESE) in nonazoospermic men. We present the limitations and advantages of SDF testing and a framework to ensure that it is appropriately utilised in clinical practice. Furthermore, we critically appraise the current literature advocating the use of TESE in nonazoospermic men.

Patient summary: This article reviews the evidence supporting the use of sperm DNA fragmentation testing in the assessment of male infertility and testicular sperm extraction in nonazoospermic men.

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1. Introduction

Infertility is defined as the inability to achieve a clinical pregnancy after 1 yr of regular, unprotected sexual intercourse [1]. The prevalence of infertility worldwide has been reported to be 9% [2]. Studies have suggested that in 50% of all infertile couples, the cause is attributable to a male factor [3,4], and a recent Human Fertilisation and Embryology Authority report noted that the most common indication for in vitro fertilisation (IVF) cycles within the UK was male factor infertility (MFI) [5]. The reported live birth rates using assisted reproductive technologies (ARTs) such as intrauterine insemination (IUI) and IVF are 11.49% and 26.96%, respectively [6]. Techniques such as intracytoplasmic sperm injection (ICSI) and intracytoplasmic morphologically selected sperm injection (IMSI) have been developed to further optimise treatment success in couples with MFI. However, these techniques have been observed to have similar live birth rates, ranging from 20% to 32% for IMSI and being 25% for ICSI [7]. Consequently, there has been an increased focus on improving ART outcomes in couples with MFI. However, the World Health Organization (WHO)-standardised semen analysis has been shown to be a poor discriminator between fertile and infertile men [8] and a poor predictor of ART outcomes. Subsequently, there has been a scientific focus on improving our understanding of the molecular mechanisms that underpin male infertility. Within this context, the contribution of sperm DNA fragmentation (SDF) to male infertility has emerged. SDF has been advocated as a novel biomarker in the diagnostic work-up of male infertility and for stratifying success rates in patients undergoing ARTs [9].

More recently, studies have advocated the use of testicular rather than ejaculated sperm at the time of ICSI, as testicular sperm may have lower SDF than ejaculated samples [10,11]. However, this model of care has the additional risks and potential complications of an operative intervention in patients who are potentially normospermic, without appropriate randomised controlled trials (RCTs) and risk-benefit analysis performed [12].

This clinical consultation guide has been developed by the European Association of Urology (EAU) Guidelines Panel on Male Sexual and Reproductive Health, with the specific aim to provide guidance regarding the rationale and indications for SDF testing in men with infertility and use of testicular sperm extraction (TESE) in nonazoospermic patients (defined as men who produce sperm in their ejaculate). The strength of the recommendations has been developed based on a modified GRADE methodology [13,14], and further information can be found on the EAU sexual and male reproductive guidelines website (http://www.uroweb.org/guideline/).

2. The role of SDF

Sperm DNA undergoes a unique and highly specific process to ensure that nuclear chromatin remains compact and stable [15]. The nuclear matrix of sperm undergoes complete decondensation such that the entire genome becomes anchored to a single structure within the base of the tail, called the nucleus annulus [16]. The degree of chromatin organisation affects epididymal maturation, transfer of genetic information to the egg, and embryo development [15]. The decondensation of the sperm nucleus occurs in the oocyte and allows the formation of the male pronucleus [17].

The pathophysiological mechanisms that underpin sperm DNA damage have been postulated to be related to defective sperm chromatin packaging [18,19], apoptosis [20,21], and oxidative stress [22–24]. The risk factors for an abnormal SDF are shown in Table 1.

There are data showing that sperm introduce the reproducing and microtubular organisation elements of the centrosome [25] and that sperm-specific phospholipase C Zeta induces calcium oscillations that stimulate egg activation and early embryonic development [26]. SDF has been hypothesised to contribute to the “late paternal effect”, which affects embryo development after fertilisation from day 2 to 5 [27]. The measurement of SDF can be direct (determining the extent of DNA damage) or indirect through determination of the susceptibility of DNA to protein denaturation [28]. Table 2 shows the different methods of measuring SDF [29–33].

There are few studies comparing the diagnostic accuracy of each SDF assay. A meta-analysis comprising of eight studies reported that the terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick end labelling (TUNEL) assay had higher diagnostic accuracy than the sperm chromatin dispersion (SCD), sperm chromatin structure assay (SCSA), and COMET assays in discriminating fertile and infertile men. However, this meta-analysis pooled data from a study [34] that included nomozoospermic patients of unproven fertility as controls. Further prospective, large-scale studies are needed, and currently there is insufficient evidence to definitively recommend one SDF assay over another at predicting ART outcomes. This is a major limitation in our current understanding of SDF testing, and there is no consensus as to which diagnostic test is the most accurate or which one should be recommended in clinical practice. There are data showing that the inter- and intraobserver coefficients of variation, computed for the SCSA, SCD, and TUNEL assays are below 10% [34–38], and interlaboratory agreement is very high ($r > 0.9$) for the SDF measured using the SCSA [33] and TUNEL assay [39]. However, there is no consensus regarding the optimal choice of an SDF assay, and there are limited data on the correlation

Table 1 – Potential risk factors for increased SDF levels

Risk factors for increased SDF levels
Varicocele [95,96]
Male genital tract infections [97]
Aging [98]
Cigarette smoking [99,100]
Chemotherapy [101,102]
Ionising radiation [102]

SDF = sperm DNA fragmentation.

Table 2 – Different methods of measuring sperm DNA fragmentation

Direct	Indirect
TUNEL assay—It measures sperm DNA damage through the attachment of dUTP to single- and double-strand DNA breaks using terminal deoxynucleotidyl transferase [29]. An advantage of this test is that it can be performed using a low sperm concentration [103]. However, the TUNEL assay lacks standardisation between laboratories [103].	COMET assay—It uses electrophoresis to separate damaged DNA (both single and double stranded), which migrate to form a comet's tail, whilst the stable double-stranded DNA makes the comet's head [30]. An advantage of this assay is that it can be performed using a low sperm concentration [103]. However, this assay has been reported to have interobserver variability [103].
ISNT assay—It labels the free 3-OH ends of the nucleotide at the DNA break; a 5′ → 3′ polymerase activity is combined with a 3′ → 5′ exonuclease activity for the elimination of precoding nucleotides and proofreading. An advantage of this test is that it is relatively simple to perform but (unlike the TUNEL assay) detects single- and double-stranded DNA breaks indistinctively [104,105].	SCD assay—It allows for acid denaturation and removal of nuclear proteins. Consequently, stable DNA (but not fragmented DNA) will produce a halo of dispersed DNA loops [32]. An advantage of this assay is that it does not require complex instrumentation, but it has been reported to have interobserver variability [32,103].
	SCSA—The degree of DNA denaturation is determined by measuring the changes in colour of acridine orange in the DNA, from green fluorescence to red fluorescence, after heat or acid treatment [33]. The SCSA assay has been standardised among laboratories, but its application requires specialist equipment and expertise [106].

dUTP = deoxyuridine triphosphate; ISNT = in situ nick translation; SCD = sperm chromatin dispersion; SCSA = sperm chromatin structure assay; TUNEL = terminal deoxynucleotidyl transferase dUTP nick end labelling.

