

Technical aspects of sperm DNA fragmentation testing, methods to select sperm with low DNA fragmentation, and usefulness of redox potential measurement in male infertility

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Response to: Henkel R. Clinical utility of sperm DNA fragmentation testing: A commentary. *Transl Androl Urol* 2017;6:S632-5.

Submitted May 01, 2017. Accepted for publication May 04, 2017.

doi: 10.21037/tau.2017.05.24

View this article at: <http://dx.doi.org/10.21037/tau.2017.05.24>

We read with interest the thought provoking commentary by Dr. Henkel (1) in response to the practice recommendations by Agarwal *et al.* (2). Dr. Henkel appropriately pointed out the shortcomings of conventional semen analysis in predicting the outcome of assisted reproductive technologies (ART), to which we concur as discussed extensively elsewhere (3-7). The author also recognized the role of sperm DNA fragmentation (SDF) tests as complementary to semen analysis. Moreover, Dr. Henkel discussed important technical issues concerning SDF tests and argued that the guideline by Agarwal *et al.* (2) could have been more detailed with regards to the technical differences among the various tests. Lastly, Dr. Henkel suggested the use of tests to measure redox potential as an alternative to SDF testing. Here, in our response to his commentary, we wish to elaborate upon the (I) technical aspects of SDF testing, (II) methods to select sperm with low DNA fragmentation for intracytoplasmic sperm injection (ICSI), and (III) usefulness of redox potential measurement in male infertility.

Although clinicians would like to have a definitive diagnostic test for male infertility with precise cutoff level above or below which they could make a decision regarding diagnosis and management, it is unlikely that the perfect test will be available in the near future (8). The reasons stem for the complexity of semen as it consists of a heterogeneous population of spermatozoa produced over

approximately 70–80 days. Furthermore, spermatozoa are mixed with and diluted by fluid secreted from accessory glands. As a result, semen quality is governed by (I) the state of testicular sperm production (II) epididymal transit, and (III) activity of accessory glands (9). To complicate matters, laboratory analysis of semen is subjected to variability due to operational and technical factors and ejaculatory abstinence period (4,10).

Despite recognizing the current limitations of SDF testing, we ponder that conventional semen analysis, i.e., sperm concentration, motility, and morphology, does not live up to the level of scrutiny or rigor that is often asked from SDF testing (8). In fact, routine semen analysis lacks the power to discriminate between fertile and infertile men, and rarely provides a clear path for management, unless the results are in extremely abnormal range (3,4,8). Given the overwhelming evidence of a negative association between SDF and male infertility and impaired reproductive outcomes, both natural and assisted, it seems sound to include SDF as an integral test of semen analysis to improve its predictive diagnostic value (6,8,11,12).

We agree with Dr. Henkel that SDF testing methods are not interchangeable. Indeed, SDF testing does measure different aspects of DNA, although these aspects are interrelated to a greater or lesser extent by the properties of the DNA molecule (13). The ideal method to measure SDF

Table 1 Summary of the effect on sperm DNA fragmentation (SDF) reduction using different strategies. Reprint with permission from Esteves *et al.* (17)

Method	SDF relative reduction (%)	SDF assay	Study
Short abstinence	25	SCD	Gosálvez <i>et al.</i> , 2011
	22	TUNEL	Agarwal <i>et al.</i> , 2016
Gradient centrifugation	22–44*	SCD	Gosálvez <i>et al.</i> , 2011
	56.6	SCD	Xue <i>et al.</i> , 2014
Swim-up	33.3	SCD	Parmegiani <i>et al.</i> , 2010
	38.1	SCD	Xue <i>et al.</i> , 2014
MACS	26.7	TUNEL	Tsung-Hsein <i>et al.</i> , 2010
	None	TUNEL	Nadalini <i>et al.</i> , 2014
PICSI	67.9	SCD	Parmegiani <i>et al.</i> , 2010
	None	SCSA	Rashki Ghaleno <i>et al.</i> , 2016
IMSI	78.1	TUNEL	Hammoud <i>et al.</i> , 2013
	None	SCD	Maettner <i>et al.</i> , 2014
Testicular sperm	79.7	SCD	Esteves <i>et al.</i> , 2015
	79.6	TUNEL	Greco <i>et al.</i> , 2005
	66.5	TUNEL	Moskovtsev <i>et al.</i> , 2010

*, combined with frequent ejaculation and short ejaculatory abstinence. MACS, magnetic-activated cell sorting; PICSI, physiologic intracytoplasmic sperm injection; IMSI, intracytoplasmic morphologically selected sperm injection; TUNEL, terminal deoxyribonucleotide transferase-mediated dUTP nick-end labeling; SCD, sperm chromatin dispersion; SCSA, sperm chromatin structure assay.

is still to be determined, so any decision to consider SDF testing should take into account the limitations of testing methods and the possible benefits for clinical outcomes, as highlighted in our recent publications (2,11,13-16). Therefore, suggesting the use of a specific assay to measure SDF is not scientifically correct until a gold standard method is established. Nevertheless, it is essential that a reliable SDF assay with a validated threshold is used (6,8).

Dr. Henkel ponders that more studies have to be conducted to widen and clarify the scope of sperm DNA testing. In fact, the number of studies published in this regard is increasing steadily; the reader will be astonished to learn that more than 2,000 articles about sperm DNA damage are indexed in PubMed, half of which was published in the last 5 years. As far as the tests to select viable sperm with reduced SDF for ART are concerned, the literature is also rich in studies comparing different methods. In a recent report, we examined some of these tests and concluded that they in general reduce the amount of sperm with SDF in the selected specimen, but none of them, alone or combined, can completely remove sperm with SDF (*Table 1*) (17).

However, a recent report indicated that among motile sperm organelle morphology examination (IMSI), physiological ICSI (PICSI) using hyaluronic acid-selected spermatozoa, frequent ejaculation, and testicular sperm, the latter was more advantageous with regards to ICSI live birth rates (18).

Lastly, Dr. Henkel pointed out that given oxidative stress is a major contributor to SDF, it would be beneficial and possibly easier to determine the redox potential either in semen or in the serum to determine that OS is causing SDF. Indeed, oxidation-reduction potential (ORP) has been advocated as a new measure of oxidative stress, as it reflects the balance between the total available oxidants and reductants in a given specimen (19). ORP seems to be a simple alternative to multiple individual markers of OS such as ROS (chemiluminescence), antioxidants (total antioxidant capacity and individual enzymatic and nonenzymatic antioxidants) and lipid peroxidation (MDA) (19-22). In addition to real-time measurement of redox capacity by ORP in semen specimens, other qualitative methods

to measure OS have been investigated. In a recent multi-center study, we analyzed nitroblue tetrazolium reactivity in human semen as a potential marker of OS (23). Despite promising results, the potential clinical value of the newly aