Chapter 24
Sperm DNA Tests Are Clinically Useful: CON

Katherine Rotker and Mark Sigman

24.1 Introduction

The diagnostic semen analysis remains the mainstay laboratory test in the evaluation of the infertile man. However, conventional semen parameters have limited diagnostic value for male fertility and are poor predictors of reproductive outcomes. There is significant overlap between semen parameters in groups of fertile and infertile men [1]. Therefore, substantial efforts have been made to identify improved diagnostic tests to provide a more accurate infertility diagnosis than by evaluation of standard semen parameters alone. Sperm DNA integrity assays have gained interest as a potential test to discriminate infertile from fertile men. Despite a growing body of literature, controversy still exists regarding the ability of these assays to provide clinically useful data in the evaluation of the infertile man. The Practice Committee of the American Society for Reproductive Medicine (ASRM), the American Urological Association (AUA) practice guidelines, and the European Society for Human Reproduction and Embryology (ESHRE) have concluded that current data doesn't support the use of sperm DNA testing on a routine basis [2–4]. In this chapter we will discuss the reasons that, despite future promise, sperm DNA damage testing in its current state is not a routinely clinically useful test.

K. Rotker (✉)
Department of Urology, UMASS Medical School & Memorial Healthcare System, Worcester, MA, USA
e-mail: katherine.rotker@umassmemorial.org

M. Sigman
Department of Surgery (Urology), Warren Alpert School of Medicine of Brown University, Rhode Island Hospital and The Miriam Hospital, Providence, RI, USA

A. Zini, A. Agarwal (eds.), *A Clinician's Guide to Sperm DNA and Chromatin Damage*, https://doi.org/10.1007/978-3-319-71815-6_24

24.2 Inherent Issues

In its most basic form, a diagnostic test should provide information that affects patient management. A successful test should ideally direct management or at least provide valuable information on prognosis. In this case, a sperm DNA test should reliably differentiate a fertile from an infertile man or provide a reliable, clinically relevant prognosis for intercourse or ART success. Not all DNA fragmentation is pathologic. Some DNA nicking occurs as part of the normal process of winding and unwinding DNA, and a certain amount of single-stranded DNA breaks may be repaired by the oocyte [5]. Current assays do not selectively differentiate clinically important DNA fragmentation from clinically insignificant fragmentation. Perhaps for this reason, although DNA damage has been associated with male infertility, fertile men also possess detectable levels of DNA damage [6]. Therefore, upper threshold levels are set, but a portion of fertile men fall over the threshold, and a portion of infertile men fall under the threshold.

The task of proving the clinical usefulness of sperm DNA testing is made more difficult by the significant heterogeneity present in the tests and thresholds. As discussed further in other chapters of this book, several assays exist to measure sperm DNA and chromatin damage. Each assay measures different aspects of sperm DNA and chromatin integrity, and some have undergone more rigorous testing than others. Furthermore, even using the same assay, sample preparation, handling, and conditions can significantly impact the final test results. Finally, not all assays have standardized and clinically relevant threshold values for the upper normal level. For these reasons, it is difficult to combine studies and make broad conclusions. Additionally, most of the evaluable studies on sperm DNA tests have poorly controlled clinical parameters including female factors, female age, and number of embryos transferred, making the ability to draw clinical conclusions difficult.

Sperm DNA integrity testing determines the percentage of cells with DNA fragmentation or chromatin defects. As this implies, not all sperm within a sample have high levels of fragmentation. Animal models have provided strong evidence that sperm DNA fragmentation is highly correlated with fertility potential and even pregnancy loss [7]. However, these experiments may or may not translate to equivalent clinical effects because unlike sperm DNA damage in humans, DNA damage in animal models is induced experimentally and is present in all spermatozoa [7]. Most assays to evaluate sperm DNA integrity involve treatment of the sample (e.g., DNA-binding dye) which makes the individual sperm unusable for advanced reproductive techniques (ART). This necessitates that the sample used for testing is different than the one utilized for ART. Put another way, current tests do not provide information on the DNA status of individual sperm used to fertilize ova.

