

The clinical utility of sperm DNA integrity testing

The Practice Committee of the American Society for Reproductive Medicine

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Sperm DNA damage is more common in infertile men and may contribute to poor reproductive performance. However, current methods for evaluating sperm DNA integrity do not reliably predict treatment outcomes, and no treatment for abnormal DNA integrity has proven clinical value. (Fertil Steril® 2008;90:S178–80. ©2008 by American Society for Reproductive Medicine.)

Mammalian fertilization and subsequent embryo development depend in part on the inherent integrity of the sperm DNA (1). Indeed, there appears to be a threshold of sperm DNA damage (DNA fragmentation, abnormal chromatin packaging, and protamine deficiency) beyond which embryo development and pregnancy are impaired (1, 2). Tests of DNA integrity have been developed and clinically applied in clinical practice. However, the data from studies that have sought to evaluate the effect of sperm DNA integrity on reproductive outcomes have not been analyzed systematically. This Committee Opinion has been prepared to summarize the current understanding of the impact of abnormal sperm DNA integrity on reproductive outcomes.

HUMAN SPERM DNA AND CHROMATIN STRUCTURE

Unlike the chromatin structure of somatic cells, sperm chromatin is very tightly compacted by virtue of the unique associations among the DNA, the nuclear matrix, and sperm nuclear proteins (3). During the later stages of spermatogenesis, histones are displaced by transition proteins and then by protamines (3). The introduction of protamines into the sperm nucleus allows the DNA strands to form toroidal (donut-shaped) structures, facilitating the tight compaction of the sperm nuclear head (3). Inter- and intramolecular disulfide cross-links between the cysteine-rich protamines allow further compaction and stabilization of the sperm nucleus, protecting sperm DNA from external stress and subsequent DNA breakage. Fertile men with normal semen parameters almost uniformly have low levels of DNA breakage, whereas infertile men, especially those with abnormal semen parameters, have increased DNA damage. Moreover, up to 8% of infertile men will have abnormal DNA integrity despite normal semen parameters (concentration, motility, and morphology) (4–6).

ETIOLOGY OF SPERM DNA DAMAGE

The etiology of sperm DNA damage, much like male infertility, appears to be multifactorial and may be due to intrinsic or external factors. Intrinsic defects that may predispose to

sperm DNA damage include protamine deficiency, mutations that adversely affect DNA compaction (7, 8), or other “DNA packaging” defects. In addition, advanced paternal age has been associated with sperm DNA damage (9). Up to 75% of potential spermatozoa complete the process of programmed cell death (apoptosis) during spermatogenesis (10). Spermatozoa that begin to undergo apoptosis but then “escape” the process (“abortive apoptosis”) also may suffer increased DNA damage (11).

High levels of reactive oxygen species are detected in the semen of 25% of infertile men, and sperm DNA damage has been associated with high levels of semen reactive oxygen species (12, 13). External factors such as heat, chemotherapeutic agents, radiation, and other gonadotoxins are associated with an increase in the percentage of ejaculated spermatozoa with DNA damage, although the exact mechanism involved has not been delineated (9, 14). Cigarette smoking (15, 16), genital tract inflammation, varicoceles (17), and hormonal deficiency (18) all have been associated with increased DNA damage in animal models or humans.

TESTS OF SPERM DNA DAMAGE

Both direct and indirect assay methods have been used to assess DNA damage. Direct methods for detecting DNA breaks include the single-cell gel electrophoresis assay (“Comet” assay) and the terminal deoxynucleotidyl transferase-mediated 2'-deoxyuridine 5'-triphosphate (dUTP)-nick end-labeling (TUNEL) assay. Indirect methods for assessing DNA damage include the sperm chromatin integrity assays (e.g., sperm chromatin structure assay, SCSA) that use chromatin and/or DNA intercalating dyes such as acridine orange to differentiate single-strand and double-stranded DNA (5, 6, 19). Several groups have suggested that sperm DNA denaturation, as measured by the SCSA test, correlates strongly with other markers of DNA damage such as TUNEL and Comet assays (6, 20, 21). Whereas TUNEL and Comet assays analyze directly the number of DNA breaks, the SCSA measures the susceptibility of sperm DNA to breakage after acid treatment using flow cytometry to determine the proportions of spermatozoa having single-strand (abnormal) and double-strand (normal) DNA. Threshold values used to define an abnormal test are shown in Table 1. Less common clinical tests for DNA damage include the sperm chromatin dispersion test that determines the susceptibility of sperm

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TABLE 1		
Common DNA integrity tests.		
Test	Abnormal threshold	Reference
SCSA (DNA fractionation index)	≥27%	23
	≥30%	24
	≥30%	25
	≥40%	5
TUNEL	≥36%	26

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DNA to acid denaturation (22), measurements of DNA oxidation that detect oxidized DNA nucleotide residues (e.g., 8-hydroxydeoxyguanosine) using high-performance liquid chromatography (4), and analysis of protamine/histone protein ratios in sperm samples.

