Sperm DNA damage assessment: a test whose time has come

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Multiple techniques have been developed to measure the amount of sperm DNA damage in an effort to identify more objective parameters for evaluation of infertile men. We now have evidence to support that integrity of sperm DNA influences a couple's fertility and helps predict the chances of pregnancy and its successful outcome. The available tests of sperm DNA damage require additional large-scale clinical trials before their integration into routine clinical practice. (Fertil Steril® 2005;84:850–3. ©2005 by American Society for Reproductive Medicine.)

Key Words: Sperm DNA damage, male infertility, assisted reproduction, pregnancy

Semen analysis is an essential component of infertility evaluation, but it may still fail to detect subtle sperm defects present in patients with male factor infertility. Although estimates vary, approximately 15% of patients with male factor infertility have normal spermiograms. It is necessary to identify diagnostic measures for male infertility that are easy to perform, relatively inexpensive, and provide an accurate diagnosis. Tests that assess sperm quality should not only identify the ability of spermatozoa to reach the oocyte but also their ability to fertilize the oocyte and activate embryo growth. The estimation of sperm DNA damage seems to fill the above need in the diagnosis of male infertility.

Male reproductive health has been under increased scrutiny recently. Specifically, numerous studies in the literature have concluded that semen quality is declining and that the incidence of testicular cancers is increasing (1). The cause behind this change has been attributed to damage in sperm chromatin. During in vivo reproduction, the natural selection process ensures that only spermatozoon with normal genomic material fertilize an oocyte. However, assisted reproduction techniques (ART) bypass this natural selection process, leading to the possibility that abnormal spermatozoa could be used to fertilize an oocyte. We can avoid this situation by quantifying the amount and type of genomic damage in sperm using well-accepted laboratory methods.

WHAT IS NORMAL SPERM DNA?

Sperm DNA is organized in a specific manner that keeps the nuclear chromatin compact and stable (2). This DNA organization not only permits the very tightly packaged genetic information to be transferred to the egg but also ensures that the DNA is delivered in a physical and chemical form that allows the developing embryo to easily access the genetic information. Fertile sperm have stable DNA, which is capable of decondensation at the appropriate time in the fertilization process and transmitting the DNA without defects.

WHAT ARE THE TYPES AND MECHANISMS OF DNA DAMAGE?

Defects in the genomic material in sperm may take the form of condensation or nuclear maturity defects, DNA breaks, DNA integrity defects, or sperm chromosomal aneuploidies (3). The causes of these defects have been attributed to disease, drug use, high fever, elevated testicular temperature, air pollution, cigarette smoking, and advanced age. The molecular mechanism of DNA damage in these diversified conditions is under intense investigation. The most important mechanisms under consideration for sperm DNA damage are abnormal chromatin packaging, reactive oxygen species (ROS) (4), and apoptosis (5, 6). It is likely that multiple mechanisms are involved, based on the clinical diagnosis responsible for DNA damage.

EVALUATION OF DNA DAMAGE

Multiple techniques can measure DNA defects in human spermatozoa (3, 7). Some new tests are under investigation (8). The ability of these techniques to accurately estimate sperm DNA damage depends on many technical and biological aspects. Some of the currently available tests evaluate the integrity of sperm DNA, including terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-nick end labeling (TUNEL), the sperm chromatin structure assay (SCSA), comet assay, in situ nick translation, and DNA breakage detection-fluorescent in situ hybridization assay (DBD-FISH). Other tests identify the packaging defects of sperm chromatin: aniline blue staining, toluidine blue staining, and chromomycin A3 staining.

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We must study the relationships among the available tests and identify those that correlate with each other and those that do not. For example, FISH assays for aneuploidies provide information on sperm DNA status, which is different from the information that is provided by the other tests. Research should continue to elucidate the nature of the abnormalities measured by each technique and, more importantly, to identify which of these abnormalities play a crucial role in spermatozoal genomic function.

EVIDENCE FOR THE ROLE OF SPERM DNA IN REPRODUCTION

The decision to integrate a new test into clinical practice is largely based on the volume and quality of published evidence. Various studies have analyzed the relationship between the degree of DNA damage and the fertilization rate, embryo cleavage rate, implantation rate, pregnancy rate, and live birth rate of offspring (9). Table 1 lists the different tests that have been used to quantify sperm DNA

TABLE 1

Studies from the literature using different tests of sperm DNA damage analysis in assisted reproduction.