between the different assays. Ribas-Maynou et al [40] compared the ability of different SDF tests (using the TUNEL, SCSA, SCD, and both the neutral and the alkaline COMET assay) to discriminate between infertile and fertile men. The authors reported a high correlation between the SCD assay and SCSA, between the SCD and TUNEL assays, and between the SCSA and TUNEL assay. Furthermore, moderate correlations were observed individually between the alkaline COMET and individual SCSA, SCD, and TUNEL assays. However, no correlation was identified between the neural COMET assay and any of the other assays. The limitation of this study was that it did not include any information pertaining to whether these cohorts of patients had male factor or unexplained infertility, and analysed only the ability of the SDF to differentiate between infertile and fertile men without any reference to whether any test was able to change the clinical diagnosis (eg, identify a male factor when there was normal sperm parameters) or management (such as ability to predict spontaneous conception or ART outcomes). It is also unclear whether the authors of this study excluded other factors that may have contributed to high SDF (such as smoking or the presence of a varicocele); whilst these factors may also contribute to infertility, it would be useful to exclude any confounding factors to confirm that the high SDF was associated with infertility per se. There is a paucity of studies analysing the correlation between different SDF assays, and the current literature is limited by small cohort sizes [41–43]. Simon et al [44] compared three SDF assays (alkaline COMET, TUNEL, and the flow cytometric chromatin evaluation [FCCE]) and ART outcomes. The authors observed an association between both the COMET and the TUNEL assay with fertilisation and implantation rate. However, the FCCE assay was not associated with either fertilisation or implantation rate. Moreover, the TUNEL and COMET assays and the TUNEL and FCCE assays showed a correlation ($r^2 = 0.126$, $p < 0.001$ and $r^2 = 0.109$, $p = 0.001$, respectively). However, this study was limited because it did not exclude female factors (such as female age) that could affect ART outcomes. Moreover, there are differences in definitions of abnormal SDF thresholds both for individual assays and amongst different assays, and there is a paucity of large-scale studies investigating a specific cut-off value for SDF. Santi et al [45] performed a receiver operating characteristic (ROC) analysis on 28 studies (utilising the COMET, SCD, SCSA, or TUNEL assay) and reported that an SDF threshold of 20% discriminated between infertile and fertile men with a sensitivity of 79% and specificity of 86%. However, this study classified normozoospermic men as fertile, and also the authors observed high study heterogeneity and highlighted that "no conclusion can be drawn about the specific SDF cut-off to be used". Therefore, large-scale prospective studies are needed to assess the correlation between different SDF assays, and these must also include outcomes such as pregnancy and live birth rate in all evaluations of the clinical utility of SDF testing. Furthermore, large-scale studies are needed to define and validate an SDF threshold with high specificity and sensitivity at discriminating a male factor in couples suffering from infertility, unexplained infertility, and recurrent pregnancy loss (RPL).

3. SDF as a diagnostic test

3.1. *Fertile versus infertile population*

There have been two meta-analyses assessing the utility of SDF testing in discriminating between fertile and infertile populations. Santi et al [45] pooled data from 28 studies investigating the use of the COMET, SCD, SCSA, and TUNEL assays. Overall, the SDF index was significantly higher in infertile men than in fertile individuals ($p < 0.001$), and this trend persisted for the SCD ($p = 0.004$), SCSA ($p < 0.001$), and TUNEL ($p < 0.001$) assays. An ROC curve analysis was performed for all the studies, and the area under the curve (AUC) was 0.844 with an SDF threshold of 20%. This

threshold value was predictive of infertility with sensitivity of 79% and specificity of 86% ($p < 0.001$). A separate ROC curve analysis was performed for the TUNEL assay (as it was the most widely used assay in the literature), and this showed an AUC of 0.844 ($p = 0.002$). The main limitation of this meta-analysis was that it included studies where the control group were normozoospermic men, healthy donors, and volunteers rather than men with proven fertility status.

Cui et al [46] performed a meta-analysis investigating the ability of SDF testing to discriminate between fertile and infertile men. The analysis comprised eight studies with 844 infertile men and 392 fertile controls, and the assays studied were TUNEL, SCD, SCSA, and COMET. The pooled sensitivity and specificity of SDF testing for infertility diagnosis were observed to be 0.80 and 0.85, respectively. The summary receiving operating characteristic curve had an AUC value of 0.9211 and the pooled diagnostic odds ratio (DOR) value was 34.66. The TUNEL assay had the highest diagnostic accuracy (sensitivity: 0.77, specificity: 0.91, AUC: 0.9506, and DOR: 56.89). However, the meta-analysis was limited because of substantial heterogeneity from non-threshold effects between the pooled studies.

3.2. Unexplained infertility

The diagnosis of unexplained infertility is made in infertile couples where the male has normal semen parameters and the female partner has normal ovulation and fallopian tube patency [47]. Unexplained infertility has been reported to affect 15–30% of couples, and the primary treatment is an ART [48]. However, there are emerging data demonstrating that the male partner, belonging to couples with unexplained infertility, may have abnormal SDF compared with fertile controls. Table 3 shows the studies reporting SDF testing in men with unexplained infertility [49–53].

The contemporary literature (Table 3) highlights that whilst the male partner of couples with unexplained infertility may have normal sperm parameters, there is an association with high levels of SDF. The importance of this finding is two-fold. Firstly, recognising that damaged sperm may cause unexplained infertility highlights not only the potential inadequacies in our current understanding of the disease, but also the diagnostic limitations of standard semen analysis. Moreover, given that the principal treatment for unexplained infertility is an ART, this also highlights shortcomings in the contemporary treatment pathway for the infertile male; this group of infertile men should be evaluated comprehensively by a urologist expert in reproductive medicine to identify any reversible causes of raised SDF (Table 1).

3.3. Recurrent pregnancy loss

The definition of RPL is inconsistent in the literature; most descriptions report the loss of either more than two or more than three consecutive pregnancies prior to 20 wk of gestation [54,55]. It has been observed that RPL affects 1–2% of couples [56], and in 40% of cases, the cause will remain unknown [57]. Where female factors have been excluded, therapeutic options for RPL couples are limited [55]. Given that sperm chromatin organisation is needed for normal embryogenesis, an excessive amount of SDF (beyond the repair capacity of oocytes) has been postulated to be contributory to RPL [15]. Indeed, this “late paternal effect” is thought to influence the activation of paternal gene expression, although whether this impacts blastocyst development, implantation, or postimplantation development remains unknown [27]. Three meta-analyses [58–60] have demonstrated an association between an abnormal SDF and an increased miscarriage rate in couples undergoing ARTs. Table 4 shows the three meta-analyses investigating the effect of SDF on RPL [61–63].

