Sperm DNA damage and its clinical relevance in assessing reproductive outcome

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Abstract

The routine examination of semen, which assesses sperm concentration, percentage motility and morphology, does not identify subtle defects in sperm chromatin architecture. The focus on the genomic integrity of the male gamete has intensified recently due to the growing concern that genetic diseases may be transmitted via assisted reproductive techniques (ART). Accordingly, the intent of this review is to describe the details of the information pertaining to mitochondrial/nuclear sperm DNA damage with an emphasis on its clinical significance and its relationship with male infertility. Assessment of sperm DNA damage appears to be a potential tool for evaluating semen samples prior to their use in ART. Testing DNA integrity may help select spermatozoa with intact DNA or with the least amount of DNA damage for use in assisted conception. In turn, this may alleviate the financial, social and emotional problems associated with failed ART attempts.

1 Introduction

Infertility affects approximately 15% of all couples trying to conceive and a male factor is the sole or contributing factor in roughly half of the cases [1]. Although semen analysis is routinely used to predict fertility, the standard measurements of sperm concentration, percentage motility and morphology may not reveal sperm defects affecting the integrity of the male genome. It is clear that abnormalities in the male genome characterized by damaged DNA may be indicative of male subfertility regardless of the routine semen parameters [2-4]. Moreover, poor semen quality has been associated with an increase in the proportion of sperm with DNA fragmentation [2, 5, 6].

Methods have been developed that can directly assess sperm nuclear and mitochondrial DNA integrity. Because sperm selection for intracytoplasmic sperm injection (ICSI) is based on motility and gross morphology, concern has been expressed that such methods bypass natural selection and could inadvertently introduce a defective paternal genome [3]. While many studies have established that DNA damage is present in sperm, the biological significance of these damaged cells in reproduction remains unclear. The current review will identify the origin; causes and clinical significance of DNA damage in male infertility and highlight its relevance in assisted reproductive programs.

2 Damage to genomic material

2.1 Mitochondrial DNA damage

Spermatozoa are particularly likely to develop different forms of mitochondrial DNA (mtDNA) abnormalities including deletions, point mutations and polymorphism. These abnormalities have been associated with a decline in semen quality, motility and male fertility [7]. MtDNA also lacks histones, which help protect against damage, therefore it is believed to have only a basic repair mechanism. Although the first study on mtDNA inheritance after ICSI suggested that human embryos eliminate the mtDNA of the injected sperm [8], another study has shown that abnormal paternal mtDNA transmission may not be uncommon when poor-quality gametes are used. It is also of interest that populations of human spermatozoa exhibiting evidence of mitochondrial dysfunction also show high rates of nuclear DNA fragmentation [9]. Abnormal sperm samples revealed high incidence of mtDNA damage, which confirms their role in male infertility [10].

2.2 Nuclear DNA damage and its origin

Many studies have detected anomalies in the nucleus of ejaculated spermatozoa [11-14]. Although the extent of DNA damage is closely related to sperm function and male infertility [12, 15], the origin of such damage is still largely controversial. Different mechanisms have been proposed to explain the presence of these anomalies in human ejaculate. Three factors may be involved in the etiology of DNA damage in the germ line: oxidative stress, deficiencies in natural processes such as chromatin packaging and abortive apoptosis.

2.2.1 Oxidative stress

Oxidative stress is caused by an imbalance between the production of reactive oxygen species (ROS) and the antioxidant capacity [16]. Virtually every human ejaculate is contaminated with potential sources of ROS [15]. Spermatozoa have only two defense mechanisms against oxidative attack of their DNA: the packaging arrangement of the DNA [17] and the seminal plasma [18].

Reactive oxygen species may lead to chromatin cross-linking [19], DNA strand breaks [20], DNA base oxidation [21], chromosome deletions, dicentrics and sister chromatid exchanges [4, 22]. Oxidative stress has also been correlated with apoptosis [23] and high frequencies of single and double DNA strand breaks [4, 5, 24]. *In vivo*, such damage may not be a cause for concern because the collateral peroxidative damage to the sperm plasma membrane would ensure that spermatozoa subjected to oxidative stress would be unable to participate in the fertilization process. However, these safeguards are clearly circumvented during the course of ICSI. Spermatozoa with DNA fragmentation may have adverse consequences if they are used for assisted reproductive techniques (ART) [2, 15, 25-28].

