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;■:■–■. ©2013 by American Society for Reproductive Medicine.)

There is a strong clinical need to distinguish fertile men from infertile men and to be able to predict the outcome of infertility procedures. The parameters of the conventional semen analysis do not reliably predict either male fertility or pregnancy after infertility treatment. Thus, researchers have sought methods to predict male fertility in a more clinically useful manner.

Mammalian fertilization and subsequent embryo development depend in part on the inherent integrity of sperm DNA (1, 2). Most sperm DNA exists bound to protamine in a dense, insoluble state more compact than that observed in somatic cell DNA (3). In this compact state DNA is protected from potentially deleterious damage during sperm transport. Only a few of the many causes of sperm DNA damage have been identified, including protamine deficiency (4), oxidative stress (5), and failure to repair DNA strand breaks (6). The association between DNA damage and diminished reproductive outcomes has led to the introduction of sperm DNA integrity testing into the clinical assessment of male fertility.

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 deoxynucleotidyl 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

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(fragmentation OR damage OR integrity) AND (pregnancy [title/abstract] OR embryo [title/abstract]) AND (Humans [mesh] AND English [language]) (204 citations). The search was restricted to MEDLINE citations published in the English language from 1966 to November 2011. Studies were eligible if they met one of the following criteria: primary evidence (clinical trials) that assessed the predictive potential using predictive statistics, meta-analyses, and relevant articles from bibliographies of identified articles.

The quality of the evidence was evaluated as follows:

Level l: Evidence obtained from at least one properly designed randomized controlled trial.

Level ll-1: Evidence obtained from well-designed controlled trials without randomization.

Level ll-2: Evidence obtained from well-designed cohort or case-control analytic studies, preferably from more than one center or research group.

Level ll-3: Evidence obtained from multiple time series with or without the intervention. Dramatic results in uncontrolled trials might also be regarded as this type of evidence.

Level lll: Opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees.

The strength of the evidence was evaluated as follows:

Level A: There is good evidence to support the recommendations, either for or against.

Level B: There is fair evidence to support the recommendations, either for or against.

Level C: There is insufficient evidence to support a recommendation, either for or against.

EVALUATING THE EVIDENCE FOR DIAGNOSTIC AND PREDICTIVE TESTS

Requirements of tests:

- 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 l 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 ll-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 ll-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.

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.

TABLE 1

Predictive value of sperm DNA integrity testing for pregnancy with IVF (22, 24–40).

Reference	Test	Sens	Spec	LR+	LR−	OR	95% CI
Boe-Hansen et al., 2006	SCSA	0.06	0.97	2.00	0.97	2.04	0.38–11.0
Borini et al., 2006	TUNEL	0.17	0.89	1.55	0.93	1.57	0.38–6.51
Bungum et al., 2007	SCSA	0.17	0.85	1.13	0.98	1.24	0.69–2.26
Check et al., 2005	SCSA	0.30	0.83	1.76	0.84	1.90	0.61–5.89
Host et al., 2000	TUNEL	0.34	0.80	1.70	0.83	1.91	0.93–3.91
Huang et al., 2005	TUNEL	0.22	0.83	1.29	0.94	1.30	0.66–2.56
Larson et al., 2000	SCSA	0.58	0.94	9.67	0.45	10.17	1.77–58.4
Larson-Cook et al., 2003	SCSA	0.17	0.98	8.50	0.85	5.08	1.24–20.8
Payne et al., 2005	SCSA	0.16	0.71	0.55	1.18	0.44	0.15–1.27
Seli et al., 2004	TUNEL	0.46	0.61	1.18	0.89	1.32	0.43–4.1
Virro et al., 2004	SCSA	0.35	0.81	1.84	0.80	2.27	1.3–3.96
Henkel et al., 2003	TUNEL	0.35	0.81	1.84	0.80	2.24	1.09–4.58
Lin et al., 2008	SCSA	0.15	0.83	0.88	1.02	0.88	0.35–2.19
Benchaib et al., 2007	TUNEL	0.07	0.86	0.50	1.08	0.46	0.11–2.0
Frydman et al., 2008	TUNEL	0.58	0.68	1.81	0.62	2.97	1.39–6.32
Tarozzi et al., 2009	CMA	0.22	0.97	7.33	0.80	10.86	0.62–191.5
Simon et al., 2011	COMET	0.95	0.80	4.75	0.06	76.00	8.69–1,714.44
Simon et al., 2010	COMET	0.82	0.50	1.64	0.36	4.50	1.79–11.92

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.

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TABLE 2

Predictive value of sperm DNA integrity testing for patients undergoing IVF/ICSI (22, 24, 25, 27–36, 38, 40, 42–44).

Reference	Test	Sens	Spec	LR+	LR−	OR	95% CI
Boe-Hansen et al., 2006	SCSA	0.36	0.57	0.84	1.12	0.76	0.21–2.73
Borini et al., 2006	TUNEL	0.71	0.75	2.84	0.39	6.55	1.77–24.3
Bungum et al., 2007	SCSA	0.30	0.63	0.81	1.11	0.74	0.42–1.31
Host et al., 2000	TUNEL	0.58	0.38	0.94	1.11	0.84	0.29–2.43
Gandini et al., 2004	SCSA	0.38	0.44	0.68	1.41	0.52	0.10–2.74
Huang et al., 2005	TUNEL	0.64	0.50	1.28	0.72	1.78	0.76–4.16
Zini et al., 2005	SCSA	0.17	0.81	0.89	1.02	0.87	0.24–3.19
Larson et al., 2000	SCSA	0.58	0.94	9.67	0.45	10.17	1.77–58.4
Larson-Cook et al., 2003	SCSA	0.17	0.98	8.50	0.85	5.08	1.24–20.8
Payne et al., 2005	SCSA	0.16	0.71	0.55	1.18	0.44	0.15–1.27
Seli et al., 2004	TUNEL	0.46	0.61	1.18	0.89	1.32	0.43–4.1
Virro et al., 2004	SCSA	0.35	0.81	1.84	0.80	2.27	1.3–3.96
Henkel et al., 2003	TUNEL	0.68	0.63	1.84	0.51	3.67	1.12–12
Lin et al., 2008	SCSA	0.26	0.77	1.13	0.96	1.21	0.45–3.23
Benchaib et al., 2007	TUNEL	0.19	0.87	1.46	0.93	1.55	0.70–3.41
Micinski et al., 2009	SCSA	0.40	0.85	2.67	0.71	3.73	0.74–18.77
Tarozzi et al., 2009	CMA3	0.49	0.27	0.67	1.89	0.34	0.09–1.29
Simon et al., 2010	COMET	0.47	0.55	1.04	0.96	1.97	0.81–4.77

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.

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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).

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