Should we evaluate and treat sperm DNA fragmentation?

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Purpose of review
The clinical utility of sperm DNA fragmentation tests needs to be revisited in light of increasing evidence of detrimental effect of sperm DNA damage on reproductive outcomes.

Recent findings
Current evidence supports the association between high sperm DNA fragmentation and poor outcomes with regards to natural conception and intrauterine insemination. The relationship between high sperm DNA fragmentation and impaired outcomes after in-vitro fertilization and intracytoplasmic sperm injection are more equivocal. However, recent studies indicate that poor sperm chromatin content is associated with an increased risk of early pregnancy loss after in-vitro fertilization and intracytoplasmic sperm injection. Several strategies are proposed to alleviate sperm DNA fragmentation and/or select sperm with higher quality chromatin content for assisted reproductive techniques. The intake of oral antioxidants, varicocele repair, use of recurrent ejaculations alone or combined with micromanipulation-based sperm selection techniques, and the use of testicular sperm for intracytoplasmic sperm injection have been attempted with promising results.

Summary
Sperm DNA fragmentation tests provide clinically relevant information for natural conception and artificial reproduction independent of those derived from conventional semen parameters. The increasing knowledge of paternal factors on pregnancy outcome and the improvement in treatment strategies should prompt routine evaluation of sperm DNA fragmentation in infertile couples.

Keywords
assisted reproductive technology, DNA integrity, infertility, sperm DNA fragmentation

INTRODUCTION

The predictive value of conventional semen analysis on male fertility potential and reproductive outcomes with assisted reproductive technology (ART) is poor [1]. There is a need to develop new markers and the importance of sperm DNA integrity in human fertility is being increasingly recognized. Sperm DNA fragmentation (SDF) tests offer an opportunity to investigate the important genetic content that is passed on to the subsequent generations [2]. SDF tests are complementary to, but distinct and more significant than the conventional semen parameters; semen analysis results only indicate the quality of sperm as a carrier of the DNA package. SDF test results also reflect, to a certain extent, sperm quality [3].

On the other hand, the fact that sperm with high DNA fragmentation can have normal motility and morphology suggests additional prognostic value of the assessment [4]. It is clearly shown that infertile men have higher levels of DNA strand breaks or other DNA defects than fertile men [5]. A higher level of SDF is also found in men with abnormal semen parameters [6] and normozoospermic partners of an infertile couple [7]. The value of SDF as an independent attribute of semen quality in addition to conventional semen analysis to the male infertility evaluation has been recently confirmed

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KEY POINTS

- There is a clear association between high SDF and decreased pregnancy rates in natural conception and IUI.
- Emerging evidence suggests a negative impact of high SDF on pregnancy outcomes in IVF and ICSI cycles.
- The widespread use of ICSI in treating couples with severe male factor infertility may result in DNA-damaged sperm mistakenly injected into the oocyte, with unclear but potentially hazardous consequences.
- Novel treatment strategies demonstrate promising results in alleviating SDF and potentially improve pregnancy outcome both naturally and with ART.
- Evaluation of SDF and determination of the underlying cause is at the best interest of infertile couples.

[8]. On the contrary, the application of intracytoplasmic sperm injection (ICSI) is increasing worldwide. The success of ICSI in achieving fertilization independent of conventional sperm parameters and levels of DNA damage casts doubt on the clinical value of SDF tests [9].

The main mechanism involved in SDF is oxidative stress-induced DNA damage during co-migration of mature sperm with reactive oxygen species (ROS)-producing immature and defective sperm through the epididymis [10,11]. Infertile men have higher oxidative parameters in the semen than fertile men [12]. The increased levels of ROS in these patients have been associated with environmental and lifestyle factors, advanced age, obesity, infection, varicocele, and other diseases [13]. Exposure of mature testicular sperm to ROS, produced either by immature and defective sperm or epithelial cells lining the epididymis, can result in sperm DNA damage before disulfide cross-linking takes place [14,15]. The sources of oxidative stress, and the relationship between oxidative stress and SDF is summarized in Fig. 1.

In this review, we evaluate the clinical impact of SDF on natural pregnancy and assisted reproductive techniques. It also addresses the possible consequences of high SDF on offspring. Then, available treatment strategies for high SDF are discussed. Lastly, the clinical utility of the current SDF tests is highlighted.