2.2.2 Sperm chromatin packaging

DNA in mammalian sperm is tightly compacted into linear arrays organized as loop domains [17]. Immature spermatozoa have high levels of DNA damage and ROS production and are likely to have alterations in protamination and chromatin packaging [29-33]. DNA fragmentation is characterized by single- and double-strand DNA breaks, which are often detected in the ejaculates of subfertile men [6]. Double-stranded breaks may also occur naturally in the male germ line in preparation for recombination and during the process of chromatin packaging [12]. These physiological strand breaks are normally resolved in the spermatid stage of spermatogenesis. Therefore, it is possible that aberrant recombination-chromatin packaging accounts for unresolved double-strand breaks in the mature human spermatozoa. However, evidence to support this contention is lacking.

2.2.3 Apoptosis

Apoptosis controls the overproduction of male gametes and restricts normal proliferation levels so that they do not surpass the supportive capacity of Sertoli cells [34, 35]. Testicular germ cell apoptosis occurs in the testis during spermatogenesis as a result of endonuclease activation. This occurs predominantly in the spermatogonia and dividing cells [12] and generates numerous DNA strand breaks in chromatin. The presence of endogenous nicks in ejaculated spermatozoa is characteristic of programmed cell death as seen in apoptosis of somatic cells. Incomplete endogenous nuclease activity creates and ligates nicks during spermiogenesis [36].

Evidence suggests that abortive apoptosis occurs in many males who exhibit poor sperm parameters [37]. In certain males, abortive apoptosis may fail in the total clearance of spermatozoa earmarked for elimination by apoptosis. Therefore, the subsequent population of ejaculated spermatozoa presents an array of anomalies that are representative of the characteristics observed in cells in the process of apoptosis.

One factor that has been implicated in sperm apoptosis is the cell surface protein, Fas [38]. Binding of Fas ligand or agonistic anti-Fas antibody to Fas kills cells by apoptosis. Men exhibiting deficiencies in the semen profile, particularly oligozoospermia, possess a large number of spermatozoa bearing Fas positivity and DNA damage [39]. These dysfunctional cells are the product of an incomplete apoptotic cascade [12]. However, in a recent publication [40], no strong correlation was evident between Fas and p53 expression and the population of sperm that were positive using the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-nick end labeling (TUNEL) assay.

3 Etiology of sperm DNA damage

A host of environmental factors and pollutants have been associated with changes in sperm parameters [41]. Most of these agents may not only disrupt hormone levels but also induce oxidative stress, which could damage sperm DNA. Smoking, alcohol, caffeine consumption and anticancer drugs have been reported to cause aneuploidy in human sperm [42].

Cigarette smoking is associated with an overall reduction in semen quality [43]. An association between DNA adducts formation in spermatozoa and cigarette smoking has been demonstrated, and cigarette smoking has been proven to affect sperm DNA integrity [44, 45]. Within an *in vitro* fertilization (IVF) program, the percentage of spermatozoa with DNA damage was significantly higher in the men who smoked than in the men who did not [5]. In human sperm, substantial oxidative modification in terms of the oxidized deoxynucleotide, 8-oxo-7, 8-dihdyro-2'deoxyguanosine (8-oxodG) has been

demonstrated in smokers [46]. These findings could possibly be explained by the increased leukocyte-induced oxidative stress on developing or mature sperm [44].

4 Assay for assessment of DNA integrity evaluation and correlation

Several assays have been developed to evaluate sperm chromatin maturity/DNA integrity (Table 1). These assays were based on the facts that defects in the chromatin structure have been shown to lead to increased DNA instability and sensitivity to denaturing stress [59]. In addition, spermatozoa from infertile men have a higher frequency of chromosomal abnormalities [60], poor DNA packing quality [31], increased DNA strand breaks [2, 6,26] and susceptibility to acid-induced DNA denaturation *in situ* than spermatozoa from fertile men [11, 61].

Table 1. Different assays employed in identifying DNA damage in spermatozoa.

