

## Current limitation and future perspective of sperm DNA fragmentation tests

Ashok Agarwal<sup>1</sup>, Chak-Lam Cho<sup>2</sup>, Sandro C. Esteves<sup>3</sup>, Ahmad Majzoub<sup>4</sup>

<sup>1</sup>American Center for Reproductive Medicine, Cleveland Clinic, Cleveland, OH, USA; <sup>2</sup>Division of Urology, Department of Surgery, Kwong Wah Hospital, Hong Kong, China; <sup>3</sup>ANDROFERT, Andrology and Human Reproduction Clinic, Referral Center for Male Reproduction, Campinas, SP, Brazil; <sup>4</sup>Department of Urology, Hamad Medical Corporation, Doha, Qatar

*Correspondence to:* Ashok Agarwal, Professor and Director, American Center for Reproductive Medicine, Cleveland Clinic, Mail Code X-11, 10681 Carnegie Avenue, Cleveland, OH 44195, USA. Email: AGARWAA@ccf.org.

*Response to:* Garrido N, Rivera R, Luján S. Clinical use of sperm DNA fragmentation analysis results, a practical example of how to deal with too much information from the literature in reproductive medicine. *Transl Androl Urol* 2017;6:S547-8.

Submitted Mar 27, 2017. Accepted for publication Mar 27, 2017.

doi: 10.21037/tau.2017.05.11

View this article at: <http://dx.doi.org/10.21037/tau.2017.05.11>

The commentary by Drs. Garrido, Rivera and Luján (1), in response to the practice recommendations by Agarwal *et al.* (2), is a valuable addition to the debate on sperm DNA fragmentation (SDF). We want to supplement their information as well as provide our opinion on several important points raised by the authors.

The authors (Garrido *et al.*) raised concern on the possible negative consequences of sperm DNA damage by stating that "...damaged DNA in sperm could lead to worse reproductive results or mid-long term health problems in offspring, given that this is the way genetic information is delivered to the next generation" (1). We certainly concur with their concern but, as opposed to the authors' view, we believe that the current evidence is not reassuring. Although it is well known that intracytoplasmic sperm injection (ICSI) has revolutionized the treatment of male infertility and allowed the most severe form of male factors to be bypassed, however, animal studies have provided unequivocal result that sperm DNA damage leads to deleterious effect on offspring (3-5). ICSI with DNA-fragmented sperm in mouse can result in premature ageing, aberrant growth, and increased incidence of tumors in the offspring (6). The possible link between SDF and defects in human offsprings is best illustrated by the effect of smoking and paternal age. Heavy smokers have higher levels of SDF (7) and this may correspond to the higher incidence of childhood cancer in the offspring of heavy smokers (8,9). Impaired sperm DNA integrity is also associated with advanced paternal age (10). The advanced paternal age has been linked to dominant genetic diseases (11),

schizophrenia (12), and birth defects (13). Despite this evidence, the relationship between SDF and genetic defect can only be fully answered by longitudinal studies with sufficient samples and duration. It is definitely too early to conclude the safety of ICSI since it was introduced into clinical practice only three decades ago. The unknown and potentially lethal consequence of passing aberrant genetic information to the next generation should be taken seriously. Correction of SDF before recommending assisted reproductive technologies (ART) should be the preferred approach as far as possible.

The presence of oocyte repair machinery for sperm DNA damage may serve as another safety check to avoid passage of defective genetic information. However, not all types of sperm DNA break, for example, extensive double stranded DNA breaks, are repairable (14). Irradiated sperm with DNA damage was shown to retain the ability to fertilize the oocyte, but the embryonic development was significantly affected in a dose-response fashion. The oocyte has the ability to repair sperm DNA damage of less than 8% (15). Even though there is a lack of human data at this time, animal studies show that female mice with defective DNA double-stranded break repair had increased frequencies of zygotes with sperm-derived chromosomal aberrations when fertilized by sperm with irradiation-induced double-stranded DNA breaks. The chromosome-type aberration, which affect both sister chromatids, resulted in high embryonic lethality (16). It is suggested that good oocyte quality may overcome the negative impact of high SDF (17). Results of a retrospective clinical study showed that the live-birth and

implantation rates during IVF/ICSI cycles in women with reduced ovarian reserve were significantly decreased when SDF exceeded 27.3%. While the risk of early abortion was increased in women with normal ovarian reserve in face of high SDF, the clinical pregnancy, live-birth and implantation rates were not affected (18). However, there is a lack of reliable biochemical or molecular marker of oocyte status. There is also no widely accepted criteria or grading method for microscopic oocyte morphological evaluation (19). Different clinical studies have used various definition of oocyte status (18). It is important to note that the use of good quality oocyte in compensating high SDF is not realistic from a clinical point of view since male and female factors often co-exist in a couple. For example, advanced age in a couple may contribute to high SDF and impaired oocyte quality simultaneously leading to poor reproductive outcomes. The use of donor oocyte or donor sperm in overcoming the male or female factor respectively in these cases is impractical and certainly not widely accepted.

Currently, SDF tests are often criticized for their inability to differentiate the exact type and nature of DNA breaks. Since sperm DNA breaks also occur in fertile males during the process of chromatin condensation (20), the unknown identity of a particular sperm DNA break may impair the clinical significance of the test result. On the other hand, the correlation between SDF and natural pregnancy/ART/miscarriage has been demonstrated despite a wide variety of testing methods employed (21). We agree that there is no single gold standard test for SDF at the moment; however the results of a recent study between two laboratories located in Cleveland, USA and Basel, Switzerland with terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay are promising as it provides a very high degree of accuracy between different laboratories when using a similar optimized test protocol, instrument and test kits (22). But it is essential to recognize the role of SDF tests as a reflection of the general status of sperm quality, which may not be reflected by conventional semen parameters (23). The unique nature of SDF test in providing information on important sperm DNA content represents the largest breakthrough in male infertility over the last two decades.

We agree with authors' viewpoint that "...any markers in sperm will hardly predict the results of combining sperm with oocytes and then endometrial receptivity...". The statement reflects the complexity of human reproduction and the inherent difficulty in research with a huge number of confounding factors. It is unlikely that SDF assay alone will accurately predict pregnancy outcomes which is widely

influenced by concomitant female factors (20,24). The negative impact of SDF on time to pregnancy was clearly illustrated by the Longitudinal Investigation of Fertility and Environment (LIFE) (25) and the Danish First Pregnancy Planner (26) studies. In addition to SDF, several semen measures and male age were identified as associated with time to pregnancy (25). It signifies that SDF should not be used in isolation. However, its important role and predictive value in conjunction of other parameters makes it a valuable tool.

Lastly, SDF tests are currently incapable to assess DNA content of a single sperm and directly assist the ICSI procedure by selecting the best spermatozoon for injection. Several treatment strategies including sperm preparation (27), sperm selection (28) and use of testicular sperm (29) have been proposed with varying success. SDF tests, although are unable to select a single best spermatozoon for reproductive purposes, it has a pivotal role in altering the management plan in selected groups of patients as suggested by Agarwal *et al.* (2).

In summary, Garrido *et al.* have written a critical review on the limitation and current status of SDF tests. Their insight on the current limitation of SDF tests sheds light on the direction of future advancement. Both the quantity and quality of publications in the field of SDF has advanced dramatically over the past 25 years. We envisage better standardization of the test in the coming years with continuous efforts of worldwide researchers and clinicians. The practice recommendations proposed by Agarwal *et al.* (2) highlighted several areas where SDF tests are potentially more useful in patient management based on the currently available evidence. Nonetheless, we believe that the application of the test should not be limited by the practice recommendations alone as a wider application of the test and more extensive clinical experience are critical in ascertaining the true significance of SDF tests in the treatment algorithm of infertile couples.

## Acknowledgements

None.