Contemporary literature (Table 4) has reported that the male partner in couples with RPL have higher SDF levels than fertile controls. Again, this is clinically important as it

Table 3 – Studies reporting on sperm DNA fragmentation in men with unexplained infertility

Study	Studies	Population	SDF assay	Results
Saleh et al (2003) [49]	Case control	23 infertile men	SCSA	DFI was significantly higher in unexplained infertility group (23%) compared with fertile controls (15%; $p = 0.02$)
		16 fertile men		
Oleszczuk et al (2012) [50]	Case control	119 infertile men	SCSA	DFI ($\geq$20%) was significantly higher in unexplained infertility couples than in the fertile group ($p = 0.005$)
		Historical cohort of 95 fertile men with normal semen parameters		
Faduola et al (2015) [51]	Case control	172 infertile	SCSA	A significantly higher DFI in the unexplained infertility cohort than in fertile controls ($p < 0.05$)
		158 fertile sperm donors		
Zandieh et al (2018) [52]	Case control	28 infertile	SCD	SDF was significantly higher in infertile men vs control ($p = 0.0004$)
		30 fertile men		
Mayorga-Torres et al (2017) [53]	Case control	23 infertile men	SCSA	DFI higher in unexplained infertility cohort (16.7 $\pm$ 5.6) vs fertile controls (14.9 $\pm$ 2.7)
		34 healthy donors of proven fertility		

DFI = sperm DNA fragmentation index; SCD = sperm chromatin dispersion; SCSA = sperm chromatin structure assay; SDF = sperm DNA fragmentation rate.

Table 4 – Meta-analyses investigating the sperm DNA fragmentation in men of couples with recurrent pregnancy loss

Study	Studies	SDF	Results
Tan et al (2019) [61]	13 studies	COMET, SCD, SCSA, and TUNEL assays	Male partners of women with RPL had a significantly higher rate of sperm DNA fragmentation than those of fertile control
	RPL defined as ≥2 failed clinical pregnancies		Overall:
	1125 participants		MD: 11.98, 95% CI 6.64–17.32, $p < 0.001$
			TUNEL:
			MD: 14.62, 95% CI 7.04–22.21, $p = 0.00$
			SCD:
			MD: 11.17, 95% CI 0.73–21.61, $p = 0.00$
			Random-effect model analysis was not performed on the COMET and SCSA assays.
McQueen et al (2019) [62]	13 studies	AO, COMET, SCSA, SCD, and TUNEL assays	Male partners of women with RPL had a significantly higher rate of sperm DNA fragmentation than those of fertile control women:
	All studies had couples with ≥2 pregnancy losses		Overall:
	Variable definition of gestation time duration		MD: 10.7, 95% CI 5.82–15.58, $p < 0.0001$
			TUNEL assay:
			MD: 14.25, 95% CI 4.86–23.64, $p = 0.003$
			SCD assay:
			MD: 3.54, 95% CI −3.30 to 10.38, $p = 0.31$
			SCSA:
			MD: 5.18, 95% CI 0.31–10.05, $p = 0.04$
			COMET:
			MD: 10.10, 95% CI 2.10–18.10, $p = 0.01$
Yifu et al (2020) [63]	27 studies	AO, COMET, SCD, SCSA, and TUNEL assays	Qualitative analysis:
	1941 participants		AO:
	RPL was defined as either ≥2 or ≥3 pregnancy losses in ≤28 wk		Sperm DNA integrity was lower in men from couples with RPL than in controls
			COMET:
			A higher DFI was associated with RPL compared with controls
			Quantitative analysis:
			TUNEL:
			Overall DFI was higher in the RPL than in the control group (MD 12.12, 95% CI 3.34–20.91, $p < 0.01$). Subgroup analysis showed that this trend persisted for RPL ≥ 2 times (MD 13.49, 95% CI 2.84–24.13, $p = 0.01$) but not for RPL ≥ 3 times (MD 9.74, 95% CI −5.33 to 24.80, $p = 0.21$).
			SCSA:
			Overall DFI was higher in patients with RPL than in controls (MD 5.40, 95% CI 1.76–9.03, $p < 0.01$). Subgroup analysis confirmed that this trend persisted for both RPL ≥ 3 times (MD 7.16, 95% CI −0.67 to 14.97, $p = 0.07$) and RPL ≥ 2 times (MD 4.13, 95% CI 0.16–8.11, $p = 0.04$)
			SCD:
			Overall DFI was higher in patients with RPL than in controls (MD 11.16, 95% CI 6.70–15.62, $p < 0.01$). Subgroup analysis confirmed that this trend persisted for both RPL ≥ 3 times (MD 13.37, 95% CI 6.25–20.50, $p < 0.01$) and RPL ≥ 2 times (MD 7.19, 95% CI 3.43–10.95, $p < 0.01$)

AO = acridine orange assay; CI = confidence interval; DFI = sperm DNA fragmentation index; MD = mean difference; RPL = recurrent pregnancy loss; SCD = sperm chromatin dispersion; SCSA = sperm chromatin structure assay; SDF = sperm DNA fragmentation rate; TUNEL = terminal deoxynucleotidyl transferase dUTP nick end labelling.

highlights that sperm with raised SDF may be contributory to RPL. Given that RPL couples are predominantly managed by gynaecologists, this highlights a further deficiency in the treatment pathway of the infertile male, and therefore SDF should be assessed in couples with RPL and a referral should be made to seek specialist andrological expertise.

4. SDF as a predictor of reproductive outcomes

4.1. *Natural pregnancy*

A meta-analysis comprising of 616 couples demonstrated that a high SDF (using the SCSA test) was negatively

associated with the ability to become pregnant by natural conception (odds ratio of 7.01 [95% confidence interval 3.68–13.36]) [59]. However, this analysis was limited, as it included only three studies and also did not provide data on live birth rates.

4.2. Assisted reproductive technologies

There are 12 meta-analyses investigating the association of SDF and ART outcomes (Table 5) [58–60,64–72].

The current literature on the use of SDF in predicting ART outcomes is conflicting. Several meta-analyses report a significant association between an abnormal SDF and lower pregnancy rates with IUI [64,66], IVF [59,60,66,71,72], and IVF and ICSI [66,67,71]. However, it is important to stress that a number of meta-analyses have observed no association between SDF and pregnancy rates using ICSI [59,60,72]. In fact, one study [70] reported that the SCSA and SCD assays had a poor predictive value for pregnancy with both IVF and ICSI cycles, whilst the TUNEL and COMET assays had a fair predictive accuracy in this clinical setting.

Few meta-analyses have analysed the impact of a high SDF on live birth rate outcomes. Osman and colleagues [68] noted that for both IVF and ICSI studies, there was a significant association between a low SDF and live birth rate (p = 0.0005) irrespective of the assay used (COMET, SCSA, and TUNEL). Similarly, Evenson and Wixon [66] reported that for ICSI and IVF, a SDF $<$ 30% (using the COMET, SCD, SCSA, and TUNEL assays) was associated with a higher chance of delivery (p = 0.06). However, Deng and colleagues [72] observed no association between SDF (using the COMET, SCD, SCSA, and TUNEL assays) and live birth rates following IVF or ICSI. These discrepancies in study findings may be attributed to differences in study populations (MFI, female factor infertility, or both) and the SDF assay utilised (types and interassay variability). However, all four meta-analyses [58–60,65] were in accordance in reporting that a high SDF was associated with a higher miscarriage rate after an ART.

