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## WHAT SHOULD BE DONE FOR MEN WITH SPERM DNA FRAGMENTATION?

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### ARTICLE SUMMARY

In an age when a small number of sperm can lead to pregnancy through in vitro fertilization or intracytoplasmic sperm injection, selecting healthy sperm is important. Sperm DNA fragmentation (SDF) is known to be higher in infertile men.

Sperm DNA damage testing methods do not have clearly standardized cut-off levels and each method has its advantages and disadvantages, making it difficult to proclaim one as a universally preferable method. It is best to select a testing method based on a patient's clinical characteristics and goals.

Physicians and researchers working with ART must continue to make efforts to obtain healthy sperm with nuclear DNA integrity to minimize the adverse effects that may arise in offspring conceived from sperm with DNA damage.

**Key word:** sperm DNA damage testing methods, sperm DNA fragmentation, infertile men.

In an age when a small number of sperm can lead to pregnancy through in vitro fertilization or intracytoplasmic sperm injection, selecting healthy sperm is important. Sperm DNA fragmentation (SDF) is known to be higher in infertile men. The male partner has a high level of SDF, he should aim to decrease SDF through lifestyle modifications, antioxidant treatment, and ensuring an appropriate duration of abstinence, and physicians need to treat the underlying diseases of such patients. If sperm DNA damage continues despite the patient's and physician's efforts, other methods, such as micromanipulation-based sperm selection or testicular sperm extraction, should be used to select healthy sperm with nuclear DNA integrity.

### INTRODUCTION

Over 15% of married couples worldwide experience fertility problems, and 50% of these cases are caused by male factor infertility [1]. Semen analysis is the most important test for evaluating male infertility, but it does not provide information regarding all functions of sperm, nor is it sufficient for predicting male fertility potential and the likelihood of success of assisted reproductive technology (ART) [2]. Furthermore, the standards for normal values in semen analysis do not reflect average values, but are instead determined using the bottom 5% as a cut-off point. In other words, it is the minimum standard for pregnancy. In fact, 15% of patients with male infertility were found to have normal semen analysis results [3]. Therefore, additional tests should be performed due to the limitations of using semen analysis results alone to evaluate male fertility potential. Research on new testing methods to evaluate sperm abnormalities has been conducted in the past 30 years, and sperm DNA integrity has emerged as an area of interest. Sperm DNA fragmentation (SDF) testing measures the quality of sperm as a DNA package carrier, and it therefore is more significant than the parameters analyzed in previous semen analyses [4]. DNA damage, such as fragmentation and denaturation, can have adverse effects on fertilization and

embryo development and can cause infertility [5]. Infertile men have a greater extent of sperm DNA damage and poorer sperm DNA integrity than fertile men, and the fertilization of DNA-damaged spermatozoa can increase the risk of genetic diseases in the offspring [6]. SDF can be observed even in men with normal semen analysis results [7]. The value of SDF as an independent index for the evaluation of semen quality has led it to be incorporated into semen analysis procedures [8]. However, as intracytoplasmic sperm injection (ICSI) has become more common worldwide, cases of successful fertilization after ICSI despite poor semen analysis results or sperm DNA damage have led to questions regarding the clinical value of SDF testing [9]. We are living in the age of ICSI, which overcomes many of the barriers of natural selection. The selection of sperm with damaged DNA when using ART can result in undesirable results, such as lower pregnancy success rates, increased rates of miscarriage, chromosomal abnormalities, and other genetic or birth defects in the offspring. Therefore, it is important in the age of ART to accurately understand and manage sperm nuclear DNA integrity. This review will discuss the clinical utility of current tests of sperm DNA damage, the effects of sperm DNA damage on offspring conceived through natural pregnancy and as a result of ART, and examine possible treatment strategies for extensive sperm DNA damage.

