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The clinical utility of sperm DNA integrity testing: a guideline

The Practice Committee of the American Society for Reproductive Medicine

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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.)

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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 pro-

portional 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

(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).

REFERENCES

1. Agarwal A, Said TM. Role of sperm chromatin abnormalities and DNA damage in male infertility. Hum Reprod Update 2003;9:331–45.
2. Ahmadi A, Ng SC. Fertilizing ability of DNA-damaged spermatozoa. J Exp Zool 1999;284:696–704.
3. Erenpreiss J, Spano M, Erenpreisa J, Bungum M, Giwercman A. Sperm chromatin structure and male fertility: biological and clinical aspects. Asian J Androl 2006;8:11–29.
4. Carrell DT, Liu L. Altered protamine 2 expression is uncommon in donors of known fertility, but common among men with poor fertilizing capacity, and may reflect other abnormalities of spermiogenesis. J Androl 2001;22: 604–10.
5. Saleh RA, Agarwal A, Sharma RK, Said TM, Sikka SC, Thomas AJ Jr. Evaluation of nuclear DNA damage in spermatozoa from infertile men with varicocele. Fertil Steril 2003;80:1431–6. Level II-2.
6. Lewis SE, Aitken RJ. DNA damage to spermatozoa has impacts on fertilization and pregnancy. Cell Tissue Res 2005;322:33–41.
7. Evenson DP, Jost LK, Marshall D, Zinaman MJ, Clegg E, Purvis K, et al. Utility of the sperm chromatin assay as a diagnostic and prognostic tool in the human fertility clinic. Hum Reprod 1999;14:1039–49.
8. Sun JG, Jurisicova A, Casper RF. Detection of deoxyribonucleic acid fragmentation in human sperm: correlation with fertilization in vitro. Biol Reprod 1997;56:602–7.
9. Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 1988;175:184–91.
10. Fernández JL, Muriel L, Rivero MT, Goyanes V, Vazquez R, Alvarez JG. The sperm chromatin dispersion test: a simple method for the determination of sperm DNA fragmentation. J Androl 2003;24:59–66.
11. Collins JA, Barnhart KT, Schlegel PN. Do sperm DNA integrity test predict pregnancy with in vitro fertilization? Fertil Steril 2008;89:823–31.
12. Fernández-Gonzalez R, Moreira PN, Pérez-Crespo M, Sánchez-Martin M, Ramirez MA, Pericuesta E, et al. Long-term effects of mouse intracytoplasmic sperm injection with DNA-fragmented sperm on health and behavior of adult offspring. Biol Reprod 2008;78:761–72.

13. Kodama H, Yamaguchi R, Fukuda J, Kasai H, Tanaka T. Increased oxidative deoxyribonucleic acid damage in the spermatozoa of infertile male patients. Fertil Steril 1997;68:519–24.

14. Spano M, Bonde JP, Hjollund HI, Kolstad HA, Cordelli E, Leter G, et al. The Danish First Pregnancy Planner Study Team. Sperm chromatin damage impairs human fertility. Fertil Steril 2000;73:43–50.

15. Zini A, Bielecki R, Phang D, Zenzes MT. Correlations between two markers of sperm DNA integrity, DNA denaturation and DNA fragmentation, in fertile and infertile men. Fertil Steril 2001;75:674–7.

16. Hu J, Zhu W, Liu W, Fan L. Factors affecting fecundity among sperm donors: a multivariate analysis. Andrologia 2011;43:155–62.

17. Evenson D, Wixon R. Meta-analysis of sperm DNA fragmentation using the sperm chromatin structure assay. Repro BiomedOnline 2006;12:466–72.

18. Giwercman A, Lindstedt L, Larsson M, Bungum M, Spano M, Levine RJ, et al. Sperm chromatin structure assay as an independent predictor of fertility in vivo: a case-control study. Int J Androl 2010;33:e221–7.

19. Simon L, Lewis SE. Sperm DNA damage or progressive motility: which one is the better predictor of fertilization in vitro? Systems biology in reproductive medicine 2011;57:133–8.

20. Muriel L, Meseguer M, Fernandez JL, Alvarez J, Remohı J, Pellicer A, et al. Value of the sperm chromatin dispersion test in predicting pregnancy outcome in intrauterine insemination: a blind prospective study. Hum Reprod 2006;21:738–44.

21. Bungum M, Humaidan P, Spano M, Jepson K, Bungum L, Giwercman A. The predictive value of sperm chromatin structure assay (SCSA) parameters for the outcome of intrauterine insemination, IVF and ICSI. Hum Reprod 2004;19:1401–8.

22. Bungum M, Humaidan P, Axmon A, Spano M, Bungum L, Erenpreiss J, et al. Sperm DNA integrity assessment in prediction of assisted reproduction technology outcome. Human Reprod 2007;22:174–9.

23. Duran EH, Morshedi M, Taylor S, Oehninger S. Sperm DNA quality predicts intrauterine insemination outcome: a prospective cohort study. Hum Reprod 2002;17:3122–8.

24. Boe-Hansen GB, Fedder J, Ersboll AK, Christensen P. The sperm chromatin structure assay as a diagnostic tool in the human fertility clinic. Human Reprod 2006;21:1576–82.