Table 5 shows that the majority of studies report SDF as a predictor of reproductive outcomes. Therefore, there is a compelling argument that the urologist should treat all potential reversible causes of SDF, which may be cost efficient, given the association between a high SDF and ART failure. However, there are discrepancies in the literature, a lack of standardisation, interlaboratory variability, multiple different assays, and also a paucity of prospective, randomised controlled studies investigating the cost benefits of SDF testing in clinical practice. Indeed, there are no large controlled cohort studies evaluating the cost benefit of performing SDF testing in conjunction with semen analysis prior to embarking upon ARTs. Furthermore, most of the treatments for high SDF are lifestyle based, such as cessation of cigarette smoking, weight loss, and optimisation of alcohol intake, and within the literature, there is a paucity of robust data reporting on the effects of these modifiable factors on live birth rates.

However, varicocele repair has been shown to improve pregnancy rates in infertile men with clinical varicoceles, MFI, and unexplained infertility [73–75]. Sakamoto et al [76] reported that the presence of a varicocele was associated with increased oxidative stress in both oligospermic and normospermic men. Moreover, microsurgical subinguinal varicocelectomy in those with grade 2/3 clinical varicoceles resulted in a significant decrease in SDF, as

Table 5 – Studies investigating the association of SDF and ART outcomes

Name	Study type	DNA fragmentation assay	Result
Evenson and Wixon (2006) [66]	SDF with IVF, IUI, ICSI	COMET, SCD, SCSA, and TUNEL assay	For IUI and IVF, a DFI of <30% was associated with a higher chance of pregnancy (p = 0.0001) and a higher chance of pregnancy and delivery (p = 0.03), respectively.
	1021 patients		For ICSI and IVF together, a DFI of <30% was not associated with a higher chance of achieving pregnancy/delivery (p = 0.06).
Collins et al (2008) [67]	SDF with both IVF and ICSI cycles	SCSA and TUNEL assay	Higher SDF was negatively associated with a chance of pregnancy via IVF and ICSI (p = 0.045).
	Meta-analysis		
	2161 cycles		
Robinson et al (2012) [58]	SDF and pregnancy loss	AO, COMET, SCSA, and TUNEL assays	A significant increase in miscarriage rate was associated with couples of men with high SDF compared with those with low SDF (p < 0.00001).
	2969 couples		
	1252 pregnancies and 225 pregnancy losses		
	15 studies on ART (IVF, IUI, ICSI) and one study on natural conception		
Zini (2011) [59]	IVF and SDF	SCSA and TUNEL assay	High SDF is associated with lower IVF pregnancy rates (p < 0.05) but no association with ICSI pregnancy rates (p = 0.65).
	1547 patients		High SDF is associated with pregnancy loss (p < 0.0001) after IVF and ICSI.
	ICSI and SDF		
	1011 patients		
	SDF and pregnancy loss after ART		
	1549 men		

Table 5 (*Continued*)

Name	Study type	DNA fragmentation assay	Result
Zhao et al (2014) [60]	IVF, ICSI, and SDF	AO, COMET, SCSA, and TUNEL assays	A significant negative association between high SDF and pregnancy rates was noted for IVF ($p = 0.008$) but not for ICSI ($p = 0.65$).
	3016 patients		A significantly increased miscarriage rate was observed in patients with high sperm DNA damage ($p < 0.0001$).
	SDF and miscarriage rate after ART		
	2756 couples and 965 pregnancies		
Osman et al (2015) [68]	IVF, ICSI, and SDF	COMET, SCSA, and TUNEL assays	For both ICSI and IVF studies, there was a significant association between low SDF and live birth rate ($p = 0.0005$). This trend persisted when each assay was investigated individually (IVF, $p = 0.01$, and ICSI, $p = 0.04$).
	998 patients		
Li et al (2016) [69]	IVF, ICSI, and SDF	SCSA and TUNEL assay	Using the TUNEL assay, high SDF was negatively associated with pregnancy rate via IVF ($p = 0.0006$) and ICSI ($p = 0.09$).
	1115 patients		Using the SCSA, high SDF was not associated with pregnancy rate via IVF ($p = 0.19$) or ICSI ($p = 0.38$).
Cissen et al (2016) [70]	IVF, ICSI, and SDF	COMET, SCD, SCSA, and TUNEL assays	The SCSA and SCD assay had poor predictive accuracy for pregnancy with both IVF and ICSI cycles.
	7672 cycles		The TUNEL and COMET assays had fair predictive accuracy for pregnancy for both IVF and ICSI cycles. For the TUNEL assay, the HSROC curve sensitivity was 0.84 (95% CI 0.75–0.90), specificity 0.24 (95% CI 0.11–0.44), and AUC 0.71 (95% CI 0.66–0.74). For the COMET assay, the HSROC curve sensitivity was 0.79 (95% CI 0.61–0.90), specificity 0.60 (95% CI 0.48–0.71), and AUC 0.73 (95% CI 0.19–0.97).
Simon et al (2017) [71]	IVF, ICSI, and SDF	COMET, SCD, SCSA, and TUNEL assays	For ICSI and IVF studies combined, high SDF was negatively associated with pregnancy rate ($p \leq 0.0001$).
	17 774 cycles		For IVF studies alone, the OR was 1.15 (95% CI 1.05–1.27 [$p = 0.0033$]).
			For ICSI studies alone, the OR was 0.89 (95% CI 0.80–0.99 [$p = 0.0254$]).
			In studies with mixed IVF and ICSI, the OR was 2.00 (95% CI 1.66–2.41 [$p < 0.0001$]).
			Subgroup analysis by SDF technique:
			TUNEL (OR: 1.85) and COMET (OR: 4.15) tests showed a closer correlation with pregnancy outcomes than SCSA (OR: 0.88) and SCD (OR: 1.16).
Deng et al (2019) [72]	IVF, ICSI, and SDF	SCD, SCSA, COMET, and TUNEL assays	The live birth rate was not significantly different between low and high SDF groups (RR: 0.89, $p > 0.05$).
	9645 couples		The clinical pregnancy rate was significantly lower in the high DFI group than in the low DFI group (risk ratio = 0.85, $p < 0.01$). Subgroup analyses confirmed this trend in the IVF subgroup but not in the ICSI subgroup.
Chen et al (2019) [64]	IUI and SDF	SCD assay and SCSA	In data pooled for both SCSA and SCD, high sperm DNA fragmentation was significantly associated with a decreased pregnancy rate: RR: 0.34 (95% CI 0.22–0.52 [$p < 0.001$]).
	2839 cycles		When separated by assay, significant association persists for the SCSA but not for SCD.
			SCSA:
			High sperm DNA fragmentation was significantly associated with a lower live birth rate: RR: 0.14 (95% CI 0.04–0.56 [$p < 0.001$]).
Sugihara et al (2020) [65]	IUI and SDF	SCSA	Low SDF was associated with a higher clinical pregnancy rate (RR: 3.30 [95% CI 1.16–9.39]).
	917 cycles		The pooled sensitivity was 94% (95% CI 0.88–0.97) and specificity 19% (95% CI 0.14–0.26).

