

Utility of sperm DNA fragmentation testing in different clinical scenarios of male reproductive abnormalities and its influence in natural and assisted reproduction

Jorge Hallak^{1,2,3,4}

¹Androscience, Science and Innovation Center in Andrology and High-Complex Clinical and Andrology Laboratory, Sao Paulo, Brazil; ²Department of Pathology, Reproductive Toxicology Unit, University of São Paulo Medical School, SP, Brazil; ³Division of Urology, Department of Surgery, Hospital das Clinicas, University of Sao Paulo Medical School, Brazil; ⁴Institute for Advanced Studies, University of Sao Paulo (IEA-USP), Brazil

Correspondence to: Jorge Hallak, MD, PhD. Androscience, Science and Innovation Center in Andrology, Rua Joaquim Floriano, 533, cjs. 903/904 Itaim-Bibi, ZIP: 04534-011, São Paulo, Brazil. Email: hallakj@androscience.com.br.

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 Jun 05, 2017. Accepted for publication Jun 26, 2017.

doi: 10.21037/tau.2017.06.29

View this article at: <http://dx.doi.org/10.21037/tau.2017.06.29>

One of the measurements of sperm quality that can be assessed with validated protocols is DNA fragmentation, the subject was profoundly dissected in the article by Agarwal *et al.* and that constitutes the backbone of this commentary (1). Authors quoted: “*Over the past decade, Sperm DNA Fragmentation (SDF) measurements have been extensively investigated and correlated with various disease entities. While SDF is increasingly being available in the urologists’ armamentarium for the evaluation of infertile men, its accurate clinical implication is still poorly understood. Few meta-analyses have been made withdrawing valuable conclusions on the significance of SDF in various contexts of male infertility, yet a precise understanding of the specific utility of such test in different clinical scenarios is still lacking.*” The abnormal sperm nuclear condensation process involves a complex sequence of events including topological rearrangements, transition of DNA-binding proteins, transcriptional alterations, nucleosomal structure loss and abnormal condensation of chromatin resulting in disturbances in the organization of genomic material in the sperm nuclei and decreasing sperm functional ability. Ultimately this reduces normal fertilization, affects early embryonic development and interferes with the primary mission of the sperm DNA which is reliable transmission of paternal genetic information (2-4). The end-result of these abnormalities is translated into the SDF test. A higher SDF level is found in men with abnormal semen parameters and normozoospermic

partners of infertile couples, and the mechanism in SDF analysis relies in oxidative stress induced DNA damage during migration of mature sperm with reactive oxygen species (ROS) producing immature and defective sperm through the epididymis and seminal tract (5). This is the rationale behind all SDF tests described in the article by Agarwal *et al.* who described that there are two types of assays that have been developed to measure SDF: those that can directly measure the extent of DNA fragmentation using probes and dyes and those that measure the susceptibility of DNA to denaturation, which occurs more commonly in fragmented DNA. They described eight standardized methods, although the most used tests are terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), the COMET assay, the sperm chromatin dispersion (SCD) test, and the sperm chromatin structure assay (SCSA), we will focus on these four for this discussion, as they are the most reliable and reproducible among the eight and as we are trying to add a reliable step into the laboratory evaluation of the infertile male and, of course reliability and ease of reproducibility are fundamental pre-requisites.

SCSA (6) is the flow cytometric measurement of the susceptibility of sperm DNA to acid-induced denaturation after staining with acridine orange, a fluorescent dye. The use of a flow cytometer allows that 5,000 to 10,000 sperm cells can be evaluated in a few seconds and thus

provides a less subjective result compared with semen analysis where only 1 to 200 cells are analyzed. In a meta-analysis, the SCSA was shown to be predictive for *in vivo* conception, intrauterine insemination (IUI) and routine in-vitro fertilization (IVF) methods. The meta-analysis revealed that it was 7.1 times (CI, 3.37, 14.91) more likely to attain a pregnancy/delivery if the DNA fragmentation index (%DFI) was <30% in couples with natural conception and IUI (n=1,962, P=0.0001). Furthermore, couples were nearly 2.0 times (CI, 1.10, 2.96) more likely to develop pregnancy following IVF if their DFI was <30% (n=375, P=0.02) (7). Another meta-analysis of six studies examining intracytoplasmic sperm injection (ICSI) and/or routine IVF reported a non-significant trend where patients were 1.7 times (CI, 0.97, 3.14) more likely to achieve a pregnancy/delivery if the %DFI was <30% (n=322, P=0.06) (8). Although SCSA results higher than cut off values, does not implicate the absence of pregnancy, they do mean a prolonged period to achieve pregnancy and a higher miscarriage rate for natural conceptions (9). It is important to mention that the protocol of SCSA is very strict and time-consuming to have it perfectly done, does not tolerate laboratory errors as minimal deviations compromise results. Also, a flow cytometer and experienced andrology technician comfortable with the methodology are required, not to mention the fundamental software provided by SCSA diagnostics (SCSA-soft®; SCSA Diagnostics, Inc., Brookings, SD, USA). However, once the laboratory achieves that level of precision, the test is extremely reliable, highly reproducible and provides a lot of useful information to guide the patient, the andrologist and the reproductive gynecologist throughout the process of natural or assisted reproduction. The SCSA is a test for infertility. A normal result is not a guarantee of fertility as it only assesses sperm DNA integrity which is one of the many essential aspects of human reproduction. It is important to note that having a DFI of <30%, does not mean that the other 70% of spermatozoa have fully normal chromatin and further, as implied by Aitken *et al.* “*This value means only that, given the physical conditions imposed on the spermatozoa to induce DNA denaturation, that 30% of the spermatozoa crossed that threshold.*” In other words, the remaining 70% of sperm may have some degree of DNA damage however, not to the extent of crossing the threshold under the conditions of sperm DNA denaturation (10). A point to support SCSA as a reliable SDF test is the simple fact that it has been around for over thirty-years, and has passed a lot of harsh test-proofs from the scientific and medical communities.