INFLUENCE OF SPERM DNA DAMAGE ON REPRODUCTIVE OUTCOMES

Male infertility is associated with poor sperm DNA integrity. Whereas 25% of sperm exhibit DNA denaturation and 28% have DNA fragmentation in infertile men, denaturation is observed in 10% and fragmentation in 13% of sperm in fertile men (4–6, 19). It has been suggested that abnormal DNA integrity may adversely affect fecundity in couples having natural intercourse and in those treated by IUI, IVF, and intracytoplasmic sperm injection (ICSI) (23–26). In addition, abnormal DNA integrity is more prevalent in couples having a history of recurrent miscarriage than in those attempting pregnancy via IVF or therapeutic donor insemination (27). Abnormal DNA integrity also has been proposed as a cause of impaired late embryo development but does not appear to be associated with poor fertilization (28). Results of a meta-analysis of studies that have compared pregnancy rates observed in populations of fertile and infertile couples having normal and abnormal sperm DNA integrity test results are shown in Table 2.

TABLE 2		
Effect of sperm DNA integrity test results on reproductive outcomes.		
Study population	No. of studies	Odds ratio (95% confidence interval) for Achieving pregnancy^a
Normal couples	2	2.08 (0.23–19.0)
IVF	3	1.06 (0.27–4.25)
ICSI	3	1.07 (0.39–2.93)
IVF and ICSI	6	1.62 (0.96–2.72)

Note: OR = odds ratio; 95% CI = 95% confidence interval.
^aOR >1 indicates pregnancy rate higher using sperm with normal DNA integrity.

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With respect to IUI outcomes, one study found no significant effect on delivery in 131 IUI cycles using the SCSA defined by the DNA fractionation index (DFI), although in an additional analysis there was a significant effect using thresholds for either DFI or highly DNA stainable cell fractions (24). A second study using the sperm chromatin dispersion test observed no correlation between test results and pregnancy rates in 100 IUI cycles (29).

THERAPIES TO REDUCE SPERM DNA DAMAGE

A limited number of studies has examined potential treatments to reduce sperm DNA damage. For the most part, the following therapeutic recommendations have been based on the results of small, uncontrolled clinical studies and experimental (animal) studies.

1. Avoidance of gonadotoxins (e.g., smoking, medications) and of hyperthermia (e.g., saunas, hot-tubs) has been recommended, based on experimental and clinical evidence that such factors may cause sperm DNA damage (16, 29, 30).
2. A small number of uncontrolled or poorly controlled studies has suggested that antioxidant vitamin supplementation may reduce sperm DNA damage (31–33). However, a definitive effect of antioxidants on semen parameters or reproductive outcomes has not been shown.
3. Treatment of a semen infection may reduce sperm DNA damage, based on clinical evidence that semen leukocyte-derived reactive oxygen species can cause sperm DNA damage (34).
4. Varicocelectomy may reduce sperm DNA damage in men with clinical varicoceles. In one study, the percentage of spermatozoa with DNA damage (assessed by SCSA) decreased from 28% (before surgery) to 25% after surgery ($P < .05$) (35).
5. Based on the observation that the testis is rich in antioxidant enzymes, it has been proposed that men with high levels of DNA damage in ejaculated sperm have sperm removed surgically from the testis for ICSI (32). However, sperm retrieval is not without complications, and there is no evidence from properly controlled studies to support a recommendation for testicular sperm retrieval for men with abnormal DNA integrity.

SUMMARY AND CONCLUSIONS

- Existing data on the relationship between abnormal DNA integrity and reproductive outcomes are limited.
- Sperm DNA damage is more common in infertile men and may affect reproductive outcomes in selected couples, including those with recurrent spontaneous miscarriage or idiopathic infertility.
- At present, the results of sperm DNA integrity testing alone do not predict pregnancy rates achieved with intercourse, IUI, or IVF and ICSI.
- Currently, there is no proven role for routine DNA integrity testing in the evaluation of infertility.
- Treatments for abnormal DNA integrity have not been shown to have clinical value.

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