

Sperm DNA fragmentation testing: ready for prime time?

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The authors present fair evidence indicating that sperm DNA fragmentation (SDF) testing is a useful diagnostic tool in male fertility evaluation. As such, they propose that SDF should be included in the evaluation of male factor fertility along with the semen analysis, and provide data for recommendation for testing under specific clinical scenarios (1). Among various available tests, the authors highlight two assays for SDF testing, the TUNEL technique (sensitive, reliable with minimal inter-observer variability, but requiring standardization between laboratories) and the SCSA (reliable but requiring more expensive instrumentation and skilled technicians). The authors quote a limited number of studies that show that SDF levels can predict the likelihood of natural pregnancy, and that higher SDF is associated with lower IUI pregnancy rates, and with lower embryo quality and pregnancy rates in the IVF/ICSI scenario. Within this context some other points warrant discussion, as elaborated in earlier publications (2-4).

How should we assess SDF with clinically meaningful tests?

Current assays to detect DNA damage in ejaculated sperm do not define the nature of the DNA lesions (5). Moreover, there is no agreement on which assay provides data that can lead to individualized management in the clinical scenario. The TUNEL assay directly measures single- and double strand DNA damage in human sperm, without the use of previous DNA denaturation steps, and as such should be recommended as a test that measures ‘real’ DNA status (as compared to “susceptibility” to certain *in vitro* incubation conditions) (5-7).

Nevertheless, it has been reported that results of sperm DNA damage tests correlate to some extent (5). In addition, and importantly, these tests do not diagnose absolute numbers of DNA breaks and/or are not able to quantify the amount or type of DNA damage in individual sperm cells. Therefore, more research is needed to determine the best test used for screening for the presence of “clinically relevant” DNA damage.

Moreover, while analysis of DNA fragmentation in the sperm populations present in the raw semen (liquefied and tested in washed or unwashed semen samples) is typically used for prediction of pregnancy in the natural or IUI setting, for ICSI the analysis of the separated elite sperm motile fractions (after gradient centrifugation or other technique) might provide better discriminatory power as those isolated spermatozoa will be the ones interacting with the egg (3,4).

Does sperm DNA damage result in dysfunctions of the male gamete?

In the human, the presence of sperm DNA damage has been associated with lower rates of *in vivo* conception, increased miscarriage, abnormal *in vitro* embryonic development, and untoward effects in offspring, including childhood cancer (7). In some animal species, although sperm with damaged DNA can successfully fertilize the oocyte (8), the use of DNA-damaged sperm reduces the rate of implantation, embryo development and the number of offspring (9). It is noteworthy that different DNA lesions may produce dissimilar effects. In addition, trans-

generational consequences have been reported including growth restriction, premature aging, abnormal behavior, and development of mesenchymal tumors (10). Therefore, we trust that novel assays may lead us to better define the nature of the human sperm DNA lesions, and to select spermatozoa without DNA damage.

Can the human oocyte repair all DNA lesions carried by the fertilizing spermatozoon?

The cell DNA repair machinery consists of homologous recombination and non-homologous end joining (11). In the murine model, radiation-induced sperm DNA lesions was shown to induce damage that persisted for at least 7 days in the fertilizing sperm. And it was the competence of the oocyte DNA repair mechanisms that determined the risks for miscarriage and frequencies of offspring with chromosomal defects of paternal origin (12). One of the surveillance mechanisms that protects cells from double strand breaks uses histone γ H2AX, an enzyme that recognizes and phosphorylates proteins at the break points. Using a human-murine heterologous ICSI model and γ H2AX, it was possible to estimate the absolute amount of double strand breaks after ICSI and remodeling of the sperm chromatin in the oocyte. This points to possible avenues to establish a sensitive single-cell analysis to study questions on sperm DNA integrity and the oocyte competence for repair in the human model (13).

To better address this issue, the types and intensity of DNA damage per sperm cell need to be further characterized. Moreover, it needs to be determined whether the oocyte competence for repair under natural conditions is similar to the one seen in oocytes following gonadotropin stimulation for IVF; because the possibility also exists that “dysfunctional” oocytes recovered after superovulation in IVF might have a compromised competence for DNA repair, therefore increasing risks for untoward effects.

How can we improve the selection of DNA intact sperm for clinical use in ICSI?

Micro-fertilization of oocytes via ICSI has become the method of choice in the IVF setting for a majority of clinical cases. At the time of ICSI, the embryologist selects the sperm to be injected based upon morphological features, as well as on the availability of the selected populations of highly motile spermatozoa. These selection methods do

not provide information about the possible inadvertent microinjection of spermatozoa with chromosomal aneuploidies and/or DNA fragmentation.

Other novel techniques are being incorporated for selection of mature spermatozoa for ICSI (14). Techniques currently being cited in the literature include the hyaluronic acid (HA) binding method based on the presence of a putative HA receptor (15), and sperm magnetic sorting with annexin V microbeads based on apoptotic markers such as the presence of externalized phosphatidylserine to the surface membrane of spermatozoa (16). The application of these methods has resulted in selection of high-quality sperm, with improved DNA integrity and cellular maturity. However, more clinical studies on safety and efficacy are needed before the implementation of these methods in ART (17). So far, none of these techniques results in the complete removal of DNA-damaged spermatozoa from the ejaculate. A major issue is that SDF evaluation in live cells is not possible with the techniques currently available. We have proposed that the evaluation of DNA integrity in morphologically normal spermatozoa after selection of the motile sperm (i.e., gradient centrifugation or swim up) is a better approach to evaluate the impact of SDF on ICSI outcome than the assessment of the total sperm population present after liquefaction in a washed or unwashed semen sample (18,19).

It is important to consider that the type and degree of sperm DNA damage (whether presence of adducts, or various degrees of single and double stranded DNA fragmentation, associated or not with genetic and/or epigenetic defects), resulting from direct oxidative damage, apoptosis, or other cause, can have a profound impact on clinical outcomes. Therefore, it will be critical to prevent the use of sperm cells with “invisible” damage in the ART setting.

To conclude, and in agreement with Agarwal *et al.* (1) the analysis of SDF has the potential to become a diagnostic tool for the evaluation of male factor fertility along with the basic semen analysis. Clinical threshold levels of SDF have been established for TUNEL and SCSA assays in unprocessed semen for natural pregnancy (20), for IUI (21,22) and for ART (19,23), but remain to be validated in larger studies. The SCSA and TUNEL assays provide data on different forms of sperm DNA damage integrity and cannot substitute for one another (24,25). The American Society for Reproductive Medicine has recently recognized the value of SDF testing but has not recommended its

routine use in the clinical setting (26). It is speculated that further studies designed to answer the unresolved issues posed herein will provide more powerful data to definitely establish the value of SDF tests in the initial steps of male infertility evaluation.

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Footnote

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

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