### SDF test

Various techniques have been used to measure damage to human sperm DNA. Currently, the most widely used testing methods are sperm nuclear DNA integrity assessment and abnormal sperm chromatin packaging assessment. Direct sperm DNA integrity assessments include the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) assay and in situ nick translation (ISNT), which use reagents that attach directly to the damaged area. Indirect types of sperm DNA integrity assessments include the sperm chromatin structure assay (SCSA) and the sperm chromatin dispersion (SCD)

assay, which indirectly measure the damaged area through protein denaturation in an acidic solution, as well as the comet assay. Sperm chromatin packaging assessment is a staining method that uses methyl green, aniline blue, toluidine blue, and chromomycin A3. This review will explain the currently most widely used DNA fragmentation assessments: the TUNEL assay, ISNT, SCSA, the comet assay, and the SCD assay.

**SCD assay** The SCD assay is a testing method based on the characteristic halo that is formed when nuclear proteins are removed after acid denaturation [36]. In other words, sperm nuclei with severe DNA damage will form a very small halo or no halo at all, while sperm with less DNA damage will disperse DNA loops and form a large halo. This is an economically feasible method that is commonly used in male infertility laboratories since it is simple, quick, has high reproducibility, and does not require complex instruments; furthermore, quality management is also straightforward [18]. However, the peripheral border of the halo with low chromatin density may sometimes not be distinguishable from the background, not all halos are on the same focal plane, which can cause errors, and since the sperm tail is not preserved, the sperm needs to be distinguished from other contaminant cells.

Clinical significance of sperm DNA damage for pregnancy In the following section, the importance of SDF testing in assessments of couples struggling with fertility issues and counseling strategies for patients with high SDF will be reviewed, on the basis of observations regarding the relationship between sperm DNA integrity and pregnancy outcomes.

**1. Natural conception:** Many studies have demonstrated that sperm DNA damage affects natural conception. Zini [37] reported that based on an SCSA analysis, high SDF made natural conception difficult. Evenson et al. [21] and Spano et al. [22] also stated that natural conception was almost impossible if SCSA found SDF of more than 30%. Furthermore, it has been reported that a higher extent of sperm DNA damage in couples planning a pregnancy without prior knowledge of their fertility could cause them to take longer to conceive naturally and reduce the likelihood of a successful pregnancy [22]. Therefore, SDF testing is relevant for male fertility through natural conception and can be applied to couples struggling with infertility with an unknown cause and used when counseling such couples regarding future methods of becoming pregnant.

**2. Intrauterine insemination** Bungum et al. [38] reported that the possibility of pregnancy using intrauterine insemination (IUI) was close to zero when the DFI was higher than 30% in SCSA and that elevated DFI levels (>30%) were a predictor of decreased pregnancy and delivery rates after IUI. Duran et al. [15] reported in another study that pregnancy was difficult when the TUNEL method indicated a proportion of sperm DNA damage of 12% or higher. On the contrary, Murie et al. [39] suggested that there was no correlation between sperm DNA damage and clinical pregnancy rates after IUI when samples were analyzed using the SCD test. Measuring sperm DNA damage is beneficial in predicting IUI results ahead of time, and when sperm DNA damage is extensive, other methods such as IVF or IVF/ICSI

should be considered.

**3. In vitro fertilization:** Much research has been conducted into the influence of SDF on the results of IVF and IVF/ICSI, but the results of these studies are complex and varied. This is due to the challenges of interpreting results from studies with heterogeneous designs and mixed protocols. Most studies reported that SDF did not affect fertilization or embryo quality, most likely because maternal regulation plays a predominant role during blastocyst development, while the effects of paternal genes are seen after the four-cell stage [40,41]. Recent studies confirmed that paternal factors and sperm DNA damage affect embryo development and early pregnancy [31,42]. Zini [37] conducted a meta-analysis of 11 studies and found a correlation between abnormal sperm DNA damage test results and low pregnancy rates. Zhang et al. [43] observed, in a meta-analysis of nine studies on IVF research, that a DFI lower than 27% was associated with higher clinical pregnancy rates.