Although to what degree is debated, some intraindividual variability exists in these tests over time. Therefore, it can be difficult to extrapolate results from a single test to future attempts at intercourse or ART. A study examined a group of couples undergoing IUI, IVF, or ICSI for more than one cycle. In those couples with an initial normal DNA fragmentation, tests in subsequent cycles showed DNA

fragmentation above the threshold in 15%. Additionally, for those with initially high DNA fragmentation scores, 37% were found to have normal scores on subsequent testing [8]. A more recent analysis in 2011 again found a high intraindividual coefficient of variation for DNA fragmentation index utilizing a sperm chromatin structure assay. In this study 11% of patients identified as being in the normal category were reassigned to the high fragmentation category in subsequent testing. Additionally, 4.4% of those identified as high DNA fragmentation initially were subsequently categorized as normal [9]. This alone would make it difficult to counsel a couple to make a significant management decision, for example, not attempting intercourse and moving straight to advanced reproductive techniques, based on an initial positive result.

The test is also limited by the fact that no proven treatment exists. Sperm DNA fragmentation has been associated with numerous environmental toxins and exposures. However, testing fails to differentiate different exposures or provide clinical information beyond what is known without testing, i.e., that one should try to avoid these exposures if possible. For example, although the DNA fragmentation percentage may be higher in a patient who smokes, one hardly needs a test to recommend that a patient stop smoking [10]. Although limited studies suggest oral antioxidant treatment decreases sperm DNA fragmentation, no convincing data exists for any treatment to improve pregnancy rates related to DNA fragmentation. Therefore, diagnosing a patient with high DNA fragmentation at this time doesn't allow for a proven treatment or intervention.

Finally, in the current healthcare environment, more attention is drawn to efficient use of resources and containment of costs. New diagnostic tests need not only to meet the burdens of sensitivity, specificity, and clinical relevance, but they must also be cost-effective.

24.3 Evidence-Based Utility

24.3.1 Intercourse

One possible arena for sperm DNA testing is in the couple planning for first-time pregnancy. A 2008 meta-analysis showed a strong association between sperm DNA damage and failure to achieve a natural pregnancy with a combined odds ratio of 7.15 [11]. In the two included studies with a median pregnancy rate of 64%, the median positive predictive value of the assay was 73%, and the median negative predictive value was 68% [12, 13]. An odds ratio of 7.15 sounds great, and this indication is often stated to be an area of greatest value for sperm DNA testing. However, when one considers the prevalence of infertility in the study populations, the clinical value is more questionable. In one of the most commonly quoted studies included, 25.6% of patients failed to achieve pregnancy. Of those patients, the sperm DNA test was abnormal in only 6.2% [12]. The sensitivity of the assay was only

19%, meaning that the test failed to identify infertility in four out of five infertile patients. Also notable was that out of the 132 patients tested, only 6 (3.7%) were found to have high DNA fragmentation and did not achieve pregnancy. This means that 96.3% paid for a test that didn't provide any benefit or guidance. Most worrisome, out of all the patients with an abnormal test, 40% still achieved pregnancy. Therefore, it would be difficult to counsel even those patients who did have an abnormal test not to try pregnancy by intercourse since they would still have a 40% success rate. A test that proves diagnostic to very few patients and provides limited prognostic ability at best to those for whom it is positive would be unlikely to change management and be clinically useful.

An additional study published in 2010 compared sperm parameters and DFI values between fertile and infertile men and found that a higher DNA fragmentation index was more common in the group of infertile men than in the fertile group. Thus, the odds ratio of being in the infertile group increased as the DFI increased. Of note, 49% of infertile men and 10% of fertile men had DFI values of >20%. As a diagnostic test, the sensitivity was only 51% with a specificity of 89%, indicating again that while there is an association between higher DFI and infertility, it falls far short of being a good diagnostic test that should be routinely used for couples trying to conceive. Moving the threshold to 30% increased the specificity (fewer false-positive results) to 96%, but sensitivity dropped to 21% [14].

