The clinical utility of sperm DNA integrity testing: a guideline

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Sperm DNA damage is more common in infertile men and may contribute to poor reproductive performance. However, current methods for assessing sperm DNA integrity do not reliably predict treatment outcomes and cannot be recommended routinely for clinical use. (Fertil Steril 2013; No. - No. -.) ©2013 by American Society for Reproductive Medicine.)

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Tests of DNA integrity have been developed and applied in clinical practice. The most commonly studied DNA integrity tests are the sperm chromatin structure assay (SCSA) (7), the deoxyribonucleotidyl transferase-mediated dUTP nick end labeling assay (TUNEL) (8), the single-cell gel electrophoresis assay (COMET) (9), and the sperm chromatin dispersion test (SCD) (10). Each of these tests provides a semi-quantitative estimate of the general state of DNA but does not provide an indication of specific DNA sequences that might be affected. For example, the SCSA utilizes flow cytometry of fluorescently labeled sperm to determine the proportion of sperm susceptible to DNA damage (red fluorescence) compared with normal sperm (green fluorescence). The TUNEL assay utilizes flow cytometry of sperm fluorescently labeled at DNA strand breaks to determine the degree of DNA damage where fluorescence intensity is proportional to the number of strand breaks. In the COMET assay, fluorescently labeled sperm cells are embedded in agarose gel, lysed to relax DNA, and electrophoresed. DNA damage is proportional to displacement between the nuclear material and the tail material. The SCD test utilizes fluorescence microscopy to distinguish cells with intact DNA (large halo) from sperm cells with damaged DNA (small or absent halo).

Numerous studies utilizing the above techniques for assessing sperm DNA integrity support the existence of a significant association between sperm DNA damage and pregnancy outcomes in both humans (11) and non-human species (12). Fertile men with normal semen parameters usually have high levels of DNA integrity, whereas infertile men, especially those with abnormal semen parameters, often have decreased DNA integrity. Moreover, a significant number of infertile men will have abnormal DNA integrity despite normal semen parameters (13–15). This Practice Committee Guideline has been prepared to assess the evidence pertaining to the clinical utility of sperm DNA integrity testing and target areas that require more study. Ideally, validation of the test must statistically determine threshold values, exclude female factors, and utilize sufficient numbers of patients to make statistically valid conclusions.

REVIEW METHODS

A systematic literature search was performed using the search strategy: sperm AND (DNA OR chromatin) AND...
Unlike predictive values, likelihood ratios are calculated in interpreting tests, likelihood ratios (LRs) are most helpful. Optimal threshold values must be determined by looking at ROC curves. Tests should be compared with a universally accepted gold standard outcome, in this case clinical pregnancy. The study population should be a population in which the test would be applied in clinical practice, in this case male infertility. The test should be a test that can be replicated accurately in the laboratory. Optimal threshold values must be determined by looking at test characteristics and optimizing sensitivity and specificity using receiver operator characteristic (ROC) curves. In interpreting tests, likelihood ratios (LRs) are most helpful as they indicate by how much a given test will raise or lower the pretest probability of the target disorder. Unlike predictive values, likelihood ratios are calculated from sensitivity (sens) and specificity (spec) and do not vary with disease prevalence. Positive likelihood ratio (LR+) = True positive/false positive rate (sens/1 – spec). Negative likelihood ratio (LR−) = False negative rate/true negative rate (1 – sens/spec).

LRs of 5–10 and 0.1–0.2 create moderate changes in pretest and post-test probabilities and may be important.

ASSESSMENT OF THE SPERM DNA INTEGRITY TESTING LITERATURE

The comprehensive literature search yielded 74 citations eligible for full review. Review articles were excluded, while meta-analyses were included in the review. Twenty studies used the TUNEL assay to assess DNA integrity while 28 employed the SCSA test. The COMET test was used in 9 papers while the SCD test was used in 5. Less commonly used assays were assessed in 5 or fewer publications. Overall, there are no Level I studies as would be expected for a predictive diagnostic clinical test. In addition, there are few high-quality prospective studies recruiting consecutive patients validating previously established cut-points with gold standard fertility outcomes. Most studies present Level II-2 evidence or less. The majority of studies are hindered by small sample size, non-consecutive recruitment of patients, variable patient populations, lack of control for female factors (particularly age), weak statistical methodology in calculating threshold values and predictive ability of tests, and use of several different methods for assessing DNA damage.

ASSOCIATION OF SPERM DNA INTEGRITY WITH REPRODUCTIVE OUTCOMES

For a diagnostic test to be clinically useful the results must be reproducible, applicable to a given patient, and change the management of the patient. For tests of DNA integrity to be clinically important there must be an association of sperm DNA damage with reproductive outcomes. The literature was reviewed to answer the following questions:

Specific Questions

Does the DNA integrity test predict male fertility with natural conception? Studies have looked at time to pregnancy (14) and fertility potential of sperm donors (16) while others compared DNA fragmentation between fertile and infertile men (7, 17–19). Overall, there is an association with increased DNA fragmentation and reduced fertility in men based on these studies. However, the number of studies is limited and available studies are Level II-2 and Level III evidence. The predictive value of these tests depends on the prevalence of abnormal tests in a population, and the appropriate population for testing has not been established. In conclusion, there is fair evidence (Level B) that increased DNA fragmentation is associated with reduced fertility; however, there is insufficient evidence (Level C) to use the test as a predictor of fertility since cut-points have not been clearly established and validated.