Technique	**Assay Principle**	**Detection method**
In situ nick translation [6, 22, 25]	Single-strand DNA breaks	Fluorescence microscopy
Acridine orange staining [74]	Differentiates between single and double stranded DNA	Fluorescence microscopy
TUNEL assay [29,30,56,80]	DNA fragmentation, single- and double-strand DNA breaks	Flowcytometry/fluorescence microscopy
Alkaline single-cell gel electrophoresis (Comet assay) [58, 81,82]	Evaluates DNA integrity, single- and double-strand DNA breaks	Fluorescence microscopy
8-oxo-7,8 dihydro-2 deoxyguanosine (8-OH-dG) [6, 24, 44]	HPLC with electrochemical detection	HPLC with electrochemical detection
Sperm chromatin structure assay (SCSA) [33, 64, 83]	Acid DNA denaturation	Flowcytometry
DNA breakage Detection-Fluorescence in situ hybridization [84, 85]	DNA breaks	Fluorescence microscopy and image analyzer
Sperm chromatin decondensation [85]	Intact spermatozoa with non-fragmented DNA produce characteristic DNA decondensation halo.	Fluorescence microscopy
Chromamycin A3 [86]	Indirect visualization of nicked, denatured DNA	Fluorescence microscopy
Toluidine blue stain [87]	The stain, which is a sensitive structural probe for DNA structure and packaging, becomes incorporated in the damaged dense chromatin.	Optical microscopy

At present, the TUNEL, comet and sperm chromatin structure assays (SCSA) are the most commonly used assays for measuring DNA fragmentation. Attempts have been made to standardize the comet assay with human sperm and establish its relationship with both SCSA and TUNEL assay data [29]. When sperm from the same aliquot were analyzed with the SCSA and TUNEL and comet assays, a significant correlation among the results from the three assays were observed. The TUNEL assay has been correlated to the outcome of IVF in humans [62]. Also, the TUNEL assay correlates with alternate semen analysis parameters including sperm concentration, motility and percentage normal morphology, although not highly [5]. The SCSA has revealed little correlation [63] although it has been strongly correlated with the dose of exposure to various toxicants, stress conditions and infertility [11].

5 Implications of sperm DNA damage on male fertility

Accumulating evidence suggests that disturbances in the organization of the genomic material in sperm nuclei are negatively correlated with the fertility potential of the spermatozoa. A number of studies have revealed that levels of DNA strand breaks are higher in infertile patients with abnormal semen parameters than in fertile subjects [62]. The same difference was also detected in men with idiopathic infertility with normal routine semen parameters that had a higher DNA fragmentation index (% DFI) [28]. One recent report found that sperm DNA denaturation had the lowest average coefficient of variation (CV), followed by motility and concentration in two consecutive samples from infertile men (CV = 21%, 24%, and 35%, respectively) [64].

The utility of sperm DNA damage as a diagnostic and prognostic tool in the human fertility clinic has been extensively studied by Evenson *et al* [11] who studied 402 samples from 165 presumably fertile couples wishing to achieve a pregnancy. The couples were followed for 12 menstrual cycles. SCSA data from the male partners of 73 couples who achieved a pregnancy during months 1-3 were used as the standard of "sperm chromatin compatible with high fertility". The data from these couples were significantly different from those of 40 couples who achieved a pregnancy in months 4-12 ($P < 0.01$) and from those of the male partners of 31 couples who did not achieve a pregnancy ($P < 0.001$). Based on logistic regression analysis, the DNA fragmentation index (%DFI) was the best predictor of whether a couple would not achieve pregnancy.

6 Correlation of DNA damage with ART

In the contemporary practice of infertility management, intrauterine insemination (IUI) often precedes the costly and rigorous options of assisted reproductive technologies, such as IVF with or without ICSI. In one study, the degree of sperm DNA fragmentation and stability and conventional semen parameters were examined to determine if they could predict IUI outcome [65]. In cycles resulting in pregnancy, the degree of DNA fragmentation after sperm preparation was significantly lower (< 12%) than those that did not result in pregnancy.

