Commentary

Commentary on sperm DNA fragmentation testing clinical guideline

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The study of physiology of reproduction, particularly in mammalian species, experienced glorious times in the second half of the twentieth century. However, with the advent of intracytoplasmic sperm injection (ICSI), the decade of 1990 appeared to forecast the demise of translational research in male reproductive biology. In fact, in its original and subsequent work, Palermo and colleagues showed that ICSI bypassed the process of natural selection of sperm (1). Then, the prevailing hope became that the only need would be merely to obtain one acceptable spermatozoon. The optimism, based on an exponential increase of successful results, has been moderated in the last decade by results indicating that certain sperm characteristics have an impact on the outcomes of assisted reproduction (2). Recent basic research findings have radically transformed our understanding of the biology of fertilization (3), underscoring the importance of cytological processes, e.g. the timing of sperm acrosomal exocytosis (4), in addition to sperm-associated epigenetic factors (5), proteomics (6) and DNA structure (7,8).

The importance of sperm DNA structure

In the nucleus of somatic cells, DNA is condensed and packaged with histones into nucleosomes. Post-translation changes may modify histones, thus regulating the degree of DNA compaction and gene expression by reducing or increasing the access of transcription factors to DNA. Conversely, in male germ cells, histones are replaced by protamines, resulting in a much higher condensation of the DNA (9). This leads the sperm to be a relatively inactive cell from a transcriptional standpoint (10). The advantage of extensive compaction is that sperm DNA is highly stable and resistant to damage; in fact, protamination deficiencies are associated with a higher risk of sperm DNA damage (11).

DNA single- and double-strand breaks occur naturally to allow the unwinding of nucleosomes; these DNA strand breaks are normally repaired, thus preventing the persistence of DNA damage in mature spermatozoa. DNA repair defects during compaction and packaging may be the underlying pathogenesis (8). Another possible etiology may be associated with incomplete apoptosis occurring in abnormal sperm, which leads to the release of reactive oxygen species (ROS) inducing sperm DNA damage (12). ROS may also cause sperm DNA damage after the spermatogenic process has finished, e.g., during epididymal transit, as suggested by the increased levels of sperm DNA damage associated with longer abstinence periods (8). External risk factors include the exposure to environmental toxins, chemicals, radiation, drugs, smoking, and varicocele (13).

How to study sperm DNA damage?

It emerges clearly from several studies that sperm DNA damage is associated with decreased fertilization rates,

embryo quality and pregnancy rates, and increased rates of spontaneous miscarriage and childhood diseases (14). Numerous laboratory determinations are able to measure sperm DNA fragmentation (SDF); however, their clinical utility has remained controversial for years (2,7,8,15).

A recent guideline sheds light into the current indications of sperm DNA testing, and give evidence-based recommendations on the management of patients with increased SDF (16). The guideline was elaborated by a panel of five urologists (Ahmad Majzoub, Sandro C. Esteves, Edmund Ko, Ranjith Ramasamy, Armand Zini) and one andrologist (Ashok Agarwal) with expertise in male infertility, and provides urologists and reproductive specialists outside the expertise of genetics with a useful guideline for deciding when SDF testing could be of clinical value.

The guideline first summarizes the existing evidence on the eight available SDF tests. Staining tests using Acridine Orange (AO), Anilin Blue (AB), Toluidine Blue (TB) or Chromomycin A3 (CMA3), as well as sperm chromatin dispersion (SCD) or Halo test are rapid, simple and inexpensive tests; as limitations, they show high inter-observer variations undermining their reproducibility. The Single Cell Gel Electrophoresis or Comet assay has the advantage of requiring small sperm numbers, so that it can be done in samples with very low sperm count; also, it is sensitive and reproducible, yet it requires experience in the observer and has high inter-observer variability. The Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) test is relatively simple and requires standard laboratory equipment (e.g., optical or fluorescent microscopy), although it can also be performed using flow cytometry; it is sensitive (can be done on few spermatozoa), reliable, and has very low inter-observer variability; its main drawback is the lack of standardization between laboratories. Finally, Sperm chromatin structure assay (SCSA®) is a flow cytometry-based assay that is able to assess large numbers of spermatozoa rapidly and robustly; its limitation is the requirement for a flow cytometer and highly trained personnel.