Technique	IVF	ICSI
Chromomycin A3	Bianchi et al., 1996 Tomlinson et al., 2001 Esterhuizen et al., 2000	Sakkas et al., 1996 Sakkas et al., 1998 Razavi et al., 2003
Aniline blue	Haidl et al., 1994 Liu et al., 1992 Hammadeh et al., 1998	Hammadeh et al., 1999 Hammadeh et al., 1996 Variant-Klun et al., 2002
TUNEL	Sun et al., 1997 Duran et al., 2002 Marchetti et al., 2002 Henkel et al., 2004 Seli et al., 2004 Henkel et al., 2003	Lopes et al., 1998 Benchaib et al., 2003 Henkel et al., 2003 Henkel et al., 2004 (14) Seli et al., 2004 Greco et al., 2005
SCSA	Larson et al., 2000 Larson-Cook et al., 2003 Saleh et al., 2003 Bungum et al., 2004 Gandini et al., 2004 Virro et al., 2004	Larson et al., 2000 Larson-Cook et al., 2003 Saleh et al., 2003 Bungum et al., 2004 Gandini et al., 2004
In situ nick translation	Sakkas et al., 1996 Tomlinson et al., 2001	Sakkas et al., 1996 Sakkas et al., 1998
Comet	Tomsu et al., 2002 (16)	Chan et al., 2001 Morris et al., 2002
Acridine orange	Claassens et al., 1992 Duran et al., 1998 Duran et al., 2002 Katayose et al., 2003 Hoshi et al., 1996	Variant-Klun et al., 2002 Angelopoulos et al., 1998 Hammadeh et al., 2001
Miscellaneous	Host et al., 2000 Filatov et al., 1999	Host et al., 2000

Note: IVF = In vitro fertilization; ICSI = intracytoplasmic sperm injection; TUNEL = terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-nick end labeling; SCSA = Sperm Chromatin Structure Assay.

Agarwal. Sperm DNA damage analysis. Fertil Steril 2005.

damage in published studies. When large reports on a specific test of sperm DNA damage are published, it seems to reflect increased interest by the research community, which may increase the chances of the test being integrated into clinical practice. However, before sperm DNA damage analysis can be used routinely in clinical practice, researchers must conduct more outcome-based studies that evaluate the tests' role in the management of male infertility. Moreover, these studies must have an adequate sample size (10). Present reports lack information on the ability of these tests in predicting normal and abnormal pregnancy outcomes (e.g., spontaneous abortions and malformations).

Two studies have used the SCSA technique to examine the relationship between DNA damage in spermatozoa and the ability of a couple to conceive naturally (11, 12). In these studies, when 30% or more of a sperm sample DNA was damaged, the female partner had difficultly conceiving. Bungum et al. (13) found that the chance of pregnancy/delivery for patients who underwent intrauterine insemination was significantly higher in the group with DNA fragmentation index (DFI) $\leq$27% and HDS $\leq$10% than in patients with DFI $>$27% or high DNA stainable (HDS) $>$10%. In the DFI $>$27% group, the results of intracytoplasmic sperm injection (ICSI) were significantly better than those of in vitro fertilization (IVF).

Henkel et al. (14) reported that, even though sperm DNA fragmentation did not correlate with the fertilization and embryo fragmentation rates, the pregnancy rates in patients who underwent IVF were significantly low when TUNEL-positive spermatozoa ($>$36.5%) were used. Pregnancy loss may occur with an increase in the degree of sperm DNA damage (TUNEL assay), and this could be the cause of unexplained pregnancy loss in some patients (15). If sperm DNA is unable to decondense after entering the ooplasma, fertilization may fail or a postfertilization failure could occur due to defective sperm DNA (e.g., poor embryo quality) (16).

FUTURE OF SPERM DNA DAMAGE ANALYSIS TESTS

Given the different types of DNA defects and inconsistency in clinical studies, it is possible that more then one test may come into clinical practice. Efforts should be made to design simple, reliable, reproducible tests, as stressed in other reports (8). These techniques need to be tested in various laboratories before they can be used routinely in clinical practice. Infertility is a very sensitive issue in society, and any new development in this field must be accurate, especially because of the hope it brings to an infertile couple. False predictions about a couple's ability to have healthy offspring that are based on a poorly designed or evaluated test may add to the frustration of the infertile couple. The tests' ability to predict abnormal reproductive outcomes should be assessed.

We must also define the fraction of defective sperm DNA, which is associated with poor fertilization, embryo quality, and pregnancy rate, using the available tests. Other issues must be addressed as well for the future development of DNA sperm damage analysis:

- Does only the quantity of a DNA defect matter, or does the quality/type/location of the DNA defect have a role in reproduction? If the latter is true, this may explain why some couples can achieve pregnancy despite high levels of sperm DNA damage.
- Is it possible that the observed lack of pregnancy in patients with little DNA damage may be due to involvement of critical chromatin material? So, in essence, should the test assess not only the quantity but also quality, type, or location of a defect in sperm DNA?
- Can any of the available tests detect important fertility-related DNA defects?
- Is it possible that in some patients, even though levels of DNA damage in the whole cell population are high, the single spermatozoa that caused fertilization may be normal and hence allows a normal pregnancy to occur? Current techniques measure the DNA status of whole cell population, not excluding the presence of a subpopulation with no significant DNA damage. So development of techniques to identify and isolate spermatozoa with intact DNA may significantly affect the future of ART. If such a technique is developed, it may render current DNA damage measurement techniques redundant.

CONCLUSION

Evidence in the literature shows that sperm DNA damage influences the fertility outcome to a variable degree. But there is no consensus on the technique that should be used to measure sperm DNA in subfertile patients. The methods used to detect sperm DNA damage should be standardized to allow comparison among different studies and to permit routine use of tests in clinical laboratories. The results of the sperm DNA damage evaluation may help physicians better counsel infertile couples about their chances of having a live birth. A new field of research is to identify the type of DNA defects that affect fertility regardless of quantity of damaged DNA and to identify and isolate spermatozoa with intact DNA for use in ART.

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