In a blinded study, Host and colleagues [66] studied DNA damage in four clinically different groups of infertile couples. DNA damage was correlated with semen parameters, the fertilization rate and IVF outcome. In group I (n=75), the female partner had tubal obstruction. Group II consisted of men with unexplained infertility (n =50). Group III consisted of men with oligozoospermia undergoing IVF with their partner (n=50). The proportion of spermatozoa having DNA strand breaks was negatively correlated with the proportion of oocytes that were fertilized after IVF in all 3 groups (r = -0.39, $P < 0.01$; r = -0.61, $P < 0.01$; r = -0.39, $P < 0.01$, respectively). Group IV consisted of men with oligozoospermia (n=50) undergoing ICSI with their partner. In the men with unexplained infertility, those who had $\leq$4% spermatozoa with DNA strand breaks had a higher pregnancy rate (58.8±28.4 *vs.* 38.2±27.6, $P < 0.05$). No association was seen between spermatozoa with DNA strand breaks and the fertilization rate in ICSI patients. It is of interest to mention that other studies found a significant negative association between the percentage of sperm with DNA fragmentation and the fertilization rate following ICSI (r = -0.23, P = 0.017) [2].

Sun *et al* [5] examined the fertilization rates in IVF, which were compared with semen parameters and DNA fragmentation damage in a total of 298 semen samples from couples undergoing IVF (n = 143) or a sperm wash procedure. A negative association was demonstrated between the results of semen analysis parameters and sperm with fragmented DNA. Most of the samples had DNA damage < 4%, and the DNA damage ranged from 5% to 40% in 27% of the samples. A significant negative correlation was seen in IVF samples between the percentage of sperm with DNA damage and the fertilization rate (P = 0.008) and embryo cleavage rate (P = 0.01).

Recently, sperm DNA fragmentation was examined in a selected group of 104 couples undergoing ART (IVF: n = 50; and ICSI: n = 54) [67]. The authors examined the relationships between the proportion of sperm with fragmented DNA examined by TUNEL assay and quality of embryos obtained embryo development and the rate of ongoing pregnancies. DNA fragmentation did not influence the fertilization rate in either IVF or ICSI. However, when the DNA fragmentation rate was divided into two categories according to threshold value of 10%, the fertilization rate was significantly higher for DNA fragmentation below 10% (84.1 *vs.* 70.7%, $P < 0.05$). No relationship was seen between the DNA fragmentation rate and embryo development. When the DNA fragmentation rate was higher than 10%, the percentage of embryos reaching blastocyst stage was always lower than 50%, whereas it reached 80% when the DNA fragmentation rate was lower than 10%.

In a prospective study [28], we examined the relationship between sperm DNA damage and ART outcomes in 33 couples with male factor infertility and whether this damage was related to seminal oxidative stress. In this study, clinical pregnancy was achieved in 27% (9/33) of couples who underwent ART [26% (5/19) with IUI, 30% (3/10) with IVF and 25% (1/4) with ICSI]. The sperm DFI was negatively correlated with sperm concentration (r = -0.31; P = 0.001), percentage motility (r = -0.47; $P < 0.001$) and normal sperm morphological forms (r = -0.40; $P < 0.0001$) [28]. A summary of the studies correlating sperm DNA damage with different ART outcomes is displayed in Table 2.

Table 2. Correlation of DNA damage and pregnancy outcome in ART.

Author	Study population	ART procedure	Type of DNA assay	Pregnancy outcome
Sun et al [5]	143	IVF	TUNEL	DNA fragmentation negatively correlated with fertilization and embryo cleavage rate.
Sakkas et al [62]	88	IVF ICSI	Chromomycin A3 Nick translation	Lower rates
Lopes et al [2]	150	ICSI	TUNEL	Fertilization rate negatively correlated with DNA fragmentation.
Larson et al [57]	24	ICSI	SCSA	DNA denaturation lower in men that initiated pregnancy. No pregnancies when DNA fragmentation >27%.
Host et al [52]	75	IVF ICSI	Direct immunoperoxidase	Negative correlation with proportion of oocytes fertilized
Tomlinson et al [14]	140	IVF	In situ nick translation (NT) Chromomycin staining	No association of DNA damage with fertilization rate. Pregnant patients had significantly lower NT levels.
Tomsu et al [63]	40	IVF	Comet	Head and tail DNA parameters useful in predicting embryo quality.
Morris et al [58]	60	IVF	Comet	DNA damage in ICSI cycles is associated with impairment of post fertilization embryo cleavage.
Duran et al [51]	119	IUI	TUNEL	No pregnancies if fragmentation >12%.
Saleh et al [28]	33	IUI IVF ICSI	SCSA	Both fertilization and embryo development negatively correlated with DNA denaturation.
Benchaib et al [53]	50 54	IVF ICSI	TUNEL	No significant difference in fertilization and embryo quality in IVF and ICSI. In ICSI pregnancy resulted when DNA fragmentation <10%. No pregnancy when DNA fragmentation >20%.