ART = assisted reproductive technology; AO = acridine orange assay; AUC = area under the curve; CI = confidence interval; DFI = sperm DNA fragmentation index ; HSROC = hierarchical summary receiver operating characteristic; ICSI = intracytoplasmic sperm injection; IUI = intrauterine insemination; IVF = in vitro fertilisation; OR = odds ratio; RR = risk ratio; SCD = sperm chromatin dispersion; SCSA = sperm chromatin structure assay; SDF = sperm DNA fragmentation rate; TUNEL = terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-nick end labelling.

measured by the TUNEL assay. Although this study is limited by its cohort size (n = 60), it suggests that oxidative stress secondary to varicoceles may cause SDF. The proposed mechanisms by which a varicocele may cause oxidative stress are scrotal hyperthermia, hypoxia, and increased reflux of adrenal and renal metabolites [77]. There is also increasing evidence that varicocele repair may improve SDF and ART outcomes [12]. Therefore, whilst the EAU Guidelines Panel on Male Sexual and Reproductive Health cannot advocate SDF testing in all infertile men with varicoceles (35–40% of infertile men), based upon the current evidence in the literature, there is a compelling argument that it should be performed in those who have failed ARTs, as a couple's chance of ART success may be improved by varicocele repair irrespective of standard semen parameters. However, further prospective studies are required to substantiate this.

The use of antioxidant therapy to reduce SDF is controversial [78], and whilst a recent Cochrane review reported that antioxidant use was associated with improved live birth and pregnancy rates in couples undergoing ARTs, when studies of high bias were removed, this trend did not persist for live birth rates [79]. Furthermore, only four studies (254 men) specifically investigated the effects of antioxidant therapy on SDF, and the pooled estimates indicated that antioxidant use was not significantly associated with lowering SDF (mean difference –5.0%, p = 0.20). The current evidence supporting antioxidant use in male infertility has serious limitations, including a serious risk of bias, high attrition rates, and small overall cohort sizes [79].

A recent randomised, multicentre, double-blind, placebo-controlled trial studied the effects of antioxidant use in a cohort of 174 men [80]. The authors observed no significant differences in sperm morphology, motility, DNA fragmentation rate, pregnancy, and live birth rate between the placebo and intervention groups. However, there were significant differences in the changes in sperm count (p = 0.021), concentration (p = 0.029), and total motile sperm count (p = 0.043) between the two cohorts, with a notable increase of these parameters in the placebo group and a decline in the antioxidant group. The results of this study are in contrast to the findings of the Cochrane review [79] and highlight the need for further well-designed large-scale studies to elucidate the effects of antioxidant therapy on male infertility.

Currently, the two groups of infertile men that are likely to benefit from SDF testing are men with unexplained infertility and those with RPL from both natural conception and ARTs. These two groups of infertile men are likely to be presumed fertile given their normal semen parameters and are unlikely to undergo any andrological assessment as they are often referred for ARTs. Therefore, in these men, SDF should be utilised as a diagnostic test to prompt a male infertility assessment and would provide an opportunity to correct for any reversible causes of SDF, which may optimise natural conception or ART outcomes. There are however conflicting data [81–83] regarding an association between implantation failure and SDF, and in these circumstances, only a male infertility assessment is advised until further evidence on the role of SDF testing in repeated implantation failure is published.

Indeed, a recent study analysing the discriminatory value of COMET scores in ART outcomes observed that this test could be used in conjunction with female age to predict those who would benefit from ICSI rather than IVF [9]. This highlights a further clinical utility for SDF testing, and further prospective trials are needed to validate this indication. Furthermore, there is sufficient evidence to support SDF testing in couples who have failed ARTs, as it can be used to counsel the couple regarding the chances of future successful ART cycles or the use of donor sperm [9].

Within this context, the EAU Guidelines Panel on Male Sexual and Reproductive Health has recommended the use of SDF testing for couples suffering from RPL either from natural conception or from ARTs and unexplained infertility [12]. Furthermore, the panel has expanded the indications for varicocele intervention to include patients with a clinical varicocele, unexplained infertility, and MFI, and those who failed ARTs and have a raised SDF. However, only a weak recommendation could be advocated, based upon the lack of prospective RCTs, and this should be a priority for future studies.

5. Role of TESE in nonazoospermic patients

In some patients, the cause for a raised SDF may remain unexplained, even after exhaustive testing (eg, varicocele treatment and exclusion of male genital infections) and lifestyle modification. There is evidence that that the passage of sperm through the seminiferous tubules and the epididymis is a potential trigger to oxidative stress, leading to high SDF [84]. This theory has partially been corroborated by studies reporting a higher SDF in ejaculated versus testicular sperm [85,86].

Three meta-analyses have compared ART outcomes in studies utilising both ejaculated and testicular sperm outside the setting of azoospermia [86,87]. Two meta-analyses have compared ART outcomes using ejaculated and testicular sperm in men with cryptozoospermia (sperm concentration <1 million/ml). Abhyankar et al [87] reported no difference in fertilisation and pregnancy rates using ICSI in men with cryptozoospermia. However, this study was criticised because it included a case report and incorrect pooling of results of pregnancy rates.

Kang et al [86] included only cohort studies, and reported a significantly higher pregnancy rate and better embryo quality, but not fertilisation or implantation rate, when using testicular compared with ejaculated sperm. Neither study reported on adverse events or complications from surgery.

Esteves et al [85] reported the only meta-analysis comparing SDF and ICSI outcomes using ejaculated and testicular sperm in men without cryptozoospermia. The authors reported a significantly higher SDF in ejaculated than in testicular sperm ($33.4 \pm 12.8\%$ vs $8.9 \pm 5.1\%$ [$p < 0.0001$]). In patients with a high SDF, clinical pregnancy and live birth rates were significantly higher with the use of testicular sperm compared with ejaculated sperm. Furthermore, the

miscarriage rate was reduced with testicular sperm-ICSI when compared with ejaculated sperm-ICSI. Only one study reported complications, with a rate of 6.2% (four cases of pain and two cases of moderate scrotal swelling).

It is important to highlight a number of limitations in the aforementioned studies. The data largely comprises of cohort studies with no RCTs. Furthermore, in the meta-analysis of Esteves et al [85], the risk of bias was moderate, and therefore the data cannot be considered comparable with well-designed RCTs. Moreover, many studies did not exclude other confounding variables such as maternal age, duration of infertility, number of oocytes retrieved, use of medications (eg, empiric treatments), lifestyle factors, presence of varicocele, any prior treatment interventions undertaken, and ovarian hyperstimulation protocols. In addition to this, different SDF assays (with potential interassay variability) and different definitions of an "abnormal" SDF were adopted. Indeed, there is a lack of data pertaining to the additional costing and complication rates associated with TESE. Additionally, in both the meta-analyses of Esteves et al [85] and Kang et al [86], studies were included where the participants acted as their own controls [10,88–90], and therefore it could be argued that without an appropriate control group, the increase in pregnancy rate observed with the use of testicular sperm may simply be a reflection of an expected increase in cumulative success rates after repeated cycles of ARTs. Furthermore, given that the procurement of ejaculated sperm is less invasive and less costly than TESE for ICSI, further data are needed to clarify how many attempts with ejaculated sperm should be made prior to embarking on surgical sperm retrieval.

Arafa et al [91] performed a prospective study comparing ICSI cycle outcomes using both ejaculated and testicular sperm from patients with a high SDF. The authors reported significantly higher pregnancy and live birth rates in ICSI cycles using testicular sperm, but this study was limited because of the small cohort size (n = 36) and the fact that the participants acted as their own controls. Moreover, no comparison was made between the SDF of testicular and ejaculated sperm, and therefore it is not clear whether the improved outcomes can be attributed to differences in SDF. Zhang et al [92] compared ICSI outcomes using ejaculated and testicular sperm in two different cohorts of infertile men with high SDF. The authors observed significantly higher pregnancy and live birth rates in ICSI cycles using testicular sperm, but again no comparison was made between the SDF of sperm used in both ejaculated and testicular ICSI cycles.