RELATIONSHIP BETWEEN SPERM DNA DAMAGE AND PREGNANCY

The importance of SDF tests as an integral part in the assessment of infertile couples is illustrated by the intimate relationship between sperm DNA integrity and pregnancy outcomes.

Natural conception

The relationship between DNA damage and natural pregnancy is demonstrated in a meta-analysis involving three studies and 616 couples. High SDF, determined by the sperm chromatin structure assay (SCSA), was associated with failure to achieve natural pregnancy with an odds ratio (OR) of 7.01 (95% CI 3.68, 13.36) [16]. In first pregnancy planners with no previous knowledge of their fertility capability, a high proportion of sperm exhibiting DNA fragmentation was associated with a longer time to achieve natural pregnancy in addition to lower fertility potential compared with low SDF [17].

Intrauterine insemination

DNA fragmentation index greater than 30% by SCSA is a predictor for decreased pregnancy and delivery rates after intrauterine insemination (IUI) with an OR of 9.9 (95% CI 2.37, 41.51) [18]. In this study, 17% of patients had an abnormal SDF index. In another study, insemination of greater than 12% terminal deoxynucleotidyl transferase-mediated fluorescein-dUTP nick-end labeling (TUNEL)-positive spermatozoa resulted in no pregnancy [19]. In contrast, an association between DNA fragmentation measured by sperm chromatin dispersion (SCD) test and clinical pregnancy rates by IUI was not observed in a study evaluating 100 treatment cycles; however, delivery rates were not reported [20].

In-vitro fertilization

The relationship between SDF and pregnancy rates after in-vitro fertilization (IVF) is more extensively studied. Notwithstanding, the interpretation of results is limited by the heterogeneous design and mixed protocols. A significant but modest OR of 1.70 (95% CI 1.30, 2.23) correlating abnormal SDF and lower pregnancy rates in IVF is suggested by a meta-analysis that pooled 11 studies and 1805 couples [16]. A more recent meta-analysis evaluating nine IVF studies showed that the odds for clinical pregnancy is higher in the group with DNA fragmentation index less than 27% (OR 1.742, 95% CI 1.382, 2.195); however, delivery rates were not analyzed and subgroup analyses indicated that SDF test method influenced the magnitude of effect size [21▪].

Intracytoplasmic sperm injection

The results of a meta-analysis on 13 IVF and ICSI studies involving 2161 treatment cycles suggest that

