

The importance of quality control and quality assurance in SDF testing

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Dr. Kadioglu and Ortac from Turkey commented about the advantages and shortcomings of SDF testing in the clinical scenarios discussed by Agarwal *et al.* (1), and provided overall supportive remarks for the utility of SDF testing in selected populations (2). Notwithstanding, the authors noted that there is no consensus as to whether or not measurement of SDF provides any clinical benefit in the assessment of the male patient. Moreover, they highlighted existing guidelines issued by the American Urological Association (AUA) and European Association of Urology (EAU), which indicate that varicocele repair is not recommended for infertile men with normal semen analysis (3,4). Lastly, the authors inquired about the interlaboratory variation of SDF testing and which test should be considered the gold standard. In our reply to Drs. Kadioglu and Ortac, we elaborate on these aspects to provide readers further insights into these concerns.

In our guidelines, we reviewed the existing evidence and provided practical recommendations graded according to the quality of the available evidence (1). The selected clinical scenarios are familiar to practicing clinicians, and all of them pose difficulties for management. Despite concurring with the authors that additional prospective studies are needed to further clarify the clinical role of SDF for the evaluation of the infertile male, there is a bulk of literature demonstrating an association between SDF and reproductive outcomes [reviewed by Cho *et al.* (5)]. Given the fact that the integrity of sperm DNA is crucial for normal fertilization, embryo development, and successful implantation, we ponder that SDF testing provides clear

information that adds to conventional semen analysis results without being superfluous (6-8).

It might be argued that the prognostic clinical value of DNA integrity testing may not affect the treatment of couples, as noted by the ASRM guidelines (9). However, new evidence has emerged particularly concerning the use of testicular in preference over ejaculated sperm for ICSI among couples whose male partners have high SDF in the neat ejaculate (10,11). Along the same lines, although the AUA and EAU guidelines regarding varicocele management advocate against surgery in the face of normal semen analysis [reviewed by Shridharani *et al.* (12)], it is worth mentioning that these guidelines based their recommendations on the grounds of routine semen analysis. It is well known that routine semen analysis is limited as a surrogate marker for male fertility (6); despite this fact, such limitations were neglected by the AUA/EAU guidelines. Added to this, the AUA guidelines are yet to update semen analysis reference ranges to the newest 2010 WHO manual (3,13). Noteworthy, clinical practice guidelines are evolving documents that should undergo periodic review and updates.

Lastly, it is important to recognize that efforts have been made to standardize SDF testing (14-18). As part of the standardization process, inter- and intra-laboratory precision, and coefficient inter- and intra-observer variation have been calculated for tests such as SCSA, SCD, and flow-cytometer TUNEL (15-22). In *Table 1*, we summarize the relevant information provided by studies as regard to standardization of the SDF testing. Overall, these tests have

Table 1 Summary of intra-laboratory and inter-laboratory correlation coefficients (r), coefficient of variation (CV), and intra- and inter-observer variation (%) for SDF assays

Methods	Intra-lab (r)	Intra-lab (CV)	Inter-lab (r)	Inter-lab (CV)	Intra-observer variation	Inter-observer variation	Study
TUNEL; benchtop flow cytometer	0.75–0.95 ⁶	0.1–5.7%	0.83–0.93 ⁷	0.2–5.2%	NR	NR	Ribeiro <i>et al.</i> , 2017
TUNEL; benchtop flow cytometer	NR	NR	NA	NA	≤3% ⁴	≤1.7% ⁵	Sharma <i>et al.</i> , 2016
TUNEL; standard flow cytometry	NR	NR	NA	NA	3.2% ²	4% ³	Sharma <i>et al.</i> , 2010
SCD*	0.91	NR	NA	NA	1% ⁹	0.21% ¹⁰	McEvoy <i>et al.</i> , 2014
SCD	NR	NR	NA	NA	6–12% ⁸	6–12% ⁸	Fernandez <i>et al.</i> , 2005
SCSA	NA	NA	0.90	≤1%	NA	NA	Giwerzman <i>et al.</i> , 2003
SCSA	NR	1.0–9.1% ¹	NA	NA	NA	NA	Giwerzman <i>et al.</i> , 1999

*, Halosperm G2 test kit. ¹, Intra-assay CV varied between 1.0% and 9.1%, and the corresponding values for the inter-assay CV was 5.2% and 8.6%. ², when absolute values were calculated, 80.0% of individual TUNEL measurement differed from the final designated values by no more than 3.2% (absolute difference); 57.1% of individual measurements in these data had a percentage difference less than 10% of the assigned value. ³, the absolute difference between an observer's designated value and the mean among 2 observers was within 4.0% in 83.3% of specimens; in 83.3% of the specimens, the percentage difference between an individual observer's designated TUNEL value and the 2 observers' average value was within 15%. ⁴, a single TUNEL measurement from a given observer was within that observer's average measurement by an absolute difference of 3% or less in 90% of cases. ⁵, the average TUNEL measurement from a given observer was within the two observers' average measurements with an absolute difference of 1.73% or less in 80% of cases. ⁶, correlation between duplicate readings obtained in each laboratory (results from two participating laboratories). ⁷, correlation coefficient between two participating laboratories reading the same set of specimens. ⁸, coefficient of variation for the estimated percentage of spermatozoa with fragmented DNA. ⁹, the average difference in the values of SDF between the two replicates was 1.02%±0.55% (absolute variation); the average percentage difference between the two replicates for each sample was 4.16%. ¹⁰, the average difference in the values of SDF between two technicians for each sample was 0.21%±0.57% (absolute difference); the average percentage difference between the two technicians for each sample was 9.56%. TUNEL, terminal deoxyribonucleotide transferase-mediated dUTP nick-end labeling) assay; SCD, sperm chromatin dispersion test; SCSA, sperm chromatin structure assay; NR, not reported; NA, not applicable; CV, coefficient of variation.