Evidence-based Indications of SDF testing

Next, using illustrative, commonly encountered, clinical scenarios, Agarwal and colleagues analyze the indications for SDF testing. One major recommendation is its use in patients with clinical varicocele and borderline to normal semen parameters, where SDF testing is helpful in the selection of varicocelectomy candidates. In these patients, blood stasis resulting from the abnormal dilatation of the pampiniform vein plexus reduces testicular blood inflow leading to hypoxia and oxidative stress, which may cause DNA damage (17). A reduction of SDF has been observed systematically, using various SDF tests, after varicocelectomy (18). Furthermore, an improvement in SDF results in these patients has been associated with an increase in pregnancy rates. The authors of the guideline conclude that SDF is recommended (grade C: based on poor quality studies, i.e., retrospective, case series, or expert opinion) in patients with grade 2/3 varicocele with normal conventional semen parameters and in patients with grade 1 varicocele with borderline/abnormal conventional semen parameters (16).

When results of studies from couples with unexplained infertility, recurrent pregnancy loss, or intrauterine insemination (IUI) failure were analyzed, the authors observed that a high DNA fragmentation index could provide a possible explanation for recurrent spontaneous abortion (RSA) and IUI failure (16). Therefore, they find it reasonable to offer SDF testing to infertile couples with RSA or prior to initiating IUI (grade C recommendation).

Concerning SDF testing in patients with recurrent failure in assisted reproductive technologies (ART), the authors advocate for further research (16). However, SDF testing can provide useful prognostic information on subsequent ART cycles and also on the use of testicular rather than ejaculated sperm when oligozoospermia, high SDF and recurrent IVF failure are associated (recommendation grade B, based on well-designed prospective, cohort studies, or C).

Finally, the authors conclude that Infertile men should be offered SDF testing when there is evidence of exposure to pollutants or of modifiable lifestyle risk factors (16). The results may be helpful to reinforce the importance of lifestyle modification, predict fertility and monitor the patient's response to intervention (grade C recommendation).

In summary, the authors provide a useful, evidence-based clinical guideline with practice recommendations for performing SDF tests in male patients during fertility evaluation.

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Footnote

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

References

1. Palermo G, Joris H, Devroey P, et al. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet 1992;340:17-8.
2. Zini A, Bach PV, Al-Malki AH, et al. Use of testicular sperm for ICSI in oligozoospermic couples: how far should we go? Hum Reprod 2017;32:7-13.
3. Okabe M. The cell biology of mammalian fertilization. Development 2013;140:4471-9.
4. Buffone MG, Hirohashi N, Gerton GL. Unresolved questions concerning mammalian sperm acrosomal exocytosis. Biol Reprod 2014;90:112.
5. Sharma A. Transgenerational epigenetics: Integrating soma to germline communication with gametic inheritance. Mech Ageing Dev 2017;163:15-22.
6. Agarwal A, Bertolla RP, Samanta L. Sperm proteomics: potential impact on male infertility treatment. Expert Rev Proteomics 2016;13:285-96.
7. Ioannou D, Miller D, Griffin DK, et al. Impact of sperm DNA chromatin in the clinic. J Assist Reprod Genet 2016;33:157-66.
8. Bach PV, Schlegel PN. Sperm DNA damage and its role in IVF and ICSI. Basic Clin Androl 2016;26:15.
9. Rathke C, Baarends WM, Awe S, et al. Chromatin dynamics during spermiogenesis. Biochim Biophys Acta 2014;1839:155-68.
10. Miller D, Brinkworth M, Iles D. Paternal DNA packaging in spermatozoa: more than the sum of its parts? DNA, histones, protamines and epigenetics. Reproduction 2010;139:287-301.
11. Aitken RJ, Bronson R, Smith TB, et al. The source and significance of DNA damage in human spermatozoa; a commentary on diagnostic strategies and straw man fallacies. Mol Hum Reprod 2013;19:475-85.
12. Aitken RJ, De Iuliis GN. On the possible origins of DNA damage in human spermatozoa. Mol Hum Reprod 2010;16:3-13.
13. Sharma R, Biedenharn KR, Fedor JM, et al. Lifestyle factors and reproductive health: taking control of your fertility. Reprod Biol Endocrinol 2013;11:66.
14. Lewis SE, John Aitken R, Conner SJ, et al. The impact of sperm DNA damage in assisted conception and beyond: recent advances in diagnosis and treatment. Reprod Biomed Online 2013;27:325-37.
15. Esteves SC. Novel concepts in male factor infertility: clinical and laboratory perspectives. J Assist Reprod Genet 2016;33:1319-35.
16. Agarwal A, Majzoub A, Esteves SC, et al. Clinical utility of sperm DNA fragmentation testing: practice recommendations based on clinical scenarios. Transl Androl Urol 2016;5:935-50.
17. Cho CL, Esteves SC, Agarwal A. Novel insights into the pathophysiology of varicocele and its association with reactive oxygen species and sperm DNA fragmentation. Asian J Androl 2016;18:186-93.
18. Miyaoka R, Esteves SC. A critical appraisal on the role of varicocele in male infertility. Adv Urol 2012;2012:597495.

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