Does the DNA integrity test predict pregnancy with intrauterine insemination (IUI)? A number of studies looked at the SCD test (20), SCSA (17, 21, 22), and TUNEL assay (23) in conjunction with intrauterine insemination. A Level II-1
study (22) showed a positive predictive value of the SCSA test with DNA fragmentation index (DFI) > 30% associated with a lower pregnancy and delivery rate. However, other studies did not confirm the cutoff for IUI and another study found no association with DNA integrity and pregnancy with IUI. In conclusion, there is insufﬁcient evidence (Level C) to recommend the use of DNA integrity tests to predict pregnancy with IUI.

Is DNA fragmentation predictive of pregnancy with in vitro fertilization (IVF)? An extensive statistical review of the studies analyzing the effect of DNA fragmentation on pregnancy with IVF was conducted (Table 1) (22, 24–40). One meta-analysis (41) showed that DNA fragmentation was associated with a modest, but signiﬁcant, reduction in IVF pregnancy rates (OR 1.7 [CI 1.3–2.23] median PPV 77%, median NPV 34%). Increased DNA fragmentation is mildly associated with IVF success overall; however, the predictive ability of the speciﬁc tests is low and lacks validation. Three studies with high (>5) LR+ included only limited numbers of subjects, did not include control groups, and did not validate the thresholds for the test (29, 30, 38). In conclusion, there is insufﬁcient evidence (Level C) to recommend routine use of DNA integrity testing for patients undergoing IVF.

Is DNA fragmentation predictive of pregnancy with IVF and intracytoplasmic sperm injection (ICSI)? An extensive statistical review of the studies testing the effect of DNA fragmentation on patients undergoing IVF/ICSI was conducted (Table 2) (22, 24, 25, 27–36, 38, 40, 42–44). Two studies with high (>5) LR+ included only limited numbers of subjects, did not include control groups, and did not validate the thresholds for the test (30, 31). A meta-analysis (11) concluded that sperm DNA fragmentation was signiﬁcantly associated with pregnancy in IVF/ICSI cycles (OR 1.44 [CI 1.03–2.03]). However, the association was mild and the predictive ability of the DNA integrity tests was weak (LR+ = 1.23, LR− = 0.81). Also, test cut-offs were not clearly established. In a more recent meta-analysis (41) pregnancy rates were found to be independent of DNA integrity test results (OR 1.15 [CI 0.9–1.55]). The analysis revealed an 11% difference in pregnancy rates among the 2 groups. Based on these results, the authors suggest that couples where the male partner has high levels of sperm DNA fragmentation proceed directly to IVF/ICSI. However, the best evidence for this recommendation should come from a randomized controlled trial where the outcome of interest is live birth rate. DNA fragmentation is not signiﬁcantly associated with IVF/ICSI success overall. In conclusion, there is insufﬁcient evidence (Level C) to recommend routine DNA integrity testing for patients undergoing IVF/ICSI.

Is DNA fragmentation predictive of pregnancy loss? A few studies have examined the association between DNA fragmentation and pregnancy loss. A meta-analysis (45) found a signiﬁcant association between DNA fragmentation and pregnancy loss after IVF or ICSI (OR 2.48 [CI 1.52–4.04]). However, there is insufﬁcient evidence (Level C) to recommend routine DNA integrity testing to predict pregnancy loss.

