

# Practice Recommendations of Sperm DNA Fragmentation Testing: Expert Commentaries by Invited Authors and Replies by Guest Editors

## Contributors from Africa

### Commentary

## Clinical utility of sperm DNA fragmentation testing: a commentary

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The andrological factor is implicated in more than 40% of couples attending infertility clinics. Considering that standard semen analysis only poorly predicts the outcome of fertilization and pregnancy in couples including assisted reproduction attempts as it only examines sperm parameters such as sperm concentration, vitality, sperm morphology and motility, it is therefore quite limited in its clinical discriminatory power (1) in about 40% of men with normal ejaculates (2). Contributing factors to this fact are the high biological variability of these parameters, essentially causing each ejaculate being unique, thus often resulting in the vague clinical diagnosis of idiopathic infertility (3). In addition, the fertilization process itself is a multifactorial process in which not only the different sperm functions such as motility, capacitation, acrosome reaction, chromatin condensation or the integrity of the genetic information, but also female and oocyte factors have to be taken into account (4). Among these conventional sperm parameters, morphology evaluated according to strict criteria exhibited a relatively low degree of variability (4). Yet, although morphology appeared as a better parameter than sperm concentration, the diagnostic value of semen analysis is limited (5). For this reason, conventional semen analysis was complemented by a panel of functional sperm parameters including sperm nuclear DNA fragmentation, which is also

regarded a parameter with low biological variation (6).

Oxidative stress, apart from incorrect chromatin modelling, endonucleases, environmental pollutants such as polychlorinated biphenyls (PCB's) or lifestyle origins like radiation, chemotherapy etc., was discovered as one major cause of sperm DNA fragmentation. Clinical studies revealed that oxidative stress has a prevalence of up to 40% in the group of unselected infertile men and up to 96% in patients with spinal cord injuries (7). Yet, although the determination of sperm nuclear DNA damage offered novel diagnostic avenues and narrowed the gap of idiopathic infertility, evaluation of sperm DNA fragmentation is still lacking relevant clinical evaluation and standardization of the technique (8). As worrying as this is, the lack of indubitable clinical criteria according to which testing of sperm DNA fragmentation should be recommended is an important point taken up in the paper by Agarwal *et al.* (9). Clarity not only in terms of a certain standardized diagnostic technique with the relevant diagnostic cut-off values, but also in terms of clinical criteria according to which the test can be recommended to the patient are essential. This is even more important since the shift of values for normality according from the fifth edition of the WHO manual (10) to its latest edition (11) caused an increase in the number of patients who are now classified as "normal" of about 15% (12). As a result, this

may lead that these patients are not being referred for proper infertility evaluation or treatment.

In this context, it is not only important to understand the etiology of sperm DNA fragmentation, but also the basis of the various available tests with their advantages, disadvantages, problems and diagnostic potential, although the latter is still controversially discussed with contrasting results reported from different groups (13,14). These aspects are addressed in the report by Agarwal *et al.* (9). Currently, there are eight tests available to analyse sperm DNA fragmentation, namely the sperm chromatin structure assay (SCSA), terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), acridine orange test (AO), aniline blue staining (AB), chromomycin A3 staining (CMA3), toluidine staining (TS), single cell gel electrophoresis assay (COMET assay), and the sperm chromatin dispersion test (SCD) with its improved version called Halosperm<sup>®</sup> assay seems to be an easy and good predictor of good embryo quality and pregnancy that has recently become popular. Yet, proper clinical evaluation remains to be established.

Basically, these different test systems can be distinguished by their principles. While the COMET assay under neutral pH conditions and TUNEL assays determine real DNA damage in terms of DNA fragmentation, the AO, SCSA, SCD (including Halosperm<sup>®</sup> assay) and the COMET assay under alkaline pH conditions rather determine the susceptibility of the DNA to denaturation. In contrast, the AB, TS and CMA3 stains probe the DNA packaging and maturity (15). The principles together with a typical picture of the evaluation are summarized with relevant advantages and disadvantages in Agarwal *et al.* (9). It needs to be added that the latter assays do neither evaluate DNA damage nor DNA fragmentation. They simply try to determine the quality of the DNA condensation, i.e., the packaging of the male genome in the protective nuclear proteins, protamines. Although DNA fragmentation and chromatin condensation are related as nicks occur during chromatin condensation in order to relax tension in the DNA molecule while compacting, these assays are determining a completely different type of parameter from those test systems that probe the DNA for fragmentation; a fact that has to be emphasized.

Another important aspect that has to be highlighted for those test systems probing the DNA integrity is that the one assay cannot replace the other assay as they are determining different aspects of DNA damage. Although certain parameters of the TUNEL assay correlated very well with the DNA fragmentation index that is

calculated in the SCSA, calculation of the concordance correlation coefficient, which contains a measurement of precision ( $\rho$ ) and accuracy ( $C_b$ ), showed only very poor comparability for the SCSA DFI with the mean channel fluorescence and the relative fluorescent activity in the TUNEL assay. In addition, for the DNA fragmentation index as percentage of the relative fluorescent activity in the TUNEL assay as well as the DNA fragmentation index as percentage of the mean channel fluorescence in the TUNEL assay, only very poor correlations could be found with SCSA parameters (16). Likewise, statistical method comparison by means of Bland-Altman plots resulted in unevenly distributed data along the abscissa. A recent study by Ribas-Maynou *et al.* (17), compared TUNEL assay, SCSA, SCD test, neutral and alkaline COMET assay, and revealed that best predicting power for the alkaline COMET assay followed by the TUNEL assay and SCSA. In contrast, the neutral COMET assay showed no predictive power indicating the importance of choosing the appropriate test system. In this regard, as well as to the choice of a suitable test, the article by Agarwal *et al.* (9) could have been more in detail and/or more specific.