Herrero et al [93] reported data from 145 couples who had failed two previous ICSI cycles using ejaculated sperm and had subsequently undergone a further ICSI cycle with either ejaculated or testicular sperm. It was observed that in men with a high SDF, the use of testicular sperm compared with ejaculated sperm improved both clinical pregnancy and live birth rates with ICSI. However, the authors failed to report the baseline characteristics between the two cohorts (including embryological and reproductive factors) and hence may have not excluded confounding variables, and also the SDF assessment of sperm from the ejaculate was not the sample used for ICSI but rather a historical sample.

Alharbi et al [94] reviewed data of 52 infertile men with a high SDF who had a failed ejaculated ICSI cycle and subsequently underwent a testicular sperm-ICSI cycle. The authors reported no significant differences in clinical pregnancy or live birth rates, but this study was limited by the small sample size and the absence of a separate control group.

Therefore, further prospective, large-scale RCTs are needed to validate the use of TESE-ICSI in this setting.

The EAU Guidelines Panel on Male Sexual and Reproductive Health does not currently advocate the routine clinical use of testicular sperm in nonazoospermic men (TESE-ICSI) outside of clinical trials. Although urologists may offer TESE-ICSI in patients with high SDF, patients should be counselled regarding the low levels of evidence for this (ie, nonrandomised controlled studies). Furthermore, testicular sperm should be used only in this setting once the common causes of high SDF have been excluded, including varicoceles, dietary/lifestyle factors, and accessory gland infections.

6. Conclusions

There is a paucity of well-designed prospective RCTs investigating the utility of testicular sperm in preference to ejaculated sperm for ARTs. Currently, the EAU Guidelines Panel on Male Sexual and Reproductive Health recommends the measurement of SDF in unexplained infertility and RPL either from natural conception or from ARTs. Whilst testicular sperm may have a role in the fertility treatment of nonazoospermic men with a raised SDF, the current evidence mitigates its use on a routine clinical basis, as no RCTs have been conducted, there is significant heterogeneity of studies, and no effective cost-benefit analysis has been undertaken to demonstrate the superiority of TESE-ICSI.

MSRH Panel recommendations for the diagnostic utility of SDF in male infertility

- SDF testing should be performed in the assessment of couples with RPL from natural conception and ARTs, or men with unexplained infertility.

  Recommendation: strong

- Varicocelectomy may be considered in men with raised DNA fragmentation with otherwise unexplained infertility or who have suffered from failed ARTs, including RPL, failure of embryogenesis, and implantation.

  Recommendation: weak

MSRH Panel recommendations for the use of TESE-ICSI in men with raised SDF

- The EAU Guidelines Panel on Male Sexual and Reproductive Health does not currently advocate the routine clinical use of testicular sperm in nonazoospermic men with

raised SDF (TESE-ICSI) outside of clinical trials. Although urologists may offer testicular sperm in patients with high DNA fragmentation, patients should be counselled regarding the low levels of evidence for this (ie, non-randomised studies).

Recommendation: strong

- Testicular sperm should be used only in this setting once the common causes of oxidative stress have been excluded, including varicoceles, dietary/lifestyle factors, and accessory gland infections.

Recommendation: weak

Conflicts of interest: The authors have nothing to disclose.

References

[1] Zegers-Hochschild F, Adamson GD, de Mouzon J, et al. International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) revised glossary of ART terminology, 2009. Fertil Steril 2009;92:1520–4.

[2] Boivin J, Bunting L, Collins JA, Nygren KG. International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. Hum Reprod 2007;22:1506–12.

[3] Thonneau P, Marchand S, Tallec A, et al. Incidence and main causes of infertility in a resident population (1 850 000) of three French regions (1988–1989). Hum Reprod 1991;6:811–6.

[4] Comhaire F. Towards more objectivity in diagnosis and management of male infertility. Blackwell Scientific; 1987. https://www.worldcat.org/title/towards-more-objectivity-in-diagnosis-and-management-of-male-infertility/oclc/221930393

[5] Fertilisation H, Authority E. Fertility treatment 2014–2016. 2014 www.hfea.gov.uk

[6] Bahadur G, Homburg R, Bosmans JE, et al. Observational retrospective study of UK national success, risks and costs for 319,105 IVF/ICSI and 30,669 IUI treatment cycles. BMJ Open 2020;10:e034566.

[7] Teixeira DM, Hadyme Miyague A, Barbosa MAP, et al. Regular (ICSI) versus ultra-high magnification (IMSI) sperm selection for assisted reproduction. Cochrane Database Syst Rev 2020;2:CD010167.

[8] Guzick DS, Overstreet JW, Factor-Litvak P, et al. Sperm morphology, motility, and concentration in fertile and infertile men. N Engl J Med 2001;345:1388–93.

[9] Nicopoullos J, Vicens-Morton A, Lewis SEM, et al. Novel use of COMET parameters of sperm DNA damage may increase its utility to diagnose male infertility and predict live births following both IVF and ICSI. Hum Reprod 2019;34:1915–23.

[10] 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;20:226–30.

[11] Esteves SC, Sánchez-Martín F, Sánchez-Martín P, Schneider DT, Gosálvez 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;104:1398–405.

[12] EAU Guidelines Committee. EAU guidelines on sexual and reproductive health. 2020 https://uroweb.org/wp-content/uploads/EAU-Guidelines-on-Sexual-and-Reproductive-Health-2020.pdf

[13] Guyatt GH, Oxman AD, Vist GE, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. BMJ 2008;336:924–6.

[14] Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Schünemann HJ. GRADE: what is "quality of evidence" and why is it important to clinicians? BMJ 2008;336:995.

[15] Agarwal A, Said TM. Role of sperm chromatin abnormalities and DNA damage in male infertility. Hum Reprod Update 2003;9:331–45.

[16] Barone JG, de Lara J, Cummings KB, Ward WS. DNA organization in human spermatozoa. J Androl 1994;15:139–44.

[17] Huret JL. Nuclear chromatin decondensation of human sperm: a review. Syst Biol Reprod Med 1986;16:97–109.

[18] Sivanarayana T, Krishna CR, Prakash GJ, et al. CASA derived human sperm abnormalities: correlation with chromatin packing and DNA fragmentation. J Assist Reprod Genet 2012;29:1327–34.

[19] Manicardi GC, Bianchi PG, Pantano S, et al. Presence of endogenous nicks in DNA of ejaculated human spermatozoa and its relationship to chromomycin A3 accessibility. Biol Reprod 1995;52:864–7.

[20] Sakkas D, Mariethoz E, St. John JC. Abnormal sperm parameters in humans are indicative of an abortive apoptotic mechanism linked to the fas-mediated pathway. Exp Cell Res 1999;251:350–5.

[21] Kim JM, Ghosh SR, Weil ACP, Zirkin BR. Caspase-3 and caspase-activated deoxyribonuclease are associated with testicular germ cell apoptosis resulting from reduced intratesticular testosterone. Endocrinology 2001;142:3809–16.