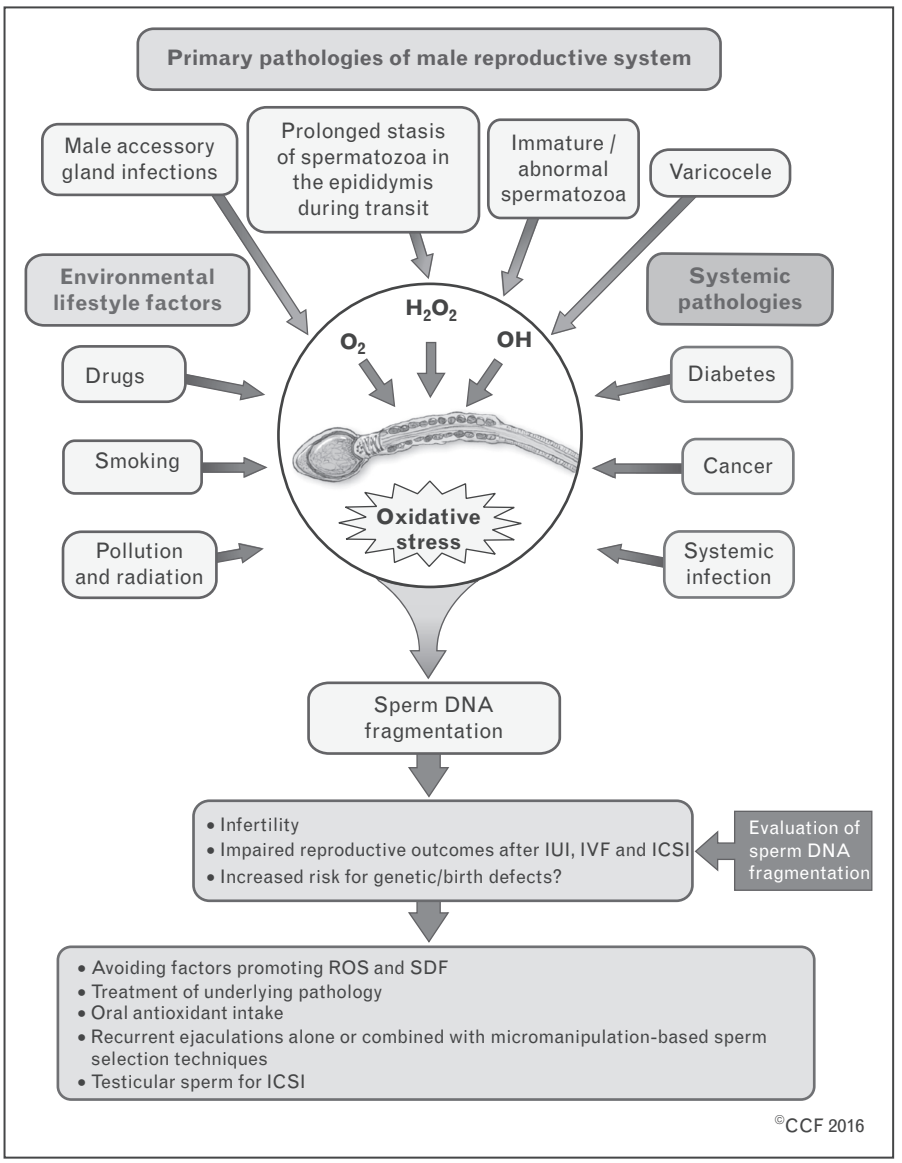

FIGURE 1. Relationship of primary pathologies of the male reproductive system, oxidative stress, and sperm DNA fragmentation (SDF). The figure depicts the possible consequences of high SDF and highlights the role of SDF testing to better manage couples facing infertility. Possible treatment strategies to overcome high SDF are indicated [75▪▪]. ROS, reactive oxygen species; ICSI, intracytoplasmic sperm injection; IUI, intrauterine insemination; IVF, in-vitro fertilization.

SDF has a significant influence on pregnancy rates by ART (log diagnostic OR 1.44, 95% CI 1.03, 2.03) [22]. Subgroup analyses showed that test accuracy was not materially affected by treatment method (IVF or ICSI). However, definition of pregnancy was heterogeneous and included clinical pregnancy, ongoing pregnancy, and live birth.

Fourteen studies involving 1171 couples were analyzed in another meta-analysis. An 11% difference in pregnancy rates was demonstrated between the groups with high and low SDF but results were not statistically significant (OR 1.15, 95% CI 0.9, 1.55) [16]. Likewise, the studies were heterogeneous in design, participants, and main outcome measures for pregnancy. Recently, a meta-analysis pooling five studies and 397 patients demonstrated no difference in clinical pregnancy rates after ICSI with SDF greater than 27% used as the cut-off for abnormal test results (OR 0.895, 95% CI 0.629, 1.273) [21▪]. Along the same lines, a recent prospective cohort study involving 156 participants suggested that clinical and ongoing pregnancy rates and cleavage-stage embryo quality were not associated with sperm DNA damage measured by the improved SCD method [23]. Contrary results have been reported in a recent study evaluating SDF by the same

aforementioned method in 165 couples undergoing ICSI. The authors found that SDF levels were associated not only with clinical pregnancy rates after elective single blastocyst transfers but also with the dynamics of embryo development evaluated by continuous time-lapse monitoring [24]. Interestingly, higher levels of SDF correlated with an increased time for the embryo to reach the blastocyst stage and adversely affected the chances of achieving pregnancy by ICSI.

Importantly, clinical and ongoing pregnancy rates are less relevant outcomes than live birth rates. Hence, the clinical validity of most existing meta-analyses should be weighed appropriately because of the lack of live birth rates as an outcome. Specifically, the association between sperm DNA damage and live birth rates was recently examined in a meta-analysis of six observational studies and 998 couples. Couples whose male partners had low SDF achieved higher live birth rates after IVF [relative risk (RR) 1.27, 95% CI 1.05, 1.52] and ICSI (RR 1.11, 95% CI 1.00, 1.23) [25▪]. The cut-off level for high DNA fragmentation in the selected studies was variable even when the same test method had been used.