7 Clinical aspects of sperm DNA damage in ART

7.1 Fertilizing ability of DNA damaged spermatozoa

The fact that the fertilization and pregnancy rates were surprisingly high after ICSI--regardless of the severity of the of sperm defect [71] --has subsequently caused debate on the possibility of using ICSI to force fertilization by abnormal spermatozoa. This may have hidden consequences for the normality of embryos and the resulting fetus [72].

7.2 Embryo quality

The influence(s) of sub-optimal sperm chromatin integrity on post-embryonic development is the subject of intense investigation. Whereas there may be some controversies about the impact of high sperm DNA fragmentation on the fertilization rate, there is a wider agreement concerning their negative effects on embryo development and pregnancy rate [48, 50, 66, 69].

It is unclear if ART can compensate for poor chromatin packaging and/or DNA damage or if sub-optimal chromatin integrity may contribute to the poor (< 20%) implantation rate seen in most ART patients [73]. Under normal circumstances, low levels of damage can be repaired by the oocyte [48, 74, 75]. DNA damage that is beyond repair will result in apoptosis and fragmentation of the early embryo or morbidity in later life [5]. This may be one of the causes of fragmentation in human embryos seen in IVF programs.

In case of genetic damage, a few embryos may reach the blastocyst stage, but natural selection will ensure that most of them will abort before growing to term [48]. Several studies have attempted to establish a correlation between sperm DNA integrity and cleavage rates and embryo quality [40, 68, 70]. Whether DNA-damaged spermatozoa can impair the process

of embryo development remains unclear. Reports indicate that damage to sperm DNA may be linked to an increase in early embryo death [12]. According to the literature, levels of embryo loss are similar following IVF and ICSI. However, Sanchez *et al* [76] found that the miscarriage rate following ICSI was greater than that expected with conventional IVF, possibly reflecting the use of genomically compromised spermatozoa that conferred irreparable DNA damage to the embryo and caused its subsequent abortion.

7.3 DNA damage in cryopreserved sperm

Cryopreservation of human spermatozoa is used extensively in artificial insemination and IVF programs. Freezing semen samples, either raw or extended, has no effect on the extent of DNA denaturation [30, 53, 77]. However, DNA samples from fertile men were more resistant to freezing damage than those of infertile men, especially if they were frozen in the presence the seminal plasma, which affords antioxidant protection [78]. The protection of DNA has important implications in the use of freeze-thawed donor spermatozoa for insemination. This is extremely relevant for individuals who may have spermatozoa banked for long-term storage prior to chemotherapy or radiotherapy. It is critical to optimize the protocols that are used to prepare and freeze sperm samples from infertile men to protect their DNA. In support of the beneficial role of the sperm preparation protocols, a general improvement in nuclear maturity may be seen in post-swim-up samples [76, 79].

7.4 DNA damage in surgically retrieved spermatozoa

Sperm extraction is frequently done in ICSI trials for men with obstructive azoospermia. A significantly high percentage of DNA breaks are seen in sperm that are surgically extracted from the epididymis or testicular tissue [80]. The breaks may be a result of the prolonged stay of the spermatozoa in the obstructed genital tract, or perhaps DNA decondensation of the chromatin is incomplete in non-ejaculated spermatozoa. This implies that these cells are sensitive to damaging or toxic agents [81]. In cases of obstructive azoospermia, it is preferable to use testicular extracted sperm rather than epididymal sperm for ICSI because testicular extracted sperm have been shown to have lower DNA fragmentation and a better development potential [80]. *In vitro* culture (48-72 h) of testicular cells in men with obstructive azoospermia has been reported to result in a decrease in the percentage of spermatozoa containing single-stranded DNA, thereby increasing the availability of double-stranded DNA spermatozoa for ICSI [82].

8 Implications of genetically damaged spermatozoa following ICSI

Sakkas *et al* [12] found that ICSI performed with sperm samples containing > 30% fluorochrome labeling and > 10% endogenous DNA nicks resulted in unfertilized oocytes in which about half of the sperm remained uncondensed. Men with poor semen parameters or those men whose sperm fail to fertilize during IVF are the usual candidates for ICSI. Therefore a significant proportion of sperm injected into the oocytes are likely to contain fragmented DNA. This may explain the inability of most clinics to achieve a fertilization rate of more than 65%-80% [83].