TUNEL cut-off values vary considerably in the literature. A threshold of 10% (8) for predicting pregnancy outcome has been proposed which is significantly lower than SCSA values of 30%. Notably, the 10% TUNEL threshold value suggested by some authors, refers to fertilization rate, not pregnancy rate, as quoted: “*No pregnancy was obtained when DNA fragmentation was higher than 20% TUNEL threshold value*”; thus, TUNEL define an absolute threshold rather than a statistical threshold (8). A literature search shows that the threshold values for TUNEL range from 12% (10), through 20%⁸ to 36.5% (11,12). SCSA results are highly correlated with TUNEL results (r=0.859, P<0.001) (13), suggesting that the sites of DNA strand break that SCSA technique measures after adding acridine orange are identical to those measured with TUNEL after the enzymatic addition of fluorochromes. In contrast, neutral COMET measures only double strand breaks and alkaline COMET measures single and double strand breaks. However, the alkaline COMET data are compromised by the existence of ‘alkaline-sensitive sites’ in sperm DNA that are not classical single strand breaks (14). Nevertheless, if a constant number of ‘alkaline-sensitive sites’ were present in all human sperm, then alkaline COMET values above that constant would likely be a measure of classical single strand breaks. Of note, the latter 36.5% TUNEL threshold established is close to the 30% SCSA threshold, so for practical purposes and standardization of cutoff values in different labs and places, can we assume that SDF close or around 30% is a referral value for this test?

SCD, or Halo test (15), as stated in the article by Agarwal *et al.* (1), “*is based on the concept that sperm with fragmented DNA do not produce the characteristic halo of dispersed DNA loops that are observed in sperm with non-fragmented DNA following acid denaturation and removal of nuclear proteins*”. Following denaturation that removes nuclear proteins from agarose-embedded sperm thereby exposing the damaged DNA, spermatozoa with intact DNA display the characteristic halos around the sperm nucleus whereas spermatozoa with damaged DNA fail to do so. A bright field microscope can be used to observe these halos after staining with eosin and azure B solution. A fluorescence microscope is used if DNA-directed fluorochromes were utilized. Despite being a simple technique not requiring complex instrumentation, this test has inter-observer subjectivity, and needs training and standardization between different labs. It also provides more useful information than conventional semen analysis. The prognostic accuracy of SDF tests depends on the precision of their technique of

implementation, and the advantage of SCD test is that it can be performed by light- microscopy so the likelihood of widespread use by andrology labs without a high degree of complexity as compared with TUNEL, COMET or SCSA, which demands more trained personnel and more sophisticated installations. On the other hand, SCD is still a relatively novel test, and more information is needed to determine its cut-off and predictive values and for IUI, IVF and ICSI outcomes. That said, the article by Agarwal *et al.* is rich in details of SDF testing for each of the most common clinical conditions encountered in infertility clinic. The practice recommendations provide reliable and convincing information to move a step forward and adopt SDF as part of the routine laboratory evaluation in combination with semen analysis and ROS. Correct SDF testing in the environment of a quality-controlled andrology lab with technical expertise is a progress that we should embrace from now on. However, particular attention should be paid to the proper use of SDF tests. Inappropriate application of SDF tests as a reckless substitute for the proper evaluation of male infertility leads to confusing results. Correct interpretation of SDF results is of utmost importance. A high SDF result may suggest the use of ICSI in view of the higher fertilization rate on one hand. On the other hand, if SDF levels are low, some reproductive specialists also suggest ICSI by arguing that it is a “good case to go straight for ICSI”. Any test should be evaluated in the context of its clinical case and the best solution to reestablish natural fertility or improve sperm quality is always preferred and more cost-effective (16-18).

Acknowledgements

Androscience, Science and Innovation Center in Andrology and High-Complex Andrology Laboratory.

Footnote

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

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Cite this article as: Hallak J. Utility of sperm DNA fragmentation testing in different clinical scenarios of male reproductive abnormalities and its influence in natural and assisted reproduction. *Transl Androl Urol* 2017;6(Suppl 4):S509-S512. doi: 10.21037/tau.2017.06.29