Pregnancy loss

The association between high SDF and an increased risk of miscarriage after ART is indicated by recent meta-analyses [26,27▪]. In one study evaluating five IVF and six ICSI studies and 1549 treatment cycles, the combined OR of 2.48 (95% CI 1.52, 4.04) indicates that SDF is predictive of pregnancy loss after ART; the ORs are independent of the type of ART used (IVF or ICSI) [26]. In another study pooling 16 papers and 2969 couples, the risk of early pregnancy loss was increased by 2.16-fold when semen specimens with high SDF were used for IVF or ICSI (95% CI 1.54, 3.03) [28]. The latest meta-analysis of 14 studies and 2756 couples also indicates that elevated SDF is associated with higher miscarriage rates in both IVF and ICSI cycles [27▪]. A positive association between recurrent spontaneous abortion and high SDF was also recently reported [29].

In summary, high SDF is associated with decreased pregnancy rates by natural conception and IUI. The association between high SDF and impaired pregnancy outcomes after IVF and ICSI is suggestive but not conclusive. There is fair evidence, however, indicating that high SDF is associated with an increased risk of early pregnancy loss after IVF and ICSI. Despite the controversy surrounding the routine use of SDF testing in the clinical evaluation of male factor infertility [30,31▪], the American Society for Reproductive Medicine (ASRM) Practice Committee recently recognized that determining the values of SDF might be clinically informative for IUI or IVF and ICSI outcomes [32].

Various SDF tests have been introduced in the past years and comparison among methods reported [32,33]. In human sciences, technological innovations often produce conflicting results; SDF tests are no exception. Such incongruities mainly reflect the inherent complexity of the human reproductive process on one hand, and inadequate study participant selection and design on the other. None of the tests has been taken up as a standard method for assessment of SDF by andrology laboratories [34]. Different assays measure different aspects of sperm chromatin. The significance of DNA damage at coding and noncoding DNA domains remains unclear. The finding of greater impact of SDF on IVF and ICSI outcomes in couples whose female partners have poor ovarian reserve suggests a modulating role of female factors [35▪▪]. Indeed, the ability of oocytes to repair sperm DNA damage makes the interpretation of pregnancy outcomes more complicated [36]. The discordance rate in SDF results among various SDF assays is another concern [37]. Notwithstanding, a variety of studies on SDF and pregnancy outcomes have shed light on our understanding of the complexity of the human reproductive process.

SDF is a normal phenomenon and is seen at low rates in fertile individuals [38]. However, the proportion of sperm with DNA fragmentation is markedly higher in infertile men with various etiology categories, including unexplained infertility [39,40]. The biological plausibility of an association between elevated SDF and lower pregnancy rates in natural conception and IUI seems to be related to the lower longevity of spermatozoa with DNA damage and the additional post-ejaculation increase in SDF. The worse reproductive outcome in IVF than ICSI might be explained by the increase in SDF *in vitro* as sperm and oocytes interact for several hours. Despite not being apparent perifertilization, the influence of damaged paternal chromatin can be observed after zygotic transcriptional activation [41]. The impact of paternal genes manifests at the stage of four to eight cells whereas maternal regulation is the major drive during blastocyst development [42,43]. The extensive involvement of the paternal component and the effects of sperm DNA damage on embryo development and early pregnancy is confirmed by recent studies [44▪,45].

SPERM DNA DAMAGE AND GENETIC/ BIRTH DEFECTS

Although ICSI revolutionized the treatment of male infertility, questions regarding the safety of ART remain. Chromosomal abnormalities are higher in

ICSI candidates [46]. Increased rate of aneuploidy has been associated with elevated SDF [47] and recurrent pregnancy loss [48]. It is also shown in mouse models that high levels of SDF can result in premature ageing, aberrant growth and behavior, and increased incidence of tumors in the offspring [49].

