Editorial

The importance of incorporating sperm DNA fragmentation testing in male infertility diagnostic routine

Sergio Garcia-Segura

Unit of Cell Biology and Medical Genetics, Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, Bellaterra, Catalunya, Spain

Correspondence to: Sergio Garcia-Segura. Unit of Cell Biology and Medical Genetics, Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, M Building, Can Domènech Avenue, Bellaterra 08193, Barcelona, Spain. Email: sergio.garcia.segura@uab.cat.

Comment on: Farkouh A, Salvio G, Kuroda S, *et al.* Sperm DNA integrity and male infertility: a narrative review and guide for the reproductive physicians. Transl Androl Urol 2022;11:1023-44.

Submitted Sep 08, 2022. Accepted for publication Oct 14, 2022.
doi: 10.21037/tau-22-572
View this article at: https://dx.doi.org/10.21037/tau-22-572

Infertility is a multifactorial disorder present in approximately 15% of couples in reproductive age, and male counterpart is known to exert an impact in about 50% of cases (1-3). Semen analysis provides relevant information for the diagnosis of the disease, but often only sperm motility and sperm count parameters are used for assessment, leading to approximately 1 in 4 infertile men do not obtain a clear diagnosis (4). In the last years, sperm DNA fragmentation (SDF) has been established as an interesting biomarker to improve semen analysis, as it has been a factor closely related to male reproductive health indicators (5-8). In fact, in the latest version of the World Health Organization (WHO) laboratory manual for the examination and processing of human semen (9), this parameter has been introduced as one of the recommended ones due to its potential, despite its technical limitations. Few fertility clinics currently perform SDF tests routinely, so specialists may have concerns about performing this test and its usefulness. The article by Farkouh and colleagues (10) discusses the pathophysiological aspects of SDF for assisting physicians in understanding the circumstances in which this parameter could be of clinical value, and proposes different analysis methods to guide physicians in incorporating this parameter into clinical practice, also providing relevant information for its interpretation and subsequent actions.

The causes of SDF are complex to define, since its origin is multifactorial and several factors probably act together. DNA breakage can occur throughout the life of the sperm, from spermatogenesis (where enzymatic damage or repair errors predominate), through the epididymis and vaginal tract [where it suffers high oxidative stress (OS)], to fertilization (where the oocyte's repair mechanisms may be defective) (8,11). As Farkouh *et al.* rightly commented (10), during spermatogenesis the enzymes responsible for creating and repairing breaks to generate genetic recombination between homologous chromosomes are the main protagonists in the generation of SDF. The roles of the Spo11 protein, which produces double-strand breaks (DSB), and the ataxia-telangiectasia mutated (ATM) protein kinase, which repairs the free ends produced by Spo11, creating chiasms, are notable (12). However, despite the fact that Farkouh *et al.* refers to the defective chromatin compaction as a cause of SDF, an ineffective packing does not cause damage by itself, but would simply be leaving the DNA more exposed, subsequently facilitating greater access to reactive oxygen species (ROS), leading to extensive DNA damage, as suggested by Aitken and De Iuliis in their double-step hypothesis (13). Thus, OS is probably the most important cause of SDF, mainly single-strand breaks (SSB). In fact, more than 30% of infertile men presents OS and it is another biomarker that offers relevant information in the diagnosis of infertility (14). In the male reproductive tract, ROS are produced by leukocytes, bacteria and the sperm themselves as products of cellular respiration and adenosine triphosphate (ATP) production. Under normal conditions, basal levels of ROS are essential to activate sperm capacitation and acrosomal reaction (15,16). When the oxygen metabolism

is defective or the antioxidant mechanisms are affected, the levels of ROS increase and the risk of them reacting chemically with cellular structures increases. ROS can produce cytoplasmic or mitochondrial membrane breaks due to lipid peroxidation, alteration of sperm capacitation, formation of mutagenic products and, frequently, DNA fragmentation (17).

When choosing an SDF analysis methodology, different aspects must be assessed such as the sensitivity and specificity of the technique, the type of damage it detects or the feasibility of implementation in the clinic. Among the different techniques proposed, the ones with greater sensibility and specificity are Terminal deoxynucleotidyl transferase dUTP Nick End Labeling (TUNEL) and Comet assays (18), of which TUNEL assay is a much more standardized technique and, therefore, easier to implement in the routine, although it requires experienced technicians and certain equipment. However, TUNEL assay detects all DNA breaks without distinction, so it does not allow us to differentiate between SSB and DSB, two types of breaks with different clinical implications. SSB is mainly associated with natural pregnancy rate, while DSB increases the risk of recurrent miscarriages and implantation failures both in natural pregnancy and in intracytoplasmic sperm injection (ICSI) cycles (8). Currently, the only technique capable of differentiating between the two types of breaks in sperm DNA is the Comet assay, which has two variants: the neutral Comet and the alkaline Comet. If a neutral pH medium is used, the DNA will maintain its double helix structure and the technique will mainly reveal DSB, since SSB keep complementary strands together and will not migrate to the anode during electrophoresis. On the other hand, if an alkaline pH medium is used, the DNA is denatured and the fragments derived from DSB or SSB could migrate towards the anode. Since the fragments resulting from DSB are usually larger than those from SSB, if the electrophoresis time is adjusted so that only the short fragments have time to migrate through agarose, the technique will reveal mainly SSB, as suggested by Ribas-Maynou *et al.* (19). This configuration has not yet been tested by different laboratories, so it is still under discussion whether the alkaline comet only detects global damage or whether it can specifically detect SSB damage under specific conditions. In addition, Comet assay is not a standardized technique and has a high inter-observer variability, although various software has recently been developed that automates the analysis and greatly reduces this bias.