In the era of ART, the integrity of sperm nuclear DNA is of paramount concern for the successful transmission of a competent paternal genome to the oocyte. How sperm DNA fragmentation will affect fertilization rates, embryo development and pregnancy rates remains unclear. Researchers have illustrated the importance of paternal influence in early embryo development and have linked increased chromosomal damage to repeated spontaneous abortions [84].

9 Strategies to reduce collateral DNA damage

Various strategies can be employed to obtain spermatozoa with good DNA integrity using sperm preparation techniques and antioxidant supplementation alone or in combination.

9.1 Separating spermatozoa with minimal DNA damage

Decreasing the percentage of spermatozoa with damaged DNA by utilizing sperm preparation techniques may be an important method for increasing ICSI fertilization rate above the current 65%-80% [2, 83]. The DNA integrity of prepared spermatozoa is always significantly higher than that of raw semen [9]. Semen samples after simple preparation techniques (e.g., density gradient centrifugation) can be enriched with spermatozoa with improved nuclear integrity [3, 47, 85, 86].

Results show that also the swim-up technique yields a population of spermatozoa that has a better DNA integrity [27]. Similarly, glass wool filtration significantly decreases the % DFI when compared with raw semen samples [85]. These data, however, urge us to reexamine the different types of sperm processing techniques to minimize sperm DNA damage.

9.2 Role of antioxidants

Ascorbic acid is the major contributor to the chain breaking antioxidant capacity of the seminal plasma [18], while alpha tocopherol can inhibit sperm lipid peroxidation *in vitro* [87]. Although ascorbic acid and alpha tocopherol--when separately supplemented--protect against DNA damage, they produce a damaging effect when added together. Administration of vitamins C (350 mg/d) and E (250 mg/d) together *in vivo* were unable to prevent DNA sperm damage occurring after ejaculation [88]. In infertile patients with high level of oxidative DNA damage, the combination of vitamins C and E with

glutathione induced only a slight increase in sperm concentration [24]. There may be a narrow physiological range in which these antioxidants can work synergistically.

Isoflavones (genistein and equol) are reported to significantly improve sperm DNA damage mediated by hydrogen peroxide [89]. Compared with ascorbic acid (10-600 mmol/L) and alpha-tocopherol (1-100 mmol/L), genistein was the most potent antioxidant, followed by equol, ascorbic acid and alpha-tocopherol when added at physiological concentrations. Genistein and equol when added in combination were more protective than when added individually. These preliminary data suggest that these compounds may play a role in antioxidant protection against sperm DNA damage. However, the beneficial effect of antioxidants is limited and controversial. The antioxidants could be useful in cases of excessive ROS production during sperm selection for IVF or IUI.

10 Conclusions

The male germ line appears to be particularly susceptible to mutagenic and promutagenic change. The mutagenic mechanisms involve replication errors that are heavily correlated with paternal age and responsible for spontaneous appearance of dominant genetic diseases. The male germ line is highly susceptible to DNA fragmentation via mechanisms that are independent of paternal age. The etiology of these strand breaks may involve aberrant recombination, oxidative stress, defective chromatin packaging and abortive apoptosis.

Oxidative stress-induced DNA damage causes pro-mutagenic change, which in its most severe form does not negatively affect the quality of the germ line as collateral oxidative damage to the plasma membrane prevents fertilization. When there is less oxidative damage, fertilization can occur, but the oocyte must repair the DNA strand breaks before the initiation of the first cleavage division. The Y chromosome is particularly vulnerable to DNA damage, partly because of its genetic structure and partly because it cannot correct double-stranded DNA deletions.

Many reports have found that the miscarriage rate following ICSI is greater than that expected with conventional IVF. This possibly reflects the use of genomically compromised spermatozoa. Further research is necessary to devise techniques to 1) identify and select sperm with undamaged DNA for ICSI or to remove sperm with damaged DNA from sperm samples and 2) to improve the pregnancy outcome after ICSI. Sperm DNA damage may be a potential tool for evaluation of semen samples prior to their use in ART. Testing sperm DNA integrity may help in selection of spermatozoa with the least amount of damage for use in assisted conception. In turn, this may alleviate the financial, social and emotional problems associated with failed ART attempts.

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