**4. Intracytoplasmic sperm injection:** Collins et al. [44] conducted a meta-analysis of 13 studies on IVF and ICSI and found that sperm DNA damage had a significant influence on pregnancy rates. A meta-analysis of 14 studies conducted by Zini [37] showed that the difference in the pregnancy rate between a group with high sperm DNA damage and a group with low sperm DNA damage was 11%, but the result was not statistically significant. However, a meta-analysis of five studies conducted by Zhang et al. [43] suggested that sperm DNA damage did not lead to a difference in the clinical pregnancy rate after ICSI. Anifandis et al. [45], in a prospective cohort study, suggested that when clinical and ongoing pregnancy rates and cleavage-stage embryo quality were measured by SCD, there was no correlation with the degree of sperm DNA damage. Wdowiak et al. [46] discovered through continuous timelapse monitoring that the degree of sperm DNA damage was related to the clinical pregnancy rate after elective single blastocyst transfer and an assessment of embryo development dynamics. The greater the extent of sperm DNA damage, the longer it took for the embryo to reach the blastocyst stage and lower the possibility of pregnancy through ICSI. However, the live birth rate is more important than the clinical and ongoing pregnancy rates. Osman et al. [47] reported that couples with low levels of male sperm DNA damage had high live birth rates after IVF and/or ICSI. However, most studies did not include the actual live birth rate after ICSI in their research, so care should be taken in interpreting their results appropriately, without overestimation or underestimation of their clinical significance.

**5. Effect on assisted reproduction results:** An analysis of the previous literature showed conflicting results regarding the correlation between sperm DNA damage and ART outcomes. According to some studies, sperm DNA damage has a major impact on pregnancy, meaning that sperm DNA testing should be included in routine clinical examinations [48]. In contrast, some other clinical reviews did not support the clinical use of sperm DNA damage tests [44,49]. In a recent study, Simon et al. [50] conducted a meta-analysis of 120 studies that analyzed ART results and sperm DNA damage. Of the 92 studies that analyzed the relationship between SDF and ART, 35 observed a significant inverse relationship

between SDF and the fertilization rate, but the other 57 studies did not find significant relationships. The inverse relationship between sperm DNA damage and the fertilization rate for each procedure was stronger in IVF (59% or 19 of 32) than in ICSI (24% or 10 of 42) or IVF and ICSI (33% or 6 of 18). An adverse effect of DNA damage on the fertilization rate was more commonly observed in IVF procedures; in contrast to ICSI, in which the most morphologically normal and motile spermatozoon is injected into the egg, the sperm that fertilizes the egg in IVF is randomly selected by sperm-oocyte interactions [51]. Of the 80 studies that analyzed SDF and embryo quality, 27 indicated that SDF had a significant effect on embryo DNA, while the other 53 studies did not show a significant relationship between these parameters. Reviewing these studies by the type of analysis, 64% of the studies that used the comet assay stated that sperm DNA damage had an adverse effect on embryo quality, and similar results were shown for 25% of the TUNEL studies, 24% of the SCSA studies, and 40% of the SCD studies. When classified by ART type, 36% of the IVF studies, 24% of the ICSI studies, and 50% of the mixed IVF and ICSI studies observed adverse effects of DNA damage. Therefore, if the studies are divided based on the type of analysis, we can see a differential association between sperm DNA damage and embryo quality. In particular, the degree of sperm DNA damage detected by the alkaline comet assay was higher than that detected using other methods, which may have been due to the sensitivity of the comet assay, which measures both single- and double-strand DNA damage through complete decondensation of sperm chromatin [31]. In an analysis of 70 studies, the odds ratio (OR) for the effect of sperm DNA damage on clinical pregnancy after ART in all studies was 1.15 (95% confidence interval [CI], 1.08–1.54;  $p < 0.0001$ ), while the corresponding OR for IVF studies was 1.15 (95% CI, 1.05–1.27;  $p = 0.0033$ ), that for ICSI studies was 0.89 (95% CI, 0.80–0.99;  $p = 0.0254$ ), and that for mixed IVF and ICSI studies was 2.00 (95% CI, 1.66–2.41;  $p < 0.0001$ ), indicating that sperm DNA damage is related to clinical pregnancy after ART. The ORs for the studies classified by testing method were as follows: TUNEL, 1.85 (95% CI, 1.52–2.26;  $p < 0.0001$ ); SCD, 1.16 (95% CI, 1.02–1.32;  $p = 0.0233$ ); comet, 4.15 (95% CI, 3.04–5.68;  $p < 0.0001$ ); and SCSA, 0.88 (95% CI, 0.80–0.96;  $p = 0.0041$ ), indicating that the TUNEL and alkaline comet tests, which directly measure DNA damage, showed a closer correlation with pregnancy outcomes than SCSA and SCD, which measure DNA damage indirectly [50].