[22] Kemal Duru N, Morshedi M, Oehninger S. Effects of hydrogen peroxide on DNA and plasma membrane integrity of human spermatozoa. Fertil Steril 2000;74:1200–7.

[23] Barroso G, Morshedi M, Oehninger S. Analysis of DNA fragmentation, plasma membrane translocation of phosphatidylserine and oxidative stress in human spermatozoa. Hum Reprod 2000;15:1338–44.

[24] Shen HM, Chia SE, Ong CN. Evaluation of oxidative DNA damage in human sperm and its association with male infertility. J Androl 1999;20:718–23.

[25] Schatten G. The centrosome and its mode of inheritance: the reduction of the centrosome during gametogenesis and its restoration during fertilization. Dev Biol 1994;165:299–335.

[26] Saleh A, Kashir J, Thanassoulas A, Safieh-Garabedian B, Lai FA, Nomikos M. Essential role of sperm-specific PLC-Zeta in egg activation and male factor infertility: an update. Front Cell Dev Biol 2020;8:28.

[27] Tesarik J, Greco E, Mendoza C. Late, but not early, paternal effect on human embryo development is related to sperm DNA fragmentation. Hum Reprod 2004;19:611–5.

[28] Cho C-L, Agarwal A, Majzoub A, Esteves SC. Clinical utility of sperm DNA fragmentation testing: concise practice recommendations. Transl Androl Urol 2017;6(Suppl 4):S366–73.

[29] Gorczyca W, Gong J, Darzynkiewicz Z. Detection of DNA strand breaks in individual apoptotic cells by the in situ terminal deoxynucleotidyl transferase and nick translation assays. Cancer Res 1993;53:1945–51.

[30] Ostling O, Johanson KJ. Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells. Biochem Biophys Res Commun 1984;123:291–8.

[31] Gold R, Schmied M, Rothe G, et al. Detection of DNA fragmentation in apoptosis: application of in situ nick translation to cell culture systems and tissue sections. J Histochem Cytochem 1993;41:1023–30.

[32] 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.

[33] 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.
[34] Sharma RK, Sabanegh E, Mahfouz R, Gupta S, Thiyagarajan A, Agarwal A. TUNEL as a test for sperm DNA damage in the evaluation of male infertility. Urology 2010;76:1380–6.
[35] Giwercman A, Richthoff J, Hjøllund H, et al. Correlation between sperm motility and sperm chromatin structure assay parameters. Fertil Steril 2003;80:1404–12.
[36] 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;84:833–42.
[37] 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;102:981–8.
[38] 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.
[39] 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;5:477–85.
[40] 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;1:715–22.
[41] Chohan KR, Griffin JT, Lafromboise M, De Jonge CJ, Carrell DT. Comparison of chromatin assays for DNA fragmentation evaluation in human sperm. J Androl 2006;27:53–9.
[42] García-Peiró A, Oliver-Bonet M, Navarro J, et al. Dynamics of sperm DNA fragmentation in patients carrying structurally rearranged chromosomes. Int J Androl 2011;34(6 Pt 2):e546–53.
[43] LeSaint C, Vingataramin L, Alix S, Phillips S, Zini A, Kadoch JI. Correlation between two sperm DNA fragmentation tests (TUNEL and SCSA) and evaluation of TUNEL assay inter-lab variability. Fertil Steril 2016;106:e297.
[44] 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;29:904–17.
[45] 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;37:315–26.
[46] Cui ZL, Zheng DZ, Liu YH, Chen LY, Lin DH, Lan FH. Diagnostic accuracies of the TUNEL, SCD, and comet based sperm DNA fragmentation assays for male infertility: a meta-analysis study. Clin Lab 2015;61:525–35.
[47] Crosignani PG, Collins J, Cooke ID, Diczfalusy E, Rubin B. Unexplained infertility. Hum Reprod 1993;8:977–80.
[48] Quaas A, Dokras A. Diagnosis and treatment of unexplained infertility. Rev Obstet Gynecol 2008;1:69–76.
[49] Saleh RA, Agarwal A, Nada EA, et al. Negative effects of increased sperm DNA damage in relation to seminal oxidative stress in men with idiopathic and male factor infertility. Fertil Steril 2003;79 (Suppl 3):1597–605.
[50] Oleszczuk K, Augustinsson L, Bayat N, Giwercman A, Bungum M. Prevalence of high DNA fragmentation index in male partners of unexplained infertile couples. Andrology 2013;1:357–60.
[51] Faduola P, Kolade CO. Sperm chromatin structure assay results in Nigerian men with unexplained infertility. Clin Exp Reprod Med 2015;42:101–5.
[52] Zandieh Z, Vatannejad A, Doosti M, et al. Comparing reactive oxygen species and DNA fragmentation in semen samples of unexplained infertile and healthy fertile men. Ir J Med Sci 2018;187:657–62.
[53] Mayorga-Torres BJM, Camargo M, Cadavid ÁP, du Plessis SS, Cardona Maya WD. Are oxidative stress markers associated with unexplained male infertility? Andrologia 2017;49:e12659.
[54] Ford HB, Schust DJ. Recurrent pregnancy loss: etiology, diagnosis, and therapy. Rev Obstet Gynecol 2009;2:76–83.
[55] Bender Atik R, Christiansen OB, Elson J, et al. ESHRE guideline: recurrent pregnancy loss. Hum Reprod Open 2018;2018, hoy004.
[56] Roman E. Fetal loss rates and their relation to pregnancy order. J Epidemiol Commun Health 1984;38:29–35.
[57] Stephenson MD. Frequency of factors associated with habitual abortion in 197 couples. Fertil Steril 1996;66:24–9.
[58] 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;27:2908–17.
[59] Zini A. Are sperm chromatin and DNA defects relevant in the clinic? Syst Biol Reprod Med 2011;57:78–85.
[60] Zhao J, Zhang Q, Wang Y, Li Y. Whether sperm deoxyribonucleic acid fragmentation has an effect on pregnancy and miscarriage after in vitro fertilization/intracytoplasmic sperm injection: a systematic review and meta-analysis. Fertil Steril 2014;102:998–1005, e8.
[61] Tan J, Taskin O, Albert A, Bedaiwy MA. Association between sperm DNA fragmentation and idiopathic recurrent pregnancy loss: a systematic review and meta-analysis. Reprod Biomed Online 2019;38:951–60.
[62] 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, e3.
[63] Yifu P, Lei Y, Shaoming L, Yujin G, Xingwang Z. Sperm DNA fragmentation index with unexplained recurrent spontaneous abortion: a systematic review and meta-analysis. J Gynecol Obstet Hum Reprod. In press. https://doi.org/10.1016/j.jogoh.2020.101740.
[64] 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;86:50–5.
[65] Sugihara A, Van Avermaete F, Roelant E, Punjabi U, De Neubourg D. The role of sperm DNA fragmentation testing in predicting intrauterine insemination outcome: a systematic review and meta-analysis. Eur J Obstet Gynecol Reprod Biol 2020;244:8–15.
[66] Evenson D, Wixon R. Meta-analysis of sperm DNA fragmentation using the sperm chromatin structure assay. Reprod Biomed Online 2006;12:466–72.
[67] Collins JA, Barnhart KT, Schlegel PN. Do sperm DNA integrity tests predict pregnancy with in vitro fertilization? Fertil Steril 2008;89:823–31.
[68] Osman A, Alsomait H, Seshadri S, El-Toukhy T, Khalaf Y. The effect of sperm DNA fragmentation on live birth rate after IVF or ICSI: a systematic review and meta-analysis. Reprod Biomed Online 2015;30:120–7.
[69] Li Z, Wang L, Cai J, Huang H. Correlation of sperm DNA damage with IVF and ICSI outcomes: a systematic review and meta-analysis. J Assist Reprod Genet 2006;23:367–76.
[70] Cissen M, van Wely M, 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.
[71] 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.