adequate precision and repeatability as shown by the low coefficient of variation, which validate the assays for SDF assessment.

To sum up, SDF tests measure the proportion of cells with DNA damage or fragmentation. Test results reflect the quality of the whole semen specimen, and offer prognostic information as regards pregnancy, both naturally and assisted. Having emerged as a complementary tool to routine semen analysis, SDF testing may enable clinicians to better evaluate, counsel, and manage the male patient and the couple as a whole, particularly in challenging clinical scenarios like varicocele and borderline/normal semen analysis, unexplained infertility, recurrent miscarriages, and failed IUI, IVF and ICSI. We advise that SDF testing should be carried out in laboratories equipped with proper instrumentation, skilled technicians, and enrolled in internal and external quality control programs.

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Footnote

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

References

1. Kadioglu A, Ortac M. The role of sperm DNA testing on male infertility. *Transl Androl Urol* 2017;6:S600-3.
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. Jarow J, Sigman M, Kolettis PN, et al. Optimal Evaluation of the Infertile Male. Available online: <http://www.auanet.org/education/guidelines/male-infertility-d.cfm> [Last accessed on 2017 Mar 2].
 4. Jungwirth A, Diemer T, Dohle GR, et al. Guidelines on Male Infertility. Available online: https://uroweb.org/wp-content/uploads/17-Male-Infertility_LR1.pdf [Last accessed on 2017 Feb 3].
 5. Agarwal A, Cho CL, Esteves SC. Should we evaluate and treat sperm DNA fragmentation? *Curr Opin Obstet Gynecol* 2016;28:164-71.
 6. Esteves SC. Clinical relevance of routine semen analysis and controversies surrounding the 2010 World Health Organization criteria for semen examination. *Int Braz J Urol* 2014;40:443-53.
 7. Esteves SC, Hamada A, Kondray V, et al. What every gynecologist should know about male infertility: an update. *Arch Gynecol Obstet* 2012;286:217-29.
 8. Majzoub A, Esteves SC, Gosálvez J, et al. Specialized sperm function tests in varicocele and the future of andrology laboratory. *Asian J Androl* 2016;18:205-12.
 9. Practice Committee of the American Society for Reproductive Medicine. Diagnostic evaluation of the infertile male: a committee opinion. *Fertil Steril* 2015;103:e18-25.
 10. 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.
 11. 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.
 12. Shridharani A, Owen RC, Elkelany OO, et al. The significance of clinical practice guidelines on adult varicocele detection and management. *Asian J Androl* 2016;18:269-75.
 13. Esteves SC, Chan P. A systematic review of recent clinical practice guidelines and best practice statements for the evaluation of the infertile male. *Int Urol Nephrol* 2015;47:1441-56.
 14. Feijó CM, Esteves SC. Diagnostic accuracy of sperm chromatin dispersion test to evaluate sperm deoxyribonucleic acid damage in men with unexplained infertility. *Fertil Steril* 2014;101:58-63.e3.
 15. Evenson DP. The Sperm Chromatin Structure Assay (SCSA®) and other sperm DNA fragmentation tests for evaluation of sperm nuclear DNA integrity as related to fertility. *Anim Reprod Sci* 2016;169:56-75.
 16. 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.
 17. Sharma R, Ahmad G, Esteves SC, et al. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay using bench top flow cytometer for evaluation of sperm DNA fragmentation in fertility laboratories: protocol, reference values, and quality control. *J Assist Reprod Genet* 2016;33:291-300.
 18. Sharma RK, Sabanegh E, Mahfouz R, et al. TUNEL as a test for sperm DNA damage in the evaluation of male infertility. *Urology* 2010;76:1380-6.
 19. McEvoy A, Roberts P, Yap K, et al. Development of a simplified method of human semen storage for the testing of sperm DNA fragmentation using the Halosperm G2 test kit. *Fertil Steril* 2014;102:981-8.
 20. Fernández JL, Muriel L, Goyanes V, et al. Simple determination of human sperm DNA fragmentation with an improved sperm chromatin dispersion test. *Fertil Steril* 2005;84:833-42.
 21. Giwercman A, Richthoff J, Hjellund H, et al. Correlation between sperm motility and sperm chromatin structure assay parameters. *Fertil Steril* 2003;80:1404-12.
 22. Giwercman A, Spano M, Lähdetie J, et al. Quality assurance of semen analysis in multicenter studies. *Asclepius. Scand J Work Environ Health* 1999;25 suppl 1:23-5.

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