**6. Pregnancy loss:** Current research on the effects of SDF on miscarriage after ART is limited; however, a recent meta-analysis investigated the correlation between high levels of DNA damage and an increased risk of miscarriage. Zini et al. [52] reported that regardless of the ART type (IVF or ICSI), sperm DNA damage was a predictive factor for pregnancy loss after ART. Robinson et al. [53] reported that IVF or ICSI using sperm with high levels of DNA damage had a 2.16 times higher risk of early pregnancy loss. Carrell et al. [54] suggested that when using the TUNEL assay, sperm DNA damage was higher in the male partners of couples that had experienced recurrent miscarriages (35%) than in the general population (22%) or normal fertile men (12%).

In a study researching 106 men in couples struggling with fertility issues who had miscarriages in the past, Check et al. [55] found that a DFI of 30% or higher was related to high miscarriage rates and low ongoing pregnancy rates. Khadem et al. [56] also reported that recurrent miscarriages and high levels of sperm DNA damage had a positive correlation. In conclusion, SDF measurements can be seen as a useful tool in predicting miscarriages related to paternal factors.

### Treatment strategies for patients with high levels of SDF

Low pregnancy success rates and high miscarriage rates are predicted even with IVF or IVF and ICSI when SDF is high in men; therefore, methods for improving sperm DNA damage prior to ART procedures and techniques for selecting sperm with better chromatin are reviewed below.

**1. Lifestyle modifications:** Physical agents such as radiation and heat, cigarette smoke, airborne pollutants, chemical agents such as anticancer drugs, sexually transmitted infections, and biological factors such as increasing male age, elevated body mass index, and diabetes are environmental and lifestyle factors known to affect sperm DNA integrity [57]. Lifestyle modification is the most fundamental, important, simple, and easy way to improve sperm quality. Men with impaired sperm quality should quit smoking and drinking; engage in exercise and manage their weight; wear loose underwear; avoid environments with high temperatures such as saunas, lower-body bathing, and high temperature workspaces; and abstain from ejaculation for an appropriate duration.

**2. Infection control:** Studies have observed male genital tract infection and inflammation in 8%–35% of cases of male infertility [58], and infections of the male genital organ are known to cause sperm DNA damage. Inflammatory cells produce reactive oxygen species, which are known to cause DNA base modifications and DNA damage [59]. White blood cells detected in semen originate from the epididymis, and male genital tract, and antioxidants in the seminal plasma scavenge the reactive oxygen species produced by the white blood cells. However, when a large quantity of reactive oxygen species is produced, the sperm DNA is damaged by oxidative stress. Such patients can be treated with medication for 2–12 weeks to decrease the amount of reactive oxygen species produced by the white blood cells in order to improve the fertility of sperm.