The possible link between sperm DNA damage and defects in offspring is illustrated by the effect of smoking and paternal age on SDF. Heavy smokers exhibit higher levels of DNA fragmentation and oxidative adduct formation in sperm [50]. And this may help to explain the suggested increase in the incidence of childhood cancer in the offspring of heavy smokers [51,52]. Sperm produced by ageing men also exhibit impaired DNA integrity [53]. Paternal age has been linked with dominant genetic diseases [54], polygenic neurological disorders such as schizophrenia [55], and birth defects such as neural tube defects [56].

The most concerning aspect is the unknown long-term consequence of a successful pregnancy with very high levels of DNA damage. It is argued that there is lack of evidence demonstrating the deleterious effect of high SDF on the human offspring. However, the unequivocal circumferential evidence from animal studies [57] and the detrimental effect of high SDF on ART outcomes are alarming. Skepticism will persist until the question of the relationship between SDF and genetic defect is answered by longitudinal studies with sufficient samples and duration.

TREATMENT OPTIONS FOR HIGH SPERM DNA FRAGMENTATION

Several strategies are proposed to alleviate SDF and/or select sperm with higher quality chromatin content for ART. The intake of oral antioxidants, varicocele repair, recurrent ejaculations alone or combined with micromanipulation-based sperm selection techniques such as magnetic cell sorting, physiological ICSI or intracytoplasmic morphologically selected sperm injection, and the use of testicular sperm for ICSI have been attempted with varying success rates.

The role of SDF testing and a summary of the possible treatment strategies to overcome high SDF are presented in Fig. 1.

Oral antioxidant therapy

The possible beneficial effect of oral antioxidants has been suggested by studies demonstrating reduction in the percentage of SDF after antioxidant therapy [58]. The clinical pregnancy and implantation rates after antioxidant therapy in couples subjected to ICSI seems to improve without differences in fertilization and cleavage rates or in embryo morphology pretreatment and posttreatment [59]. Although oral antioxidant intake has been commonplace, its effects to alleviate ROS-induced SDF are limited and many patients persist with high SDF after therapy [60]. In a study comparing antioxidants to placebo or no treatment, SDF rates were found to be reduced by only 13.8% (95% CI 10.4%, 17.7%) [61]. Additional studies are required involving careful selection of patients with high levels of oxidatively induced sperm DNA damage and a standardized treatment regimen.

Varicocele repair

Oxidative stress is the central element in the pathophysiology of varicocele. Elevated ROS can inflict damage to both nuclear and mitochondrial DNA, thus resulting in base modification, strand breaks, and chromatin cross-link [62]. Elevated SDF is confirmed in men with varicocele [63]. A meta-analysis of six studies including 177 patients evaluated the effect of varicocelectomy on sperm DNA damage. The authors reported that varicocelectomy improves sperm DNA integrity with a mean difference of −3.37% (95% CI −4.09%, −2.65%) [64]. Because of the low magnitude of the effect size, further research is needed to elucidate the clinical significance of varicocelectomy on sperm DNA damage.

Sperm selection

Human sperm is heterogeneous with regards to DNA damage. Isolation of sperm for ART that possess low levels of DNA damage is an attractive option. Density gradient centrifugation [65], sequential density gradient centrifugation and washing [66], glass wool filtration [65], and electrophoretic sperm isolation [67] are some of the techniques attempting to isolate sperm populations with less SDF. Hyaluronic acid-binding method [68], sperm magnetic sorting [69,70] and high magnification microscopy [71] are among the other studied techniques. Processing and selection of sperm and embryos might partially explain the abrogation of the likely adverse effect of sperm DNA damage on reproductive outcomes with ICSI [72]. Yet, none of these methods, alone or combined, has been unequivocally proven to be of clinical value to bypass the potential detrimental effect of abnormal SDF on ART outcomes. Current sperm selection techniques are limited by the fact that none of them completely deselect sperm with DNA damage or aneuploidies [73].

