Clinical Utility of Sperm DNA Integrity Tests

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Abstract

Tests of sperm DNA integrity are being used increasingly in the evaluation of infertile men with the premise that these tests may better diagnose the infertility and predict reproductive outcomes. Indeed, a systematic review of the literature allows us to conclude that sperm DNA damage is associated with lower natural, IUI, and IVF pregnancy rates. By contrast, studies to date have not shown a clear association between sperm DNA and chromatin defects and pregnancy outcomes after ICSI. In couples undergoing IVF or ICSI, there is also evidence to show that sperm DNA damage is associated with an increased risk of pregnancy loss. A limitation of the systematic reviews and meta-analyses is that they do not address an important feature of the clinical studies on sperm DNA damage, the often marked heterogeneity of the individual study characteristics. Although the clinical utility of tests of sperm DNA damage remains to be established, the data suggest that there is clinical value in testing couples prior to assisted reproductive technologies – ARTs (IUI, IVF, and ICSI) and in those couples with recurrent abortions. Large, well-designed prospective studies are needed before testing becomes a routine part of patient care.

Keywords

Sperm DNA integrity tests • DNA integrity tests • Infertility in men • In vitro fertilization • Sperm chromatin defects

Clinical Utility of Sperm DNA Tests

The relationship between sperm chromatin/DNA damage and pregnancy outcomes has been examined by systematic reviews and meta-analyses [1–3]. The strength of these systematic reviews is

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the improved precision of the summary estimates compared with the individual study estimates of the relationship between sperm DNA defects and pregnancy outcomes. On the contrary, a weakness of meta-analyses (particularly on this topic) is the fact that it combines studies with highly variable study characteristics: data collection (prospective or retrospective), population characteristics (unselected, male factor), female inclusion/exclusion criteria, laboratory expertise in assessment of sperm DNA/chromatin damage, sperm DNA/chromatin test type, and sperm DNA test cutoff.

The recommendations for sperm DNA testing are based on (1) systematic reviews and meta-analyses of the relevant studies, (2) the characteristics of sperm DNA testing (e.g., sensitivity, positivity rate), and (3) disease prevalence (e.g., pregnancy, pregnancy loss).

Screening Test for First Pregnancy Planners

The data from three studies [4–6] show that sperm DNA damage is associated with a reduced probability of natural pregnancy (combined OR 7.01, 95% CI 3.68, 13.36, $p < 0.0001$). Remarkably, the three studies [4–6] report very similar associations between sperm DNA damage and natural pregnancy rate (with ORs of 6.54, 6.82, and 7.59, respectively, see Table 37.1). An analysis of the three studies reveals a median pregnancy rate of 53%, with a median positive predictive value (PPV) of 83% and a median negative predictive (NPV) of 58% associated with sperm DNA testing [4–6]. As such, the analysis predicts that in populations with an overall pregnancy rate of 53% (at 6–12 months of follow-up), the pregnancy rate is 17% when there is a positive test for sperm DNA damage and at 58% when the test result is normal. Therefore, testing for sperm DNA damage can discriminate between pregnancy rates of 17% and 58%. However, because the prevalence of a positive test in this context (first pregnancy planners) is low (<10%) and 17% of couples with a positive test will achieve a pregnancy, *indiscriminate sperm DNA testing in this context is not advocated. Clinicians may choose to test first pregnancy planners, but they should understand the predictive value and limitations* (e.g., *sensitivity, specificity) of the sperm DNA test in this context and discuss these issues with the patients.*

Couples with Mild Male-Factor Infertility: IUI Candidates

Data from one valid IUI study show that sperm DNA damage is related to a significantly reduced IUI pregnancy rate (OR 9.9, 95% CI, 2.37, 41.51, $p < 0.0001$) [7]. In the Bungum et al. study, the overall IUI pregnancy rate is 20%, the PPV is 97%, and the NPV is 24% [7]. Therefore, in populations with an IUI pregnancy rate of 20%, a positive test for sperm DNA damage predicts the pregnancy rate to be 3% and a normal test result predicts the pregnancy rate to be 24%. Therefore, testing for sperm DNA damage prior to IUI can differentiate between pregnancy rates of 3% and 24%. *According to the Bungum* et al. *study, couples with high levels of sperm DNA damage should proceed to IVF and/or ICSI rather than IUI.* However, it is important to note that the sensitivity and prevalence of a positive test in

Table 37.1 Selected diagnostic properties of studies on sperm DNA damage and natural pregnancy

Study	*n*	%hDFI	Sens	Spec	PPV	NPV	OR	(95% CI)
Evenson et al. [4]	144	7	0.19	0.96	0.60	0.81	6.54	(1.72, 24.92)
Spano et al. [6]	215	13	0.23	0.96	0.86	0.55	7.59	(2.54, 22.67)
Giwercman et al. [9]	257	12	0.21	0.96	0.83	0.58	6.82	(2.52, 18.47)