**3. Oral antioxidant therapy:** Fifteen percent of reproductive-age couples experience fertility issues, and 50% of those cases involve issues associated with the male partner [1]. However, many male patients do not know the causes of oligoasthenoteratozoospermia. Oxidative stress is known to be an important factor that causes male infertility by damaging sperm DNA [60–62]. Greco et al. [63] found that patients who took antioxidants before an ICSI procedure did not show differences in the fertility rate, cleavage rate, or embryo morphology, but had higher clinical pregnancy and implantation rates. However, Tremellen et al. [64] argued that the positive effects of antioxidant therapy on SDF caused by reactive oxygen species are limited and that sperm DNA damage remains high in many patients even after treatment. Imamovic Kumalic and Pinter [60] conducted a meta-analysis of 32 studies published between 2000 and 2013

related to male infertility and antioxidants and found vitamin E, vitamin C, selenium, coenzyme Q10, N-acetylcysteine, zinc, and L-carnitine to be effective. Among these, vitamin C and vitamin E were most effective in reducing DNA fragmentation, and zinc and selenium had similar effects as well. However, additional research is needed since there are no standardized treatment guidelines for male infertility patients with high levels of oxidative damage.

4. **Varicocele repair:** Varicocele is caused by abnormal dilatation of the pampiniform plexus veins and occurs in 15% of men. This is one of the most common causes of male infertility that can be repaired through surgical procedures. It is found in 35% of patients with male infertility and in 70% of secondary infertility patients [65]. Higher levels of SDF were observed in patients with varicocele [66]. Elevated levels of reactive oxygen species damage both the nuclear and mitochondrial DNA, causing base modifications, strand breaks, and chromatin cross-links, as well as increasing sperm DNA damage [67]. Surgical repair improved semen parameters and was more cost-effective than IVF/ICSI [65]. Among the surgical methods, microsurgical varicocelectomy through the subinguinal approach was the most effective method [65].

5. **Micromanipulation-based sperm selection:** Many studies have investigated techniques for selecting sperm with less DNA damage when performing ART. Some such methods include density gradient centrifugation, electrophoretic sperm isolation using a cell sorter, a hyaluronic acid-binding method, sperm magnetic sorting, and high-magnification microscopy. Sperm and embryo treatment and selection are known to decrease adverse ICSI reproductive outcomes caused by sperm DNA damage, and many fertility clinics currently perform these methods. However, there is no definitive clinical evidence that any of these methods can avoid the potentially harmful effects of abnormal sperm on ART outcomes. Sperm selection technologies face limitations because none of the current techniques can completely prevent the selection of sperm with DNA damage or aneuploidy [68].

6. **Testicular sperm:** Testicular sperm tend to have less DNA damage and better DNA integrity than ejaculated sperm. However, due to some genetic and epigenetic risks, doubts have been raised regarding the use of testicular sperm [69]. A retrospective analysis of neonatal data on births by sperm injection from obstructive and nonobstructive azoospermic men showed no significant differences in short-term neonatal outcomes; likewise, there were no significant differences in

the congenital malformation rate in offspring of ICSI from testicular sperm [70]. Esteves et al. [71] found testicular sperm to have a three to five times lower proportion of DNA fragmentation than ejaculated sperm. In a recent prospective comparative study on the use of testicular sperm for ICSI in 172 patients with high levels of sperm DNA damage, it was found that the proportion of testicular sperm DNA damage (8.3%) was five times lower than that of ejaculated sperm DNA damage (40.9%). Better reproductive outcomes were shown when testicular sperm was used for ICSI in the patient group that continued to show oligozoospermia and high levels of sperm DNA damage even after antioxidant treatment. The birth rate in the ICSI group that used testicular sperm was 46.7%, while the birth rate in the ICSI group that used ejaculated sperm was 26.4%; moreover, the relative risks for miscarriage and birth were 0.29 and 1.76, respectively, suggesting that testicular sperm had better outcomes [71]. Furthermore, based on several recent meta-analyses, clinical pregnancy rates were low and miscarriage risks were high when IVF/ICSI was performed with ejaculated sperm with high levels of DNA damage; therefore, using testicular sperm for the next ICSI cycle may be more effective for couples that have high levels of SDF and a history of failed IVF/ICSI [72].