## Footnote

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

## References

1. Garrido N, Rivera R, Lujan S. Clinical use of sperm

- DNA fragmentation analysis results, a practical example of how to deal with too much information from the literature in reproductive medicine. *Transl Androl Urol* 2017;6:S547-8.
2. 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.
  3. Trasler JM, Hales BF, Robaire B. Chronic low dose cyclophosphamide treatment of adult male rats: effect on fertility, pregnancy outcome and progeny. *Biol Reprod* 1986;34:275-83.
  4. Trasler JM, Doerksen T. Teratogen update: paternal exposures-reproductive risks. *Teratology* 1999;60:161-72.
  5. Lewis SE, Aitken RJ. DNA damage to spermatozoa has impacts on fertilization and pregnancy. *Cell Tissue Res* 2005;322:33-41.
  6. Fernández-Gonzalez R, Moreira PN, Pérez-Crespo M, et al. Long-term effects of mouse intracytoplasmic sperm injection with DNA-fragmented sperm on health and behavior of adult offspring. *Biol Reprod* 2008;78:761-72.
  7. Fraga CG, Motchnik PA, Wyrobek AJ, et al. Smoking and low antioxidant levels increase oxidative damage to sperm DNA. *Mutat Res* 1996;351:199-203.
  8. Ji BT, Shu XO, Linet MS, et al. Paternal cigarette smoking and the risk of childhood cancer among offspring of nonsmoking mothers. *J Natl Cancer Inst* 1997;89:238-44.
  9. Lee KM, Ward MH, Han S, et al. Paternal smoking, genetic polymorphisms in CYP1A1 and childhood leukemia risk. *Leuk Res* 2009;33:250-8.
  10. Schmid TE, Eskenazi B, Baumgartner A, et al. The effects of male age on sperm DNA damage in healthy non-smokers. *Hum Reprod* 2007;22:180-7.
  11. Crow JF. The origins, patterns and implications of human spontaneous mutation. *Nat Rev Genet* 2000;1:40-7.
  12. Sapos A, Rasmussen F, Harrison G, et al. Paternal age and schizophrenia: a population based cohort study. *BMJ* 2004;329:1070.
  13. McIntosh GC, Olshan AF, Baird PA. Paternal age and the risk of birth defects in offspring. *Epidemiology* 1995;6:282-8.
  14. García-Díaz M, Domínguez O, López-Fernández LA, et al. DNA polymerase lambda (Pol lambda), a novel eukaryotic DNA polymerase with a potential role in meiosis. *J Mol Biol* 2000;301:851-67.
  15. Ahmadi A, Ng SC. Fertilizing ability of DNA-damaged spermatozoa. *J Exp Zool* 1999;284:696-704.
  16. Marchetti F, Essers J, Kanaar R, et al. Disruption of maternal DNA repair increases sperm-derived chromosomal aberrations. *Proc Natl Acad Sci U S A* 2007;104:17725-9.
  17. Meseguer M, Santiso R, Garrido N, et al. Effect of sperm DNA fragmentation on pregnancy outcome depends on oocyte quality. *Fertil Steril* 2011;95:124-8.
  18. Jin J, Pan C, Fei Q, et al. Effect of sperm DNA fragmentation on the clinical outcomes for in vitro fertilization and intracytoplasmic sperm injection in women with different ovarian reserves. *Fertil Steril* 2015;103:910-6.
  19. Balaban B, Barut T, Urman B. Assessment of oocyte quality. In: *Practical manual of in vitro fertilization*. Nagy ZP, Varghese AC, Agarwal A, editors. New York, USA: Springer, 2012:105-19.
  20. Balhorn R. Sperm chromatin: an overview. In: *Sperm Chromatin: biological and clinical applications in male infertility*. Zini A, Agarwal A, editors. New York, USA: Springer, 2011:3-18.
  21. Agarwal A, Cho CL, Esteves SC. Should we evaluate and treat sperm DNA fragmentation? *Curr Opin Obstet Gynecol* 2016;28:164-71.
  22. Ribeiro S, Sharma R, Gupta S, et al. Inter- and intra-laboratory standardization of TUNEL assay for assessment of sperm DNA fragmentation. *Andrology* 2017;5:477-85.
  23. Irvine DS, Twigg JP, Gordon EL, et al. DNA integrity in human spermatozoa: relationships with semen quality. *J Androl* 2000;21:33-44.
  24. Esteves SC, Sharma RK, Gosálvez J, et al. A translational medicine appraisal of specialized andrology testing in unexplained male infertility. *Int Urol Nephrol* 2014;46:1037-52.
  25. Buck Louis GM, Sundaram R, Schisterman EF, et al. Semen quality and time to pregnancy: the Longitudinal Investigation of Fertility and the Environment Study. *Fertil Steril* 2014;101:453-62.
  26. Spanò M, Bonde JP, Hjøllund HI, et al. Sperm chromatin damage impairs human fertility. The Danish First Pregnancy Planner Study Team. *Fertil Steril* 2000;73:43-50.
  27. Zhang XD, Chen MY, Gao Y, et al. The effects of different sperm preparation methods and incubation time on the sperm DNA fragmentation. *Hum Fertil (Camb)* 2011;14:187-91.
  28. Bradley CK, McArthur SJ, Gee AJ, et al. Intervention improves assisted conception intracytoplasmic sperm injection outcomes for patients with high levels of sperm

- DNA fragmentation: a retrospective analysis. *Andrology* 2016;4:903-10.
29. Esteves SC, Sánchez-Martín F, Sánchez-Martín P, et al. Comparison of reproductive outcome in oligozoospermic

men with high sperm DNA fragmentation undergoing intracytoplasmic sperm injection with ejaculated and testicular sperm. *Fertil Steril* 2015;104:1398-405.

**Cite this article as:** Agarwal A, Cho CL, Esteves SC, Majzoub A. Current limitation and future perspective of sperm DNA fragmentation tests. *Transl Androl Urol* 2017;6(Suppl 4):S549-S552. doi: 10.21037/tau.2017.05.11