Commentary

Commentary: the value of testing sperm DNA fragmentation in infertile men

Damayanthi Durairajanayagam

Discipline of Physiology, Faculty of Medicine, Sungai Buloh Campus, Universiti Teknologi MARA, Selangor, Malaysia

Correspondence to: Damayanthi Durairajanayagam. Discipline of Physiology, Faculty of Medicine, Sungai Buloh Campus, Universiti Teknologi MARA, Jalan Hospital, 47000 Sungai Buloh, Selangor, Malaysia. Email: damayanthi.d@gmail.com.

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Over the past 20 years, the necessity and usefulness of clinically testing for DNA fragmentation in spermatozoa has been increasingly researched and debated. Studies have shown that sperm with impaired DNA integrity are related to prolonged time to pregnancy, significantly lower conception rates both naturally and after intrauterine insemination (IUI), as well as increased pregnancy loss. High levels of sperm DNA damage have also been linked to poor integrity of the embryonic genome and impaired embryo development. However, both scientists and clinicians remain divided as to whether the potential information that the measurement of immature chromatin or fragmented DNA in spermatozoa may offer, in addition to that provided by routine semen analysis, is actually relevant in a clinical setting. In a recent paper, a select team of authorities in the field of male infertility recommended a practice guideline pertaining to the clinical value of sperm DNA fragmentation assessment (1). The group employed an evidence-based approach to describe clinical scenarios that most warranted sperm DNA testing as well as the management of patients with high sperm DNA fragmentation.

In the initial part of the paper, the authors examined the different tests used for measuring sperm DNA fragmentation, comprising those that (I) measure the extent of DNA damage following denaturation [sperm chromatin structure assay (SCSA), sperm chromatin dispersion (SCD)/Halo test, acridine orange assay, Comet assay]; (II) directly measure single- (TUNEL) or double-stranded (TUNEL, Comet) breaks in DNA; and (III) measure abnormalities in the sperm chromatin structure/integrity [aniline blue staining, toluidine blue staining, chromomycin A_3 (CMA_3) staining)]. With the availability of various methods for assessing the levels of DNA integrity, due consideration should be given as to whether these tests (I) reveal DNA damage of the similar type as the target sites for each test may not correspond precisely, (II) are sensitive, reliable and comparable, and (III) have standardized protocols and are reproducible (2).

The authors reported that commonly used sperm DNA fragmentation testing methods such as the TUNEL and Comet assays offer an advantage as they are sensitive, reliable or reproducible and require comparatively fewer sperm. However, depending on the method of assessment, the possible setbacks of running sperm DNA fragmentation tests are high inter-laboratory (e.g., acridine orange assay, aniline blue staining) or inter-observer (e.g., toluidine blue staining, CMA_3 staining, SCD, Comet assay) variability, costly equipment and the need for highly skilled technical expertise (e.g., SCSA test using flow cytometry). Lately however, a comprehensive protocol along with quality control steps for measuring sperm DNA fragmentation by TUNEL assay using a user-friendly bench top flow cytometer was reported (3). These researchers presented a simple, reproducible and economical method to determine sperm DNA fragmentation, with which they were able to obtain a very high specificity level (91.6%) and positive predictive value (90%) at a cutoff point of 16.8%. In a meta-analysis measuring the diagnostic accuracy of sperm DNA fragmentation test in infertile males, the TUNEL assay was

reported to have higher precision compared to the Comet and SCD assays respectively (4).

In the subsequent section of their paper, the experts proposed several different scenarios in which sperm DNA fragmentation testing would be recommended. These cases were specifically selected to illustrate typical clinical scenarios that fertility specialists often come across in their practice, namely scenario 1: varicocele; scenario 2: a combination of unexplained infertility, recurrent pregnancy loss and IUI failure; scenario 3: IVF and/or ICSI failure; and scenario 4: normal or borderline semen analysis with risk factors. Within each scenario, the experts provided clear justifications for testing sperm DNA fragmentation in the respective group of patients as well as provided grading of these recommendations based on the quality of available evidence. The quality of evidence for scenarios 1, 2 and 4 were based on studies of poorer quality, while that of scenario 3 were based on a mix of studies that were well-designed as well as those of poorer quality.

Undoubtedly, there are limitations to the current pool of published studies regarding the value of DNA fragmentation testing. Among them is the poor accuracy and reproducibility of sperm DNA fragmentation assessments due to lack of standardized protocols for the different tests, intra-assay variability, inter-observer variability, and variability in the chosen cut-off values. Studies relating the value of DNA fragmentation testing to the outcome of assisted reproduction, for example, have weaknesses such as inclusion of couples with wide heterogeneity (e.g., male factor infertility, past failed assisted reproductive technology (ART), currently on ART) and varied timing of when the DNA fragmentation tests were performed (i.e., before, during or after the ART procedure) (5). Well-designed, adequately powered studies with live birth outcomes will certainly help increase the quality of evidence, provided there is sufficient allocation of funding and time directed towards achieving this (6). Perhaps with the accumulation of more robust clinical evidence, the diagnostic/prognostic value of sperm DNA fragmentation testing in infertile males would increase.

In the current practice guideline paper, the experts recommend the use of sperm DNA fragmentation testing for the following purposes: (I) to aid in the selection of candidates who would benefit the most from varicocelectomy based on their clinical varicocele grade and semen parameters; (II) to offer IVF/ICSI as an earlier option to infertile couples with recurrent spontaneous abortion or who were planning for IUI; (III) to serve as a prognostic test for subsequent ART cycles in patients with recurrent ART failure; and (IV) to strengthen lifestyle modification measures and monitor the patient's response to such interventions in order to better predict their fertility potential. Based on these recommendations, it seems that the additional information offered by sperm DNA fragmentation test results would be inclined to change current practice. Thus, this timely practice guideline serves as an important reference point for urologists and fertility practitioners on when sperm DNA fragmentation testing would probably best serve the patient.

Spermatozoa are complex cells that must successfully undergo a series of events before completing the fertilization stage. As such, it is very probable that more than one test would be required in order to obtain the information necessary for successful prediction of the male fertility potential (7). At present, semen analysis (which is not without its own limitations) continues to serve as the initial, fundamental laboratory evaluation of the infertile male. While the analysis of semen offers information on the functionality of the seminiferous tubules, epididymides, and accessory sex glands, it does not provide potentially beneficial knowledge about sperm dysfunction (8). Sperm DNA fragmentation testing evaluates the genetic content and helps detect molecular abnormalities of the male gamete. This knowledge is critical as the paternal genome impacts fertilization and the quality of early embryonic development (9). Therefore, sperm DNA fragmentation testing is likely to provide a more accurate analysis of the male fertility potential, and when supplemented with conventional semen analysis, could improve its clinical value.

This novel practice guideline examines the various methods of testing sperm DNA integrity and highlights the potential benefit of incorporating sperm DNA fragmentation testing as a treatment strategy for specified groups of patients. It is a significant addition to the existing body of literature on the impact of sperm DNA integrity on the male fertility potential. The panel of experts endorses the clinical utility of sperm DNA fragmentation testing in the evaluation of infertile couples.

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Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

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