Testicular sperm

Spermatozoa retrieved from the testis of men with high proportion of ejaculated sperm with DNA fragmentation tend to have better DNA quality. The incidence of DNA fragmentation is three-fold to five-fold lower in testicular sperm than ejaculated sperm [74,75▪▪]. The use of testicular sperm for ICSI was evaluated in a recent prospective comparative study involving 172 patients with elevated SDF. SDF was five-fold lower in testicular sperm compared with ejaculated sperm (40.7 ± 9.9 versus $8.3 \pm 5.3\%$, $P < 0.001$). And the use of testicular sperm for ICSI in a group of patients with oligozoospermia and persistently high SDF levels in their ejaculates, even after oral antioxidant therapy, was associated with better reproductive outcome [75▪▪]. For the testicular sperm-ICSI group versus the ejaculated sperm-ICSI group, respectively, the live birth rates were 46.7 and 26.4% ($P = 0.007$), with a RR of 1.76 (95% CI 1.15, 2.70) favoring testicular sperm.

CLINICAL UTILITY

SDF tests provide invaluable clinical information and should be included in the armamentarium of an infertility specialist. Evidence is supportive of an association between lower chances of pregnancy both naturally and by IUI in cases of high SDF. Likewise, an association between high SDF and early pregnancy loss after IVF and ICSI is noted. High SDF in couples with recurrent pregnancy loss suggests a paternal effect as the causative factor. SDF may be also considered in the assessment of idiopathic and unexplained infertility given the strong association between high SDF and reduced natural pregnancy. Test results can guide management and aid in monitoring intervention outcomes (Fig. 1).

Success in ART has led some practitioners to ignore suboptimal sperm quality. Disregarding the excessive costs, which are not inconsequential, and potential risks borne by the female partner undergoing ovarian stimulation and IVF, the need for repeated cycles and the inherent risks related to the health of the offspring is not acceptable. It would seem a grave disservice to a couple not to offer the option of SDF assessment in the face of current evidence. The clinical utility of SDF tests will certainly expand in light of further research in the area.

DISCUSSION

An argument against the use of SDF tests is the lack of test standardization with clear cut-off levels. At present, each SDF test has its advantages and shortcomings and none of them is universally accepted. Test standardization will definitely improve its clinical utility and facilitate future research. But it is important to realize that in the context of a complex reproductive system, a single 'magic' test with a clear cut-off is probably not available for humans. It is unlikely that the result of the dynamic interaction among multiple confounding factors can be concluded by a single test. SDF tests are unique in providing important assessment of genetic content in male gamete. SDF tests should be considered as a piece of an important jigsaw puzzle complementary to, but different from the information provided by conventional semen analysis. Given the prognostic value of SDF tests illustrated by numerous studies, regardless of the testing method used, assessment of sperm DNA damage has an unequivocal role in the assessment of fertility potential of an individual [76].

The unclear long-term consequences of transmitting defective genes, particularly in cases of extremely high SDF treated with ICSI, should not be overlooked. Genetic or epigenetic damage associated with the use of DNA-damaged spermatozoa for assisted conception rarely manifests as an obvious phenotype in the immediate offspring. No comfort can be taken because genetic defect is cumulative and may affect future generations. It may require millions of ICSI children and several generations before we can draw any firm conclusion on the long-term safety of the procedure. Because DNA damage in a single live cell cannot be assessed with current techniques, it is possible that sperm with normal appearance but with DNA fragmentation be mistakenly selected to fertilize the oocyte in ICSI. It would therefore seem rational to attempt to determine the cause of DNA damage in the patient with high SDF and offer a strategy to either alleviate sperm DNA damage or improve reproductive outcomes.

With respect to the question posted to us, ''Should we evaluate and treat sperm DNA fragmentation'', our answer is ''YES''. We strongly believe that the wider application of SDF tests will help clinicians to better manage infertile couples.

CONCLUSION

Fertility, and more precisely reproductive outcome, is a multifactorial phenomenon that involves the participation of two gametes evolving from each partner. With respect to the male factor, sperm DNA and its ability to produce a stable balanced genome are crucial to promote normal embryonic growth. SDF testing has emerged as a useful clinical marker of fertility potential, and we advocated its

inclusion in the routine male infertility workup. Determination of the levels of SDF can provide valid information to the understanding of certain andrological conditions and help clinicians to better manage couples facing infertility. A series of standardization practices should be implemented in andrology laboratories willing to master the application of SDF and ensure its validity as a biomarker of male infertility and pregnancy prediction.

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Conflicts of interest

There are no conflicts of interest.

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