%hDFI proportion of samples with high sperm DNA fragmentation index (DFI); *Sens* sensitivity; *Spec* specificity; *PPV* positive predictive value; *NPV* negative predictive value; *OR* odds ratio; *CI* confidence interval

this context (couples with mild male-factor infertility) are low (<20%) and these recommendations are derived from only one reliable study [7]. As such, *additional IUI studies are needed before routine testing is recommended prior to initiating IUI treatments.*

Couples with Severe Male-Factor Infertility: IVF or ICSI Candidates

Data from more than 20 studies (11 evaluable – see Table 37.2) demonstrate that sperm DNA damage is associated with a modest but significant reduction in the IVF pregnancy rate (combined OR of 1.70, 95% CI 1.30, 2.23, $p<0.05$) [7–17]. Further analysis of the 11 evaluable IVF studies (with a median pregnancy rate of 33%) reveals a median PPV of 77% and median NPV of 34%. In clinical terms, this means that in populations with an overall IVF pregnancy rate of 33%, a positive test for sperm DNA damage predicts the IVF pregnancy rate to be 23% and 34% if the test is negative. As such, couples with sperm DNA damage may choose to proceed to ICSI, where pregnancy rates are independent of test results (see below). However, *the clinical value of an 11% difference in IVF pregnancy rates (23% vs. 34%, with positive and negative test result, respectively) is modest, and it may be hard to justify routine testing in this setting.* However, clinicians may want to test select couples (e.g., with failed IVF) so as to better counsel these couples in future ART cycles.

Data from more than 20 studies (14 evaluable – see Table 37.3) have evaluated the relationship between sperm DNA integrity and pregnancy rates after IVF/ICSI. As with IVF studies, these ICSI studies are quite heterogeneous. In keeping with a recent analysis [1], the results of this updated meta-analysis on ICSI studies indicate that sperm DNA damage is not related to ICSI pregnancy rates (combined OR of 1.15, 95% 0.90, 1.55, $p=0.65$) [7–10, 13–22]. *These data suggest that sperm DNA testing is not clinically valuable in predicting ICSI outcomes.* Perhaps the most concerning aspect of these findings is the unknown long-term consequence (i.e., postnatal health) of a successful pregnancy with high levels of DNA damage.

Testing couples with severe male-factor infertility enrolled in IVF or ICSI may also be valuable because sperm DNA damage is associated with a significantly higher rate of pregnancy loss after IVF or ICSI (combined OR of 2.48, 95% CI; 1.52, 4.04, $p<0.0001$) [3]. Data derived from these studies (PPV and NPV) indicate that in populations with an overall rate of pregnancy loss of 18%, the rate of pregnancy loss is estimated at

Table 37.2 Selected diagnostic properties of 11 studies on sperm DNA damage and pregnancy after IVF

Study	*n*	Assay	%hDD	Sens	Spec	PPV	NPV	OR	(95% CI)
Filatov et al. [11]	176	CC	41	0.46	0.88	0.96	0.21	6.34	(1.82, 22.08)
Host et al. [14]	175	TUNEL	30	0.34	0.79	0.77	0.37	1.92	(0.92, 4.04)
Henkel et al. [13]	208	TUNEL	69	0.35	0.81	0.81	0.35	2.24	(1.09, 4.58)
Huang et al. [15]	217	TUNEL	19	0.22	0.83	0.50	0.57	1.30	(0.66, 2.56)
Boe-Hansen et al. [9]	139	SCSA	5	0.06	0.97	0.86	0.29	2.43	(0.28, 20.83)
Borini et al. [10]	82	TUNEL	16	0.17	0.89	0.85	0.23	1.66	(0.33, 8.28)
Lin et al. [16]	137	SCSA	16	0.15	0.83	0.45	0.51	0.88	(0.35, 2.19)
Benchaib et al. [8]	84	TUNEL	10	0.07	0.86	0.50	0.32	0.46	(0.11, 2.00)
Bungum et al. [7]	388	SCSA	16	0.17	0.86	0.71	0.34	1.24	(0.69, 2.26)
Frydman et al. [12]	117	TUNEL	44	0.58	0.68	0.64	0.35	2.97	(1.39, 6.32)
Tarozzi et al. [17]	82	CMA3	17	0.22	0.97	0.97	0.28	10.86	(0.62, 191.5)

%hDD proportion of samples with high sperm DNA damage; *Sens* sensitivity; *Spec* specificity; *PPV* positive predictive value; *NPV* negative predictive value; *OR* odds ratio; *CC* chromatin compaction; *TUNEL* terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling; *SCSA* sperm chromatin structure assay; *CMA3* chromomycin A3

Table 37.3 Selected diagnostic properties of 14 studies on sperm DNA damage and pregnancy after ICSI