## CONCLUSION

Sperm DNA damage testing methods do not have clearly standardized cut-off levels and each method has its advantages and disadvantages, making it difficult to proclaim one as a universally preferable method. The human reproductive system is complex, which makes it impossible to define a clear and universal cut-off level for various testing methods, and it is difficult to predict the outcome of dynamic interactions among several factors that may disturb fertility using a single test. Thus, it is best to select a testing method based on a patient's clinical characteristics and goals. Since sperm DNA damage tests provide genetic information from male reproductive cells, in contrast to the simple information that previous semen analysis parameters provide, more research on methods for selecting sperm with undamaged DNA in ART should be conducted in the future. Furthermore, physicians and researchers working with ART must continue to make efforts to obtain healthy sperm with nuclear DNA integrity to minimize the adverse effects that may arise in offspring conceived from sperm with DNA damage.

## РЕЗЮМЕ

**ЧТО ДОЛЖНО БЫТЬ ПРЕДПРИНЯТО В ОТНОШЕНИИ МУЖЧИН  
С ФРАГМЕНТАЦИЕЙ ДНК СПЕРМАТОЗОИДОВ?****Доктор Прамод Кумар Баджадж (Pramod Kumar Bajaj)**Sperm Processor Pvt. Ltd.  
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В возрасте, когда небольшое количество сперматозоидов может привести к беременности через оплодотворение *in vitro* или внутрицитоплазматическую инъекцию сперматозоидов, отбор здоровой спермы играет важную роль. Известно, что у бесплодных мужчин фрагментация ДНК сперматозоидов (ФДС) выше.

Методы исследования повреждения ДНК сперматозоидов не имеют четко стандартизированных уровней отсечки. Каждый метод имеет свои преимущества и недостатки, поэтому невозможно использовать какой-то один универсально предпочтительный метод. Лучше всего выбрать метод исследования, основанный на клинических характеристиках и целях пациента.

Врачи и исследователи, работающие с ВРТ, должны использовать сперму с целостной цепочкой ДНК в целях снижения побочных воздействий, которые могут возникнуть у потомства, зачатого с использованием спермы с повреждением ДНК.

**Ключевые слова:** методы исследования повреждения ДНК сперматозоидов, фрагментация ДНК сперматозоидов, бесплодные мужчины.

## ТҮЙІНДЕМЕ

**СПЕРМАТОЗОИДТАРДЫҢ ДНҚ ФРАГМЕНТАЦИЯСЫ (БӨЛІНУ)  
БАР ЕРКЕКТЕРГЕ ҚАТЫСТЫ НЕ ІСТЕУ КЕРЕК?****Доктор Прамод Кумар Баджай (Pramod Kumar Bajaj)**Sperm Processor Pvt. Ltd.  
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Сперматозоидтың аз мөлшері *in vitro* ұрықтандыру немесе сперматозоидты цитоплазма ішілік енгізу арқылы жүктілікке әкелетін жаста, сау сперманы таңдау маңызды рөл атқарады. Бедеулігі бар еркектерде сперматозоидтың ДНҚ фрагментациясы (СДФ) жоғары екендігі белгілі.

Сперматозоидтың ДНҚ зақымдануын зерттеу әдістері нақты кесілген стандарттық деңгейге ие емес. Әрбір әдіс өзінің артықшылықтары мен кемшіліктеріне ие, сондықтан қандай да бір әмбебап қолайлы әдісті қолдану мүмкін емес. Науқастың клиникалық сипаттамалары мен мақсаттарына негізделген зерттеу әдісін таңдаған дұрыс.

ҚРТ-мен жұмыс істейтін дәрігерлер мен зерттеушілер ДНҚ-сы зақымданған ұрықтан туындаған ұрпақтарда пайда болатын жанама әсерлерді азайту үшін ДНҚ-ның толық тізбегі бар сперманы қолдануы керек.

**Түйін сөздер:** сперматозоидтың ДНҚ зақымдануын зерттеу әдістері, сперматозоидтың ДНҚ фрагментациясы, бедеу еркектер.