Finally, the sperm DNA test is most accurate when used during the month of attempted conception. In both the studies of pregnancy by intercourse, predictive ability was best if the test was done close to the time of intercourse and performed more poorly when it was performed further ahead of time. This makes the assay impractical for couples attempting conception by intercourse each month. Take, for example, a couple newly considering pregnancy. If diagnosed with high levels of fragmentation, the sample could differ the following month, and they would still have a reasonable chance of conception. Therefore, the couple should continue to attempt conception by intercourse for 12 months just as they would if they had never had the test performed rendering the test clinically useless in this scenario.

24.3.2 Intrauterine Insemination

Another possible indication for sperm DNA testing would be in the infertile couple who has failed intercourse and is determining the utility of IUI versus advancing to IVF or ICSI. A number of studies have found an association between sperm DNA damage [15, 16] and lower IUI pregnancy rates, but in only one study was an odds ratio able to be calculated [17]. In this study, an odds ratio of 9.9 was derived, and a PPV of 97% and a NPV of 24% were calculated. However, the sensitivity of the assay was only 20.7%, meaning that the test again failed to identify four out of five couples that did not conceive by IUI. Out of all samples tested, abnormal results were obtained in only 17%, but 80% of patients didn't go on to conceive with IUI. The test would have to be performed on 83.4% of people for whom it would

have no diagnostic or prognostic value in order to benefit only 16.6% of people. In this study, the sample used for IUI was the same sample utilized for testing raising concern about the accuracy of testing performed remotely. In clinical practice, there would need to be time after the test resulted in order for it to affect management decisions. Additionally, this is based on a single study. More studies are needed for a proper statistical evaluation and would certainly be necessary before adding sperm DNA testing to routine clinical practice.

24.3.3 IVF and ICSI

The literature regarding sperm DNA damage and its effect on IVF/ICSI outcome is controversial at best, and numerous studies and a number of recent meta-analyses have been performed. Each meta-analysis reaches slightly different conclusions suggesting that if any difference does exist in IVF/ICSI outcomes between men with high and normal levels of DNA damage, it is likely slight and very unlikely to be clinically significant enough to warrant the additional cost of testing.

In one of the earliest meta-analyses, Li et al. concluded that the SCSA assay had no significant effect on the chance of clinical pregnancy after IVF or ICSI treatment and that the TUNEL assay was associated with significant decreases in the chance of IVF clinical pregnancy but was not associated with changes in IVF fertilization, ICSI fertilization, or ICSI clinical pregnancy [18]. A total of eight articles met inclusion/exclusion criteria at that time (five utilizing TUNEL assay and three utilizing SCSA), and for the only significant finding, the clinical pregnancy rate using IVF was 27.75% for those with positive sperm DNA testing and 43.11% for those with a negative test (RR 0.68, 95% CI 0.54–0.85, $p = 0.0006$). A meta-analysis published in 2008 included 13 studies in analysis and found a small but statistically significant association between sperm DNA integrity test results and pregnancy in IVF and ICSI cycles but concluded the difference was "not strong enough to provide a clinical indication for routine use of these tests in infertility evaluation of men" [19]. The studies included varied widely, and the sensitivity of the tests ranged from 6% to 71%, and the specificity ranged from 38% to 98%. The American Society for Reproductive Medicine performed a meta-analysis in 2013 and concluded that "existing data do not support a consistent relationship between abnormal DNA integrity and reproductive outcomes" [2]. Another meta-analysis of 21 articles in 2014 found no association between DFI and clinical pregnancy by IVF or ICSI [20].