**TABLE 1**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Test</th>
<th>Sens</th>
<th>Spec</th>
<th>LR+</th>
<th>LR−</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boe-Hansen et al., 2006</td>
<td>SCSA</td>
<td>0.06</td>
<td>0.97</td>
<td>2.00</td>
<td>0.97</td>
<td>2.04</td>
<td>0.38–11.0</td>
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<td>Borini et al., 2006</td>
<td>TUNEL</td>
<td>0.17</td>
<td>0.89</td>
<td>1.55</td>
<td>0.93</td>
<td>1.57</td>
<td>0.38–6.51</td>
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<td>Bungum et al., 2007</td>
<td>SCSA</td>
<td>0.17</td>
<td>0.85</td>
<td>1.13</td>
<td>0.98</td>
<td>1.24</td>
<td>0.69–2.26</td>
</tr>
<tr>
<td>Check et al., 2005</td>
<td>SCSA</td>
<td>0.30</td>
<td>0.83</td>
<td>1.76</td>
<td>0.84</td>
<td>1.90</td>
<td>0.61–5.89</td>
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<tr>
<td>Host et al., 2000</td>
<td>TUNEL</td>
<td>0.34</td>
<td>0.80</td>
<td>1.70</td>
<td>0.83</td>
<td>1.91</td>
<td>0.93–3.91</td>
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<tr>
<td>Huang et al., 2005</td>
<td>TUNEL</td>
<td>0.22</td>
<td>0.83</td>
<td>1.29</td>
<td>0.94</td>
<td>1.30</td>
<td>0.66–2.56</td>
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<td>Larson et al., 2000</td>
<td>SCSA</td>
<td>0.58</td>
<td>0.94</td>
<td>9.67</td>
<td>0.45</td>
<td>10.17</td>
<td>1.77–58.4</td>
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<td>Larson-Cook et al., 2003</td>
<td>SCSA</td>
<td>0.17</td>
<td>0.98</td>
<td>8.50</td>
<td>0.85</td>
<td>5.08</td>
<td>1.24–20.8</td>
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<tr>
<td>Payne et al., 2005</td>
<td>SCSA</td>
<td>0.16</td>
<td>0.71</td>
<td>0.55</td>
<td>1.18</td>
<td>0.44</td>
<td>0.15–12.7</td>
</tr>
<tr>
<td>Seli et al., 2004</td>
<td>TUNEL</td>
<td>0.46</td>
<td>0.61</td>
<td>1.18</td>
<td>0.89</td>
<td>1.32</td>
<td>0.43–4.1</td>
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<tr>
<td>Virro et al., 2004</td>
<td>SCSA</td>
<td>0.35</td>
<td>0.81</td>
<td>1.84</td>
<td>0.80</td>
<td>2.27</td>
<td>1.3–3.96</td>
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<td>Henkel et al., 2003</td>
<td>TUNEL</td>
<td>0.35</td>
<td>0.81</td>
<td>1.84</td>
<td>0.80</td>
<td>2.24</td>
<td>1.09–5.48</td>
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<tr>
<td>Lin et al., 2008</td>
<td>SCSA</td>
<td>0.15</td>
<td>0.83</td>
<td>0.88</td>
<td>1.02</td>
<td>0.88</td>
<td>0.35–2.19</td>
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<td>Benchabli et al., 2007</td>
<td>TUNEL</td>
<td>0.07</td>
<td>0.86</td>
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<td>1.08</td>
<td>0.46</td>
<td>0.11–2.0</td>
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<td>Frydman et al., 2008</td>
<td>TUNEL</td>
<td>0.58</td>
<td>0.68</td>
<td>1.81</td>
<td>0.62</td>
<td>2.97</td>
<td>1.39–6.32</td>
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<tr>
<td>Tarozzi et al., 2009</td>
<td>CMA</td>
<td>0.22</td>
<td>0.97</td>
<td>7.33</td>
<td>0.80</td>
<td>10.86</td>
<td>0.62–191.5</td>
</tr>
<tr>
<td>Simon et al., 2011</td>
<td>COMET</td>
<td>0.95</td>
<td>0.80</td>
<td>4.75</td>
<td>0.06</td>
<td>76.00</td>
<td>8.69–71.14</td>
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<tr>
<td>Simon et al., 2010</td>
<td>COMET</td>
<td>0.82</td>
<td>0.50</td>
<td>1.64</td>
<td>0.36</td>
<td>4.50</td>
<td>1.79–11.92</td>
</tr>
</tbody>
</table>

Note: Sens = sensitivity; Spec = specificity; LR+ = positive likelihood ratio; LR− = negative likelihood ratio; OR = odds ratio; CI = conﬁdence interval; SCSA = sperm chromatin structure assay; TUNEL = terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling assay; CMA = chromomycin A3; COMET = single-cell gel electrophoresis assay.


**SUMMARY**

- Existing data do not support a consistent relationship between abnormal DNA integrity and reproductive outcomes.
- At present, the results of sperm DNA integrity testing alone do not predict pregnancy rates achieved through natural conception or with IUI, IVF, or ICSI. However, further research may lead to validation of the clinical utility of these tests.
RECOMMENDATION

There is insufficient evidence to recommend the routine use of sperm DNA integrity tests in the evaluation and treatment of the infertile couple (Level C).

Acknowledgments: This report was developed under the direction of the Practice Committee of the American Society for Reproductive Medicine as a service to its members and other practicing clinicians. Although this document reflects appropriate management of a problem encountered in the practice of reproductive medicine, it is not intended to be the only approved standard of practice or to dictate an exclusive course of treatment. Other plans of management may be appropriate, taking into account the needs of the individual patient, available resources, and institutional or clinical practice limitations. The Practice Committee and the Board of Directors of the American Society for Reproductive Medicine have approved this report.

This document was reviewed by ASRM members and their input was considered in the preparation of the final document. The following members of the ASRM Practice Committee participated in the development of this document. All Committee members disclosed commercial and financial relationships with manufacturers or distributors of goods or services used to treat patients. Members of the Committee who were found to have conflicts of interest based on the relationships disclosed did not participate in the discussion or development of this document.


REFERENCES