Once assessed, actions to reduce the patient's SDF are certainly limited, although in many cases are sufficient to achieve reproductive success. The main strategy is to reduce OS through the elimination of the risk factors (such as obesity, varicocele, short abstinence or hormone therapy, among others) or the provision of antioxidants, which its effect is still in controversy. Farkouh and collaborators (10) rightly propose advanced sperm selection as an alternative that, despite being in optimization phases, already produces very significant results in reducing SDF in specific assisted reproductive technologies procedures. Despite the clear implication of the SDF on reproductive success, the limitations in the treatments to reduce it may cause doubts when implementing this assessment in the diagnosis of the disease. But it must be remembered that, given that infertility is a multifactorial disorder, a good diagnosis involves gathering as much information as possible about the causes to choose the best reproductive strategy. Currently, many reproductive clinics do not make a good diagnosis and prematurely start ICSI cycles that do not achieve a childbirth. A clear example is found in patients with high levels of DSB and an increased risk of recurrent miscarriages or implantation failures, where with an SDF analysis included in semen assessment, could avoid the time and money wasting of several ICSI cycles by applying, perhaps, some advanced sperm selection technique before ICSI cycle such as microfluidic chips, that reduce the number of sperm with this type of damage (20). For this reason, articles are needed that encourage physicians to incorporate this type of analysis into their seminal evaluation routine in order to optimize the diagnosis of the disease.

Acknowledgments

Funding: Author's research group was funded by Generalitat de Catalunya (No. 2017SGR1796).

Footnote

Provenance and Peer Review: This article was commissioned by the editorial office, *Translational Andrology and Urology*. The article did not undergo external peer review.

Conflicts of Interest: The author has completed the ICMJE uniform disclosure form (available at https://tau.amegroups.com/article/view/10.21037/tau-22-572/coif). The author

has no conflicts of interest to declare.

Ethical Statement: The author is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

1. de Kretser DM. Male infertility. Lancet 1997;349:787-90.
2. Boivin J, Bunting L, Collins JA, et al. International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. Hum Reprod 2007;22:1506-12.
3. Agarwal A, Mulgund A, Hamada A, et al. A unique view on male infertility around the globe. Reprod Biol Endocrinol 2015;13:37.
4. Poongothai J, Gopenath TS, Manonayaki S. Genetics of human male infertility. Singapore Med J 2009;50:336-47.
5. Lewis SE, Simon L. Clinical implications of sperm DNA damage. Hum Fertil (Camb) 2010;13:201-7.
6. Aitken RJ, Smith TB, Jobling MS, et al. Oxidative stress and male reproductive health. Asian J Androl 2014;16:31-8.
7. Agarwal A, Parekh N, Panner Selvam MK, et al. Male Oxidative Stress Infertility (MOSI): Proposed Terminology and Clinical Practice Guidelines for Management of Idiopathic Male Infertility. World J Mens Health 2019;37:296-312.
8. Ribas-Maynou J, Benet J. Single and Double Strand Sperm DNA Damage: Different Reproductive Effects on Male Fertility. Genes (Basel) 2019;10:105.
9. World Health Organization. WHO laboratory manual for the examination and processing of human semen. Sixth edit. Geneva, Switzerland: WHO: World Health Organization Licence: CC BY-NC-SA 3.0 IGO, 2021.
10. Farkouh A, Salvio G, Kuroda S, et al. Sperm DNA integrity and male infertility: a narrative review and guide for the reproductive physicians. Transl Androl Urol 2022;11:1023-44.
11. Sakkas D, Alvarez JG. Sperm DNA fragmentation: mechanisms of origin, impact on reproductive outcome, and analysis. Fertil Steril 2010;93:1027-36.
12. Keeney S, Lange J, Mohibullah N. Self-organization of meiotic recombination initiation: general principles and molecular pathways. Annu Rev Genet 2014;48:187-214.
13. Aitken RJ, De Iuliis GN. On the possible origins of DNA damage in human spermatozoa. Mol Hum Reprod 2010;16:3-13.
14. Wagner H, Cheng JW, Ko EY. Role of reactive oxygen species in male infertility: An updated review of literature. Arab J Urol 2017;16:35-43.
15. Aitken RJ, Paterson M, Fisher H, et al. Redox regulation of tyrosine phosphorylation in human spermatozoa and its role in the control of human sperm function. J Cell Sci 1995;108:2017-25.
16. de Lamirande E, Gagnon C. Human sperm hyperactivation and capacitation as parts of an oxidative process. Free Radic Biol Med 1993;14:157-66.
17. Agarwal A, Cho CL, Esteves SC, et al. Reactive oxygen species and sperm DNA fragmentation. Transl Androl Urol 2017;6:S695-6.
18. Ribas-Maynou J, García-Peiró A, Fernández-Encinas A, et al. Comprehensive analysis of sperm DNA fragmentation by five different assays: TUNEL assay, SCSA, SCD test and alkaline and neutral Comet assay. Andrology 2013;1:715-22.
19. Ribas-Maynou J, García-Peiró A, Abad C, et al. Alkaline and neutral Comet assay profiles of sperm DNA damage in clinical groups. Hum Reprod 2012;27:652-8.
20. Pujol A, García-Peiró A, Ribas-Maynou J, et al. A microfluidic sperm-sorting device reduces the proportion of sperm with double-stranded DNA fragmentation. Zygote 2022;30:200-5.

Cite this article as: Garcia-Segura S. The importance of incorporating sperm DNA fragmentation testing in male infertility diagnostic routine Transl Androl Urol 2022;11(10):1371-1373. doi: 10.21037/tau-22-572