[72] 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.
[73] Yamamoto M, Hibi H, Hirata Y, Miyake K, Ishigaki T. Effect of varicocelectomy on sperm parameters and pregnancy rate in patients with subclinical varicocele: A randomized prospective controlled study. J Urol 1996;155:1636–8.
[74] Kroese AC, de Lange NM, Collins J, Evers JL. Surgery or embolization for varicoceles in subfertile men. Cochrane Database Syst Rev 2012;10:CD000479.
[75] Kim KH, Lee JY, Kang DH, Lee H, Seo JT, Cho KS. Impact of surgical varicocele repair on pregnancy rate in subfertile men with clinical varicocele and impaired semen quality: a meta-analysis of randomized clinical trials. Korean J Urol 2013;54:703–9.
[76] Sakamoto Y, Ishikawa T, Kondo Y, Yamaguchi K, Fujisawa M. The assessment of oxidative stress in infertile patients with varicocele. BJU Int 2008;101:1547–52.
[77] Jensen CFS, Østergren P, Dupree JM, Ohl DA, Sønksen J, Fode M. Varicocele and male infertility. Nat Rev Urol 2017;14:523–33. http://dx.doi.org/10.1038/nrurol.2017.98.
[78] 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;62:387–95.
[79] Smits RM, Mackenzie-Proctor R, Yazdani A, Stankiewicz MT, Jordan V, Showell MG. Antioxidants for male subfertility. Cochrane Database Syst Rev 2019;3:1–235.
[80] Steiner AZ, Hansen KR, Barnhart KT, et al. The effect of antioxidants on male factor infertility: the Males, Antioxidants, and Infertility (MOXI) randomized clinical trial. Fertil Steril 2020;113:552–60, e3.
[81] 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;19:1401–8.
[82] Coughlan C, Clarke H, Cutting R, et al. Sperm DNA fragmentation, recurrent implantation failure and recurrent miscarriage. Asian J Androl 2015;17:681–5.
[83] Speyer BE, Pizzey AR, Ranieri M, Joshi R, Delhanty JDA, Serhal P. Fall in implantation rates following ICSI with sperm with high DNA fragmentation. Hum Reprod 2010;25:1609–18.
[84] Xie P, Keating D, Parrella A, et al. Sperm genomic integrity by TUNEL varies throughout the male genital tract. J Urol 2020;203:802–8.
[85] 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:. http://dx.doi.org/10.1016/j.fertnstert.2017.06.018, 456–67.e1.
[86] Kang YN, Hsiao YW, Chen CY, Wu CC. Testicular sperm is superior to ejaculated sperm for ICSI in cryptozoospermia: an update systematic review and meta-analysis. Sci Rep 2018;8:1–9.
[87] Abhyankar N, Kathrins M, Niederberger C. Use of testicular versus ejaculated sperm for intracytoplasmic sperm injection among men with cryptozoospermia: a meta-analysis. Fertil Steril 2016;105, 1469–75.e1.
[88] Ben-Ami I, Raziel A, Strassburger D, Komarovsky D, Ron-El R, Friedler S. Intracytoplasmic sperm injection outcome of ejaculated versus extracted testicular spermatozoa in cryptozoospermic men. Fertil Steril 2013;99:1867–71.
[89] Bendikson KA, Neri QV, Takeuchi T, et al. The outcome of intracytoplasmic sperm injection using occasional spermatozoa in the ejaculate of men with spermatogenic failure. J Urol 2008;180:1060–4.
[90] Hauser R, Bibi G, Yogev L, et al. Virtual azoospermia and cryptozoospermia-fresh/frozen testicular or ejaculate sperm for better IVF outcome? J Androl 2011;32:484–90.
[91] Arafa M, AlMalki A, AlBadr M, et al. ICSI outcome in patients with high DNA fragmentation: Testicular versus ejaculated spermatozoa. Andrologia 2018;50:e12835.
[92] 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.
[93] 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;7:281–7.
[94] Alharbi M, Hamouche F, Phillips S, Kadoch J, Zini A. Use of testicular sperm in couples with SCSA-defined high sperm DNA fragmentation and failed intracytoplasmic sperm injection using ejaculated sperm. Asian J Androl 2020;22:348–53.
[95] Saleh RA, Agarwal A, Sharma RK, Said TM, Sikka SC, Thomas AJ. Evaluation of nuclear DNA damage in spermatozoa from infertile men with varicocele. Fertil Steril 2003;80:1431–6.
[96] Zini A, Blumenfeld A, Libman J, Willis J. Beneficial effect of microsurgical varicocelectomy on human sperm DNA integrity. Hum Reprod 2005;20:1018–21.
[97] Gallegos G, Ramos B, Santiso R, Goyanes V, Gosálvez J, Fernández JL. Sperm DNA fragmentation in infertile men with genitourinary infection by Chlamydia trachomatis and Mycoplasma. Fertil Steril 2008;90:328–34.
[98] Petersen CG, Mauri AL, Vagnini LD, et al. The effects of male age on sperm DNA damage: An evaluation 2,178 semen samples. J Bras Reprod Assist 2018;22:323–30.
[99] Taha EA, Ezz-Aldin AM, Sayed SK, Ghandour NM, Mostafa T. Smoking influence on sperm vitality, DNA fragmentation, reactive oxygen species and zinc in oligoasthenoteratozoospermic men with varicocele. Andrologia 2014;46:687–91.
[100] Taha EA, Ez-Aldin AM, Sayed SK, Ghandour NM, Mostafa T. Effect of smoking on sperm vitality, DNA integrity, seminal oxidative stress, zinc in fertile men. Urology 2012;80:822–5.
[101] Chatterjee R, Haines GA, Perera DM, Goldstone A, Morris ID. Testicular and sperm DNA damage after treatment with fludarabine for chronic lymphocytic leukaemia. Hum Reprod 2000;15:762–6.
[102] 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;25:1877–83.
[103] 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 2016;5:935–50.
[104] Schulte RT, Ohl DA, Sigman M, Smith GD. Sperm DNA damage in male infertility: etiologies, assays, and outcomes. J Assist Reprod Genet 2010;27:3–12.
[105] Muriel L, Segrelles E, Goyanes V, Gosálvez J, Fernández JL. Structure of human sperm DNA and background damage, analysed by in situ enzymatic treatment and digital image analysis. Mol Hum Reprod 2004;10:203–9.
[106] Majzoub A, Agarwal A, Esteves SC. Sperm DNA fragmentation: overcoming standardization obstacles. Transl Androl Urol 2017;6 (Suppl 4):S422–4.