Is Sperm DNA Integrity Assessment Useful?

**YES**

Tests of sperm DNA integrity have been used increasingly in the evaluation of the infertile man. These tests have been developed to further our understanding of spermatogenesis and sperm function, and to potentially provide a more accurate infertility diagnosis than by evaluation of standard sperm parameters alone. Conventional sperm parameters such as sperm concentration, motility and morphology exhibit a high degree of biological variability and are only fair measures of fertility potential. Sperm DNA integrity tests have also been developed in the hope that they may predict pregnancy outcome after assisted reproductive technologies (ARTs), as conventional sperm parameters are poor predictors of ART outcomes.

Animal models have played a major role in providing strong evidence that sperm DNA fragmentation is highly correlated with male fertility potential. Moreover, experimental studies have demonstrated that sperm DNA damage is associated with poor reproductive outcomes (lower pregnancy rates, chromosomal abnormalities, pregnancy loss, reduced longevity and birth defects) after ARTs. These experiments have raised some concerns regarding the use of DNA damaged sperm in the context of ARTs. However, these observations 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.

Several assays have been developed to measure sperm DNA and chromatin damage. The most common are SCSA (sperm chromatin structure assay), COMET (single cell gel electrophoresis) assay, TUNEL (Terminal deoxynucleotidyl transferase-mediated dUTP Nick End-Labeling) assay and SCD (sperm chromatin dispersion) assay. It is important to consider that some assays have undergone more rigorous testing than others, that different assays measure different aspects of sperm DNA and chromatin integrity, and that these assays do not selectively differentiate clinically important from insignificant damage. Moreover, sample preparation and handling, and assay conditions (eg accessibility of the dye or enzyme to the sites of damaged DNA) can impact the final test results. To provide clinically relevant information, an upper normal level (cut-off) of the percentage of cells with DNA fragmentation or chromatin defect has been well defined for SCSA, TUNEL and COMET assays but not for others. Clinical samples with assay results above the established threshold are considered to have high levels of DNA damage.

A large number of clinical studies have clearly shown that infertile men have substantially higher levels of sperm DNA damage than fertile men and some specific clinical conditions have been associated with a higher prevalence of elevated sperm DNA fragmentation test. For example, advanced paternal age, varicocele, gonadotoxic (eg cancer) therapy, genital tract infection, spinal cord injury and febrile illness have been associated with high levels of sperm DNA damage. As such, it may be reasonable to test men with these conditions whether they are presenting for first pregnancy planning or prior to initiating ARTs. Moreover, several prospective studies indicate that sperm DNA damage in men with unknown fertility status is associated with a lower probability of conception (odds ratio ~7) and a prolonged time to pregnancy, and that sperm DNA testing is a better predictor of pregnancy than conventional sperm parameters in this context.

A systematic review of studies correlating sperm DNA test results and reproductive outcomes after ARTs indicated that sperm DNA damage is associated with lower intrauterine insemination (IUI) (odds ratio ~9) and conventional in vitro fertilization (IVF) pregnancy rates (odds ratio ~1.6–1.9) but does not impact intracytoplasmic sperm injection (ICSI) pregnancy rates. However, the widespread clinical application of sperm DNA tests in predicting IUI and IVF pregnancy has not been firmly established despite an already large number (40 to 50) of clinical studies because most studies are relatively small (each study has reported on roughly 100 to 200 ART cycles) and the study characteristics are heterogeneous.

During the last decade several clinicians have observed a higher rate of spontaneous pregnancy...
loss in men with sperm DNA damage, and a recent systematic review suggests that this damage is clearly associated with an increased risk of pregnancy loss (after an established natural and IVF or ICSI pregnancy). Although the mechanism(s) responsible for the pregnancy loss is unknown, these data are cause for concern because similar results have been reported in experimental studies and there is uncertainty regarding the long-term reproductive outcomes (eg postnatal health) when a pregnancy is established with DNA damaged sperm. Furthermore, what is remarkable about these data is that, to date, no other sperm test has been linked to pregnancy loss and/or postnatal health.

In summary, the available data clearly indicate that sperm DNA damage is associated with male factor infertility and support sperm DNA testing as an adjunct to conventional semen analysis in couples with failed IVF or IUI, recurrent pregnancy loss and unexplained infertility, particularly if there is an additional clinical indication (eg advanced paternal age). Establishing the level of sperm DNA damage may help guide infertility treatment in these couples.

Ashok Agarwal
Center for Reproductive Medicine
Department of Urology
Cleveland Clinic
Cleveland, Ohio

NO

The value of a diagnostic test resides in its ability to provide information that assists or changes management. As more attention focuses on the cost of health care, clinicians need to give added thought before ordering diagnostic tests such as assessment of sperm DNA integrity, an assay promoted as an important adjunct in the assessment of the infertile couple. Sperm DNA undergoes a compaction process that protects the DNA from damage. While defects in sperm DNA integrity may take the form of chromat in tertiary structural defects, nuclear protein defects or fragmentation of the DNA backbone, most studies have examined fragmentation.

Some DNA breaks normally occur during spermatogenesis but an excess of fragmentation has been associated with infertility. The assays that measure sperm DNA fragmentation indicate the percent of sperm demonstrating fragmentation with common thresholds between 20% and 30% of sperm with fragmentation. Those with fragmentation values higher than this are associated with worse reproductive outcomes. Current tests do not differentiate clinically significant from insignificant fragmentation and do not provide information on the DNA status of individual sperm used to fertilize ova.

Despite more than a decade of use, significant controversy remains about the value and role of the test in the treatment of the infertile male. There is little disagreement that higher levels of sperm DNA fragmentation are found in men with a variety of conditions including infertility; exposure to chemotherapy, radiation therapy and air pollution; and smoking. However, this association alone is not sufficient reason to use the test in the clinical scenario.

What do the data show? Pregnancy rates by intercourse are lower for men with high levels of DNA fragmentation. Similar findings have been found for IUI and IVF but not for ICSI. In addition, miscarriage rates may be higher for those with high levels of sperm DNA fragmentation.

While there is an association between increased sperm DNA fragmentation and pregnancy intercourse, studies documenting this are limited. Pregnancy rates for men with abnormal sperm DNA fragmentation are as high as 40% with no clear cutoff. Individual assay results may vary significantly and thus, the test is more accurate when used during the month of attempted conception, which makes this assay impractical for couples attempting conception by intercourse. Couples with high levels of fragmentation should continue to attempt conception by intercourse for 12 months just as they would if they had never had the test performed.

Data on IUI are limited and conflicting. Some studies have found significantly lower pregnancy rates for men with high levels of fragmentation but other studies have found no relationship. Because the IUI data are limited and largely not replicated, it is unclear if the results from studies from 1 institution are even applicable to the general population. For IVF, meta-analyses have generally shown a poor predictive ability with only a minimal effect on pregnancy rates (approximately 5% difference). In general, pregnancy rates by ICSI have not correlated fragmentation levels, indicating this test has no role in those couples undergoing ICSI.

Another aspect to examine when evaluating use of sperm DNA testing is the implications of abnormal results for management. Because of the association of smoking with decreased fecundity, one does not need DNA fragmentation testing to recommend that patients stop smoking. While limited studies suggest oral antioxidant treatment decreases sperm DNA fragmentation, there are no convincing data that these treatments improve pregnancy rates related to decreased DNA fragmentation.

Despite considerable research, a lack of standardization of techniques and thresholds remains between assays and often between laboratories. While it is well known that the standard semen
analysis is an imperfect test of fertility, sperm DNA fragmentation testing is not a replacement. As a test to diagnose infertility, the sensitivity of sperm DNA fragmentation is extremely low because most couples who are unable to conceive have normal levels of sperm DNA fragmentation. As a routine test in the infertile couple, the assay adds expense to the health care system and does not benefit most couples. Practice guidelines from the American Urological Association and the American Society of Reproductive Medicine recommend against the routine use of sperm DNA testing.7,8

At this point, sperm DNA fragmentation testing does not fulfill the criteria of a useful diagnostic test in the evaluation of the infertile male. The techniques and thresholds are not standardized; test characteristics, specifically sensitivity and specificity, remain suboptimal for a diagnostic test; and the tests do not differentiate clinically significant from insignificant fragmentation and cannot evaluate individual sperm used for fertilization. While most people agree that excess sperm DNA fragmentation is not a good thing, this assay needs to be dramatically improved before it should be considered useful as a routine assay.

Mark Sigman
Division of Urology
Brown University
Providence, Rhode Island

REFERENCES