Chapter 32
Sperm DNA Testing: Where Do We Go from Here?

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32.1 Introduction

The assessment of male fertility potential traditionally depends on the semen analysis, and the most important parameters of this analysis are sperm concentration, motility, and morphology. Unfortunately, the clinical value of these parameters in the diagnosis of male infertility remains limited [1]. While some authors recognize the importance of semen parameters in the assessment of male fertility potential [2, 3], others question the prognostic value of this test [4–6]. Moreover, with the introduction of intracytoplasmic sperm injection (ICSI), the clinical importance of the semen analysis has declined [7].

The genomic integrity of the spermatozoon is essential for the accurate transmission of genetic information and for the proper development and maturation of the embryo [8, 9]. Animal models of sperm chromatin and DNA damage have clearly shown that sperm DNA fragmentation (e.g., experimentally induced damage) is associated with reduced male fertility potential [10–13]. These experimental studies have shown that sperm DNA damage is associated with adverse reproductive outcomes after ARTs, lower pregnancy rates, chromosomal abnormalities, pregnancy loss, reduced longevity, and birth defects [14–17]. These studies have raised concerns regarding the potential adverse outcomes associated with the use of DNA-damaged sperm in the context of human assisted reproduction.

A large number of tests have been developed to measure sperm chromatin and DNA damage in human spermatozoa [18, 19]. These tests were developed with the hope that they might further our understanding of sperm nuclear architecture, accurately measure sperm chromatin and DNA damage, and be valuable tools in clinical

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practice. To date, the studies show that sperm DNA tests may be good markers of male fertility potential. Prospective studies of couples with unknown fertility status have shown that sperm DNA damage is associated with a lower probability of conception (odds ratio = ~7) and a prolonged time to pregnancy [20–23]. These studies also reveal that sperm DNA test results may be better predictors of pregnancy than conventional sperm parameters in this context [23].

Several systematic reviews of studies correlating sperm DNA test results and reproductive outcomes after ARTs have shown that sperm DNA damage is associated with lower intrauterine insemination (IUI) (odds ratio = ~9) and conventional in vitro fertilization (IVF) pregnancy rates (odds ratio = ~1.6–1.9) [19, 24–27]. In contrast, systematic reviews have shown that the relationship between sperm DNA damage and intracytoplasmic sperm injection (ICSI) pregnancy rates is weak (OR = ~1.3) [19, 24–27]. Several systematic reviews have also shown that sperm DNA damage is associated with an increased risk of pregnancy loss after an established natural, IVF, or ICSI pregnancy [28, 29].

The widespread clinical application of sperm DNA tests in the evaluation of infertile men and in the management of couples enrolled in IUI and IVF treatment cycles has not been firmly established despite a large number of clinical studies (40–50 relevant studies). One of the important reasons for the poor acceptance of sperm DNA tests in the evaluation of infertile men is the marked heterogeneity of the study characteristics. Studies on sperm DNA damage and reproductive outcomes differ in their design (prospective, retrospective, case-control) and in patient (e.g., female factors) and cycle characteristics (e.g., day of embryo transfer). Moreover, it is difficult to compare studies because they use one of several sperm DNA tests (e.g., SCSA (sperm chromatin structure assay), comet assay (also known as single-cell gel electrophoresis), TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling) assay).

Another reason sperm DNA tests have not been widely utilized in clinical practice is the limited understanding of what the individual assays actually measure [30]. All of the assays require some preparation of the sperm nucleus (variable degree of nuclear decondensation) prior to addition of an enzyme or dye that permits detection of the target sites (e.g., sites of damaged DNA). As such, it remains unclear if a test measures real damage or damage induced by the assay conditions. Ultimately, it is believed that all sperm DNA tests provide an indirect measure of DNA damage (e.g., SCSA, TUNEL) because the assay conditions alter the chromatin state [9, 31, 32]. It is the unique property of the sperm nucleus (i.e., with a tightly packaged chromatin) that limits the accessibility of assay reagents to all areas of the genome and complicates the correct interpretation of assay results [33]. The limited and variable accessibility of reagents to potentially damaged sites in the sperm DNA and chromatin is one reason that the precise nature, location, and clinical significance of sperm DNA damage remain poorly understood.

The lack of consensus on what is considered an acceptable assay and/or assay conditions has been another reason for the limited utilization of these assays in the clinic [30, 34]. Similarly, the lack of standardized protocols for these assays is

another worry voiced by many clinicians. This has led to some concern regarding precision, reproducibility, and repeatability of the various assays. Another important weakness of these studies is the fact that multiple cutoffs or thresholds have been used, even for the same assay (e.g., 15% or 30% for DFI using the SCSA). The variability of DNA damage thresholds has led to some confusion and misinterpretation of test results [35]. Moreover, the thresholds for many of these tests have not been adequately validated (not adequately powered or not using appropriate control populations).

The biological variability of sperm DNA tests is also an important point to remember when interpreting sperm DNA test results and using these results in clinical decision-making. It has been shown that tests of sperm DNA damage exhibit a small to moderate degree of biologic variability (coefficient of variation (CV) in the range of 10–30%) such that one may need to repeat the assay to confirm the result [36–40]. Several studies have shown that sperm DNA test results can be influenced by sexual abstinence, with longer abstinence periods being associated with higher levels of sperm DNA damage [41, 42]. Finally, external factors (e.g., fever, infections, medications) can also affect sperm DNA integrity [43–45].

Given the important clinical and biological uncertainties of sperm DNA testing, additional work in this area is much needed. In the future, basic studies should be aimed at improving our understanding of the nature of sperm chromatin and DNA damage and what it is that the various sperm DNA tests truly measure. We should also establish standardized sperm DNA assay protocols that provide reproducible results across different laboratories. Future clinical studies evaluating the relationship between sperm DNA damage and reproductive outcomes should be designed as prospective, controlled trials with well-defined populations. These studies should help establish validated and clinically relevant sperm DNA damage thresholds. Ultimately, such studies will help establish the clinical value of sperm DNA tests as markers of male fertility potential.

32.2 Conclusions

A large number of clinical studies (over 50 relevant studies to date) have shown that sperm DNA damage is associated with reproductive outcomes. However, the widespread clinical application of sperm DNA tests in the evaluation of the infertile man has not been firmly established due to a number of limiting clinical and biological factors. The factors responsible for the limited acceptance of sperm DNA tests in the evaluation of infertile men include the marked heterogeneity of clinical studies, the incomplete understanding of sperm chromatin and DNA damage, the lack of standardized sperm DNA test protocols, and the biological variability of these assays. Future studies should be aimed at improving our understanding of the nature of sperm chromatin and DNA damage and, ultimately, help establish the clinical value of sperm DNA tests in the evaluation of infertile men.

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