Study	*n*	Assay	%hDD	Sens	Spec	PPV	NPV	OR	95% CI
Hammadeh et al. [20]	60	ABlue	44	0.50	0.71	0.82	0.35	2.40	(0.72, 7.96)
Host et al. [14]	61	TUNEL	59	0.57	0.38	0.58	0.36	0.79	(0.28, 2.25)
Henkel et al. [13]	54	TUNEL	48	0.68	0.63	0.79	0.50	3.67	(1.12, 12.0)
Gandini et al. [19]	22	SCSA	41	0.31	0.44	0.44	0.31	0.36	(0.06, 2.08)
Huang et al. [15]	86	TUNEL	57	0.64	0.50	0.55	0.60	1.80	(0.76, 4.27)
Zini et al. [22]	60	SCSA	18	0.17	0.81	0.46	0.51	0.87	(0.23, 3.22)
Check et al. [18]	104	SCSA	28	0.29	0.76	0.72	0.34	1.34	(0.52, 3.43)
Boe-Hansen et al. [9]	47	SCSA	38	0.36	0.57	0.67	0.28	0.76	(0.21, 2.72)
Borini et al. [10]	50	TUNEL	60	0.71	0.75	0.90	0.45	7.36	(1.67, 32.4)
Benchaib et al. [8]	218	TUNEL	17	0.19	0.87	0.72	0.37	1.55	(0.70, 3.41)
Bungum et al. [7]	223	SCSA	33	0.29	0.61	0.52	0.37	0.65	(0.37, 1.14)
Lin et al. [16]	86	SCSA	24	0.26	0.77	0.52	0.52	1.21	(0.45, 3.23)
Micinski et al. [21]	50	SCSA	35	0.40	0.85	0.91	0.28	3.73	(0.74, 18.77)
Tarozzi et al. [17]	50	CMA3	56	0.49	0.27	0.61	0.18	0.34	(0.09, 1.29)

%hDD proportion of samples with high sperm DNA damage; *Sens* sensitivity; *Spec* specificity; *PPV* positive predictive value; *NPV* negative predictive value; *OR* odds ratio; *ABlue* aniline blue; *TUNEL* terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling; *SCSA* sperm chromatin structure assay; *CMA3* chromomycin A3

37% when the test is positive and 10% when it is negative. The difference between a pregnancy loss rate of 37% and 10% may be valuable to patients and clinicians. Although the effect of DNA damage on pregnancy loss should be discussed with patients prior to undergoing ART, many couples will proceed with these treatments regardless of sperm DNA test results and the impact on pregnancy loss.

Couples with Pregnancy Loss After IVF or IVF/ICSI

The prevalence of a positive test, sensitivity and specificity of sperm DNA testing in the context of pregnancy loss after IVF and ICSI are and 25, 40, and 85%, respectively [3]. This indicates that sperm DNA damage is a minor cause of pregnancy loss after IVF and ICSI (based on the low prevalence and low sensitivity). However, if the test is positive, it suggests that the sperm DNA damage (or male-factor) may be the cause of the pregnancy loss (based on the high specificity). In this setting, it may be advisable to evaluate or reevaluate the male and correct any potential male factor (e.g., varicocele) that may contribute to the DNA damage.

Guidelines on Clinical Value of Sperm DNA Tests

The ASRM (American Society for Reproductive Medicine) has published guidelines on the clinical utility of sperm DNA integrity tests in 2006 and again in 2008 [23, 24]. Based on their evaluation of the existing literature (up to 2006 in both the 2006 and 2008 reports), they conclude the following:

1. Existing data on the relationship between abnormal DNA integrity and reproductive outcomes are limited.
2. 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.
3. At present, the results of sperm DNA integrity testing alone do not predict pregnancy rates achieved with intercourse, IUI, or IVF and ICSI.
4. Currently, there is no proven role for routine DNA integrity testing in the evaluation of infertility.
5. Treatments for abnormal DNA integrity have not been shown to have clinical value.

Although these guidelines provide clinicians with a fair assessment of the value of sperm DNA tests (based on literature up to 2006), more recent studies have added to our understanding of this test and the data suggest that there may be value in testing couples prior to ARTs.

Summary

Tests of sperm DNA and chromatin integrity are being used in the evaluation of infertile men. To date, the clinical studies on sperm DNA and chromatin defects allow us to conclude that sperm DNA damage is associated with lower natural, IUI, and IVF pregnancy rates, but not with ICSI pregnancy rates. Moreover, sperm DNA damage is associated with an increased risk of pregnancy loss in those couples undergoing IVF or ICSI. Although the clinical utility of tests of sperm DNA/chromatin damage remains to be firmly established, the data suggest that there is clinical value in testing couples with recurrent abortions or prior to initiating ART cycles.

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