On the other hand, despite more knowledge has been gained in terms of the etiology of sperm DNA fragmentation and the identification of oxidative stress as a major factor contributing to this condition, little has been published to give the clinician a guide at hand according to which a test for DNA fragmentation should be requested. The aim for such effort has to be the patient's interest so as to properly diagnose him in order to find the best possible treatment and also to keep the cost for the patient as low as possible. In this regard, the article, however, is the first giving this urgently needed practical guidance. However, more studies have to be conducted to widen and clarify the scope of sperm DNA testing. These efforts should then include new non-consumptive tests that can be used in the preparation to select "good quality" sperm for ICSI like the polarization microscopy where the birefringence of light is used to evaluate the organelle structure of the male germ cell. Applying this technique, Gianaroli *et al.* (18) report significantly higher implantation, clinical pregnancy and ongoing pregnancy rates.

Finally, in the light of oxidative stress being a major contributor to sperm DNA fragmentation, it will be beneficial and possibly easier if the redox potential either in semen or in serum can be determined so to say as a factor that causes sperm DNA fragmentation. Currently, we know

that during oxidative stress the balance between oxidation and reduction shifts towards the oxidative conditions which leads to the said damages. Therefore, doctors prescribe antioxidants as therapy as a counterbalance and positive results with decreased values for sperm DNA fragmentation have been reported in most studies (19). Yet, an overdose of antioxidants may lead to so-called reductive stress which is regarded as dangerous as oxidative stress. The problem scientists are facing is that it is still unknown where the balance between oxidation and reduction is. Also, does that balance physiologically shift depending on the individual situation a body is in. A possible first step addressing this issues might be the determination of the oxidation-reduction potential (20) which provides an easy and quick method that does not consume the spermatozoa that may be used for insemination purposes. Further studies, however, have to be conducted in order to strengthen this relationship and establish clinical cut-off values.

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### Footnote

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

### References

- Guzick DS, Overstreet JW, Factor-Litvak P, et al. Sperm morphology, motility, and concentration in fertile and infertile men. *N Engl J Med* 2001;345:1388-93.
- van der Steeg JW, Steures P, Eijkemans MJ, et al. Role of semen analysis in subfertile couples. *Fertil Steril* 2011;95:1013-9.
- Hendin BN, Kolettis PN, Sharma RK, et al. Varicocele is associated with elevated spermatozoal reactive oxygen species production and diminished seminal plasma antioxidant capacity. *J Urol* 1999;161:1831-4.
- Henkel R, Maaß G, Bödeker R-H, et al. Sperm function and assisted reproduction technology. *Reprod Med Biol* 2005; 4:7-30.
- Alvarez C, Castilla JA, Martínez L, et al. Biological variation of seminal parameters in healthy subjects. *Hum Reprod* 2003;18:2082-8.
- Zini A, Kamal K, Phang D, et al. Biologic variability of sperm DNA denaturation in infertile men. *Urology* 2001;58:258-61.
- Padron OF, Brackett NL, Sharma RK, et al. Seminal reactive oxygen species and sperm motility and morphology in men with spinal cord injury. *Fertil Steril* 1997;67:1115-20.
- 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.
- 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.
- World Health Organization. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. Cambridge University Press, Cambridge, UK, 1999.
- World Health Organization, Department of Reproductive Health and Research. WHO laboratory manual for the examination and processing of human semen. 5th edn. ed. Switzerland; WHO Press, 2010.
- Murray KS, James A, McGeady JB, et al. The effect of the new 2010 World Health Organization criteria for semen analyses on male infertility. *Fertil Steril* 2012;98:1428-31.
- 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.
- Simon L, Zini A, Dyachenko A, et al. A systematic review and meta-analysis to determine the effect of sperm DNA damage on in vitro fertilization and intracytoplasmic sperm injection outcome. *Asian J Androl* 2017;19:80-90.
- Henkel R. DNA based sperm assessment. In: Kruger TF, van der Spuy Z, Kempers RD (eds); *Advances in Fertility Studies and Reproductive Medicine—IFFS 2007*; Juta, Cape Town, South Africa, 2007;284-92.
- Henkel R, Hoogendijk CF, Bouic PJ, et al. TUNEL assay and SCSA determine different aspects of sperm DNA damage. *Andrologia* 2010;42:305-13.
- 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.
- Gianaroli L, Magli MC, Ferraretti AP, et al. Birefringence characteristics in sperm heads allow for the selection of

- reacted spermatozoa for intracytoplasmic sperm injection. *Fertil Steril* 2010;93:807-13.
19. Zini A, Al-Hathal N. Antioxidant therapy in male infertility: fact or fiction? *Asian J Androl* 2011;13:374-81.
20. Agarwal A, Roychoudhury S, Bjugstad KB, et al. Oxidation-reduction potential of semen: what is its role in the treatment of male infertility? *Ther Adv Urol* 2016;8:302-18.

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