25. Borini A, Tarozzi N, Bizzaro D, Bonu MA, Fava L, Flamigni C, et al. Sperm DNA fragmentation: paternal effect on early post-implantation embryo development in ART. Human Reprod 2006;21:2876–81.

26. Check JH, Graziano V, Cohen R, Krotec J, Check ML. Effect of an abnormal sperm chromatin structural assay (SCSA) on pregnancy outcome following (IVF) with ICSI in previous IVF failures. Arch Androl 2005;51:121–4.

27. Host E, Lindenberg S, Smidt-Jensen S. The role of DNA strand breaks in human spermatozoa used for IVF and ICSI. Acta Obstetricia et Gynecologica 2000;79:559–63.

28. Huang CC, Lin DP, Tsao HM, Cheng TC, Liu CH, Lee MS. Sperm DNA fragmentation negatively correlates with velocity and fertilization rates but might not affect pregnancy rates. Fertil Steril 2005;84:130–40.

29. Larson KL, DeJonge CJ, Barnes AM, Jost LK, Evenson DP. Sperm chromatin structure assay parameters as predictors of failed pregnancy following assisted reproductive techniques. Hum Reprod 2000;15:1717–22.

30. Larson-Cook KL, Brannian JD, Hansen KA, Kasperson KM, Aamold ET, Evenson DP. Relationship between the outcomes of assisted reproductive techniques and sperm DNA fragmentation as measured by the sperm chromatin structure assay. Fertil Steril 2003;80:895–902.

31. Payne JF, Raburn DJ, Couchman GM, Price TM, Jamison MG, Walmer DK. Redefining the relationship between sperm deoxyribonucleic acid fragmentation as measured by the sperm chromatin structure assay and outcomes of assisted reproductive techniques. Fertil Steril 2005;84: 356–64.

32. Seli E, Gardner DK, Schoolcraft WB, Moffatt O, Sakkas D. Extent of nuclear DNA damage in ejaculated spermatozoa impacts on blastocyst development after in vitro fertilization. Fertil Steril 2004;82:378–83.

33. Virro MR, Larson-Cook KL, Evenson DP. Sperm chromatin structure assay (SCSA) parameters are related to fertilization, blastocyst development, and ongoing pregnancy in in vitro fertilization and intracytoplasmic sperm injection cycles. Fertil Steril 2004;81:1289–95.

34. Henkel R, Kierspel E, Hajimohammad M, Stalf T, Hoogendijk C, Mehnert C, et al. DNA fragmentation of spermatozoa and assisted reproduction technology. Reproductive biomedicine online 2003;7:477–84.

35. Lin MH, Kuo-Kuang Lee R, Li SH, Lu CH, Sun FJ, Hwu YM. Sperm chromatin structure assay parameters are not related to fertilization rates, embryo quality, and pregnancy rates in in vitro fertilization and intracytoplasmic sperm injection, but might be related to spontaneous abortion rates. Fertil Steril 2008;90:352–9.

36. Benchaib M, Lornage J, Mazoyer C, Lejeune H, Salle B, Francois Guerin J. Sperm deoxyribonucleic acid fragmentation as a prognostic indicator of assisted reproductive technology outcome. Fertil Steril 2007; 87:93–100.

37. Frydman N, Prisant N, Hesters L, Frydman R, Tachdjian G, Cohen-Bacrie P, et al. Adequate ovarian follicular status does not prevent the decrease in pregnancy rates associated with high sperm DNA fragmentation. Fertil Steril 2008;89:92–7.

38. Tarozzi N, Nadalini M, Stronati A, Bizzaro D, Dal Prato L, Coticchio G, et al. Anomalies in sperm chromatin packaging: implications for assisted reproduction techniques. Reprod Biomed Online 2009;18:486–95.

39. Simon L, Lutton D, McManus J, Lewis SE. Sperm DNA damage measured by the alkaline Comet assay as an independent predictor of male infertility and in vitro fertilization success. Fertil Steril 2011;95:652–7.

40. Simon L, Brunborg G, Stevenson M, Lutton D, McManus J, Lewis SE. Clinical significance of sperm DNA damage in assisted reproduction outcome. Human Reprod 2010;25:1594–608.

41. Zini A. Are sperm chromatin and DNA defects relevant in the clinic? Systems biology in reproductive medicine 2011;57:78–85.

42. Gandini L, Lombardo F, Paoli D, Caruso F, Eleuteri P, Leter G, et al. Full-term pregnancies achieved with ICSI despite high levels of sperm chromatin damage. Human Reprod 2004;19:1409–17.

43. Zini A, Meriano J, Kader K, Jarvi K, Laskin CA, Cadesky K. Potential adverse effect of sperm DNA damage on embryo quality after ICSI. Hum Reprod 2005;20:3476–80.

44. Micinski P, Pawlicki K, Wielgus E, Bochenek M, Tworkowska I. The sperm chromatin structure assay (SCSA) as prognostic factor in IVF/ICSI program. Reprod Biol 2009;9:65–70.

45. Zini A, Boman JM, Belzile E, Ciampi A. Sperm DNA damage is associated with an increased risk of pregnancy loss after IVF and ICSI: systematic review and meta-analysis. Hum Reprod 2008;23:2663–8.