Commentary

Sperm DNA fragmentation: the evolution of guidelines for patient testing and management

Thomas B. Pool

Fertility Center of San Antonio, San Antonio, TX, USA

Correspondence to: Thomas B. Pool, PhD, HCLD (ABB), Scientific Director. Fertility Center of San Antonio, 4499 Medical Drive, Ste 200, San Antonio, Texas 78229 USA. Email: rpool@fertilitysa.com.

Comment on: 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.

Submitted Jan 30, 2017. Accepted for publication Feb 04, 2017.
doi: 10.21037/tau.2017.03.48
View this article at: http://dx.doi.org/10.21037/tau.2017.03.48

Sperm DNA fragmentation (SDF) represents a perplexing biological phenomenon that can, at an elevated level, interfere with either initiating and/or maintaining pregnancy. Although we are in our third decade of studying this problem, few investigators have attempted to assess the value of the various available assays for SDF with the specific aim of amalgamating them into practice guidelines for managing SDF. Agarwal, in collaboration with urological experts from North and South America, has done just that, employing a number of clinical scenarios involving SDF to produce some evidence-based, practical guidelines for patient management (1). But if in our third decade of investigation, why are management guidelines so difficult to come by for patients with elevated SDF? First, it should be emphasized that SDF is an exceedingly complex process involving not one, but a number of causative mechanisms that can generate a variety of insults to the integrity of sperm DNA. Sakkas and Alvarez (2) have described six main mechanisms that can damage both sperm nuclear as well as mitochondrial DNA. Included in this list are testicular apoptotic processes during spermatogenesis, aberrations during the events of chromatin re-modeling, induction by exogenous caspases and nucleases plus damage by chemo- and radiotherapy as well as environmental toxicants. Perhaps the most robust events occur in the epididymis in response to insult by reactive oxygen species. That epididymal and ejaculated sperm have higher amounts of DNA fragmentation than testicular sperm is now well documented (3-5). What is not known are the testicular events that render sperm labile to chemical radicals traversing the epididymis. Secondly, the insults can produce single-stranded breaks, double-stranded breaks and/or nucleotide damage. Not all are detected by the same tests, the different types of damage are repaired to different degrees by processes resident in the oocyte and thus, do not have the same prognosis. Likely though, it is the tests used to detect and measure SDF, along with their varying levels of complexity, that introduce the bulk of the conflicting data and conclusions common in the SDF literature.

As Agarwal *et al.* demonstrate in concise tabular form, there are at least eight tests for SDF, each with attendant advantages and disadvantages. The predictive value of these, assuming proper execution and quality control, depends upon the type of DNA damage, the percent of sperm with fragmentation, the extent of damage per sperm, whether there is combined fragmentation and nucleotide damage, whether the lesions affect exons or introns and the effectiveness of the oocytes in repairing DNA damage in zygote genomic DNA contributed by the fertilizing sperm (2). Of interest, SDF fails to affect the fertilizing capacity of sperm (6), so testing is performed to either visualize the state of sperm DNA via differential staining of fragmented versus intact DNA, to reveal the physical restrictions fragmentation places upon DNA dispersion, to measure differential electrophoretic mobility of intact versus fragmented DNA or measure nucleotide incorporation into DNA nicks. A very recent review and meta-analysis by Cissen *et al.* (7) examined the value of SDF measures in predicting the chance of ongoing pregnancy with *in vitro* fertilization (IVF) or

intracytoplasmic sperm injection (ICSI). Of 658 studies reviewed, 30 had data incorporated into the meta-analysis. In general, their results supported the conclusion that SDF tests had reasonable to good sensitivity but limited to low specificity, acknowledging that a wide variety of other factors may also affect IVF/ICSI production of ongoing pregnancy. The predictive value of sperm chromatin structure assay (SCSA) was determined from the analysis of 14 studies, of which 11 were prospective, whereas five were included for sperm chromatin dispersion test (SCD), nine for terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) and four for COMET, all of which were prospective. Whereas the TUNEL and comet assays had fair discriminatory capacity, SCSA and SCD had poor predictive value. Furthermore, meta-regression indicated that TUNEL, SCD and comet assays showed no predictive value between IVF and ICSI cases, a finding that differed from SCSD, which did. They conclude that SDF tests have limited capacity to predict the chance of pregnancy in assisted reproductive technology (ART) and are therefore not recommended for routine application to couples undergoing IVF/ICSI. Other studies are in conflict with Cissen *et al.* For example, Zhao *et al.* (8) saw a negative influence of SDF upon patients undergoing IVF but not ICSI in a meta-analysis that included nearly 3,000 couples.

Despite these controversial findings, SDF testing decidedly has its place in ART. It can direct the appropriate patient towards ICSI or testicular biopsy and testicular sperm extraction (TESE). It can well shed light upon the path for successful continued management in fertility therapy; that SDF can adversely influence embryogenesis, even in euploid embryos, is known (9-11). Agarwal *et al.* have been judiciously conservative in making their recommendations and have included only clinical scenarios where evidence allows. Additionally, they have not relied on just one test or measure of SDF. This is important in that different tests measure different aspects of DNA damage such as single-strand breaks, double-strand breaks and protamine loss. Although the strength of the evidence leading to the suggested guidelines is moderate to low, it is the complicated nature of the DNA fragmenting process and fragmented state, as well as the SDF testing, that makes it so and to date, it remains the best evidence available. Is there a clinical presentation where the urologist or andrologist would rely solely upon the results of SDF testing? Not according to these guidelines or other conclusions from the current literature. As the authors suggest, it is an additional tool, nothing more.

Acknowledgements

None.

Footnote

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

References

1. 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.
2. Sakkas D, Alvarez JG. Sperm DNA fragmentation: mechanisms of origin, impact on reproductive outcome, and analysis. Fertil Steril 2010;93:1027-36.
3. Ollero M, Gil-Guzman E, Lopez MC et al. Characterization of subsets of human spermatozoa at different stages of maturation: implications in the diagnosis and treatment of male infertility. Hum Reprod 2001;16:1912-21.
4. Greco E, Scarselli F, Iacobelli M et al., Efficient treatment of infertility due to sperm DNA damage by ICSI with testicular spermatozoa. Hum Reprod 2005;20:226-30.
5. Suganuma R, Yanagimachi R, Meistrich ML. Decline in fertility of mouse sperm with abnormal chromatin during epididymal passage as revealed by ICSI. Hum Reprod 2005;20:3101-8.
6. Aitken RJ, Gordon E, Harkiss D et al. Relative impact of oxidative stress on the functional competence and genomic integrity of human spermatozoa. Biol Reprod 1998;59:1037-46.
7. Cissen M, Wely MV, Scholten I, et al. Measuring sperm DNA fragmentation and clinical outcomes of medically assisted reproduction: a systematic review and meta-analysis. PLoS One 2016;11:e0165125.
8. Zhao J, Zhang Q, Wang Y, 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;102:998-1005.e8.
9. Shoukir Y, Chardonnens D, Campana A, et al. Blastocyst development from supernumerary embryos after

intracytoplasmic sperm injection: a paternal influence? Hum Reprod 1998;13:1632-7.
10. Seli E, Gardner DK, Schoolcraft WB, et al. Extent of nuclear DNA damage in ejaculated spermatozoa impacts on blastocyst development after in vitro fertilization. Fertil Steril 2004;82:378-83.
11. Borini A, Tarozzi N, Bizzaro D, et al. Sperm DNA fragmentation: paternal effect on early post-implantation embryo development in ART. Hum Reprod 2006;21:2876-81.

Cite this article as: Pool TB. Sperm DNA fragmentation: the evolution of guidelines for patient testing and management. Transl Androl Urol 2017;6(Suppl 4):S409-S411. doi: 10.21037/tau.2017.03.48