In contrast, Zhao et al. performed a meta-analysis in 2014 where 16 studies met inclusion criteria. They found a significant decrease in pregnancy rates in patients with high DNA damage using all techniques combined (IUI/IVF/ICSI) with a small odds ratio of 0.81 (95% CI 0.70–0.95; $p = 0.008$). When stratified by a type of procedure, an association was found with IVF but not ICSI [21]. A meta-analysis in 2016 also concluded that "there is sufficient evidence in the existing literature suggesting that sperm DNA damage has a negative effect on clinical pregnancy following IVF and/or ICSI treatment" [22]. This included 41 studies in the analysis and

found a combined odds ratio of all studies (IVF, ICSI, and IVF/ICSI combined) utilizing a random effects model to be 1.84 (95% CI 15–2.27, $p = 0.0001$). Notably, this robust effect was only noted for studies utilizing the TUNEL, comet, and SCD assays. No effect was noted in the studies utilizing the SCSA assay. The inclusion of the SCD and comet assays differentiates this analysis from the ASRM evaluation which primarily included studies utilizing SCSA and TUNEL assays. This points out that the prognostic significance may very well depend on the type of assay utilized. This issue needs to be clarified since assays such as the SCSA are the most commonly employed ones in clinical practice. Until more data is accumulated, the value of these tests for IVF and ICSI remains questionable. In addition, even if the assays are used to predict lower ART success, management is not affected since couples will still proceed with ICSI as the test does not predict inability to conceive but a slightly lower pregnancy rate by ART in those with high DNA fragmentation.

24.3.4 Pregnancy Loss

A theoretical risk exists that successful fertilization with DNA-damaged sperm may cause de novo mutations in the offspring, despite the ability of the oocyte and embryo to repair this DNA damage [5]. No relationship has been found between level of sperm DNA fragmentation and characteristics of children born after ART [23]. In addition, current testing does not allow for evaluation of the DNA integrity in the individual sperm that is utilized for ART.

However, the relationship between sperm DNA fragmentation and recurrent pregnancy loss has been examined. An early meta-analysis looked at the association between high DNA fragmentation and pregnancy loss and included 11 studies. It found that sperm DNA damage was statistically significantly associated with pregnancy loss when IVF/ICSI studies were combined with an odds ratio of 2.48 (95% CI 1.52–4.04, $p = <0.0001$). With an overall pregnancy loss rate of 18%, the median positive predictive value was 37%, and the median negative predictive value was 90% [24]. A more recent meta-analysis included 14 publications and found a significant association between DNA damage and miscarriage rate with a slightly lower combined OR of 2.28 (95% CI 1.55–3.35, $p = <0.001$) for IVF/ICSI studies combined [21].

The major issue is what clinically one would do with these results, other than cause anxiety, which in itself has been implicated in poor pregnancy outcomes [25]. Take, for example, a couple considering IVF/ICSI. Using current data, those with a negative test would still have a pregnancy loss rate of 10% but would be reassured. In fact, 60% of all pregnancy loss cases would have a negative test (sensitivity 40%). However, those with a positive test would have a 37% chance of pregnancy loss. This means that 63% of couples with a positive test would still go on to have a viable, term pregnancy with IVF/ICSI. One can hardly tell a couple not to proceed to IVF/ICSI because they have high DNA fragmentation if they have a two-thirds chance that any pregnancy will go to term. If data on effective treatments for DNA

fragmentation accumulate and clearly show a positive effect, this may be the one area where the test could be useful in this very selected population. However, testing in its current state is not clinically useful for this concern.

24.4 Conclusion

As a routine test in the infertile couple, sperm DNA testing adds expense to the healthcare system and does not provide a clinical benefit for most couples. The techniques and thresholds are not standardized, and the results are variable over time. With suboptimal sensitivity and specificity, the tests do not differentiate clinically significant from insignificant fragmentation and cannot evaluate individual sperm used for ART. Finally, with no proven treatment, the test fails to change management. Despite the potential, at this point, DNA fragmentation testing does not meet the criteria of a clinically useful diagnostic test in the evaluation of the infertile male.

References

1. Guzick DS, et al. Sperm morphology, motility, and concentration in fertile and infertile men. N Engl J Med. 2001;345(19):1388–93.
2. Practice Committee of the American Society for Reproductive Medicine. The clinical utility of sperm DNA integrity testing: a guideline. Fertil Steril. 2013;99(3):673–7.
3. Barratt CL, et al. Sperm DNA: organization, protection and vulnerability: from basic science to clinical applications—a position report. Hum Reprod. 2010;25(4):824–38.
4. American Urological Association. The optimal evaluation of the infertile male: best practice statement reviewed and validity confirmed; 2011. Accessed 1 Aug 2016. Available from: http://www.auanet.org/education/guidelines/male-infertility-d.cfm
5. Ahmadi A, Ng SC. Fertilizing ability of DNA-damaged spermatozoa. J Exp Zool. 1999;284(6):696–704.
6. Zini A, et al. Correlations between two markers of sperm DNA integrity, DNA denaturation and DNA fragmentation, in fertile and infertile men. Fertil Steril. 2001;75(4):674–7.
7. Fernandez-Gonzalez R, 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(4):761–72.
8. Erenpreiss J, et al. Intra-individual variation in sperm chromatin structure assay parameters in men from infertile couples: clinical implications. Hum Reprod. 2006;21(8):2061–4.
9. Oleszczuk K, Giwercman A, Bungum M. Intra-individual variation of the sperm chromatin structure assay DNA fragmentation index in men from infertile couples. Hum Reprod. 2011;26(12):3244–8.
10. Potts RJ, et al. Sperm chromatin damage associated with male smoking. Mutat Res. 1999;423(1–2):103–11.
11. Evenson DP, Wixon R. Data analysis of two in vivo fertility studies using sperm chromatin structure assay-derived DNA fragmentation index vs. pregnancy outcome. Fertil Steril. 2008;90(4):1229–31.
12. Evenson DP, et al. Utility of the sperm chromatin structure assay as a diagnostic and prognostic tool in the human fertility clinic. Hum Reprod. 1999;14(4):1039–49.

13. Spano M, et al. Sperm chromatin damage impairs human fertility. The Danish first pregnancy planner study team. Fertil Steril. 2000;73(1):43–50.
14. Giwercman A, et al. Sperm chromatin structure assay as an independent predictor of fertility in vivo: a case-control study. Int J Androl. 2010;33(1):e221–7.
15. Duran EH, et al. Sperm DNA quality predicts intrauterine insemination outcome: a prospective cohort study. Hum Reprod. 2002;17(12):3122–8.
16. Muriel L, et al. Value of the sperm chromatin dispersion test in predicting pregnancy outcome in intrauterine insemination: a blind prospective study. Hum Reprod. 2006;21(3):738–44.
17. Bungum M, et al. Sperm DNA integrity assessment in prediction of assisted reproduction technology outcome. Hum Reprod. 2007;22(1):174–9.
18. Li Z, et al. Correlation of sperm DNA damage with IVF and ICSI outcomes: a systematic review and meta-analysis. J Assist Reprod Genet. 2006;23(9–10):367–76.
19. Collins JA, Barnhart KT, Schlegel PN. Do sperm DNA integrity tests predict pregnancy with in vitro fertilization? Fertil Steril. 2008;89(4):823–31.
20. Zhang Z, et al. Sperm DNA fragmentation index and pregnancy outcome after IVF or ICSI: a meta-analysis. J Assist Reprod Genet. 2015;32(1):17–26.
21. Zhao J, et al. Whether sperm deoxyribonucleic acid fragmentation has an effect on pregnancy and miscarriage after in vitro fertilization/intracytoplasmic sperm injection: a systematic review and meta-analysis. Fertil Steril. 2014;102(4):998–1005 e8.
22. Simon L, et al. A systematic review and meta-analysis to determine the effect of sperm DNA damage on in vitro fertilization and intracytoplasmic sperm injection outcome. Asian J Androl. 2017;19(1):80–90.
23. Bungum M, et al. Spermatozoa DNA damage measured by sperm chromatin structure assay (SCSA) and birth characteristics in children conceived by IVF and ICSI. Int J Androl. 2012;35(4):485–90.
24. Zini A, et al. 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(12):2663–8.
25. Terzioglu F, et al. The effect of anxiety and depression scores of couples who underwent assisted reproductive techniques on the pregnancy outcomes. Afr Health Sci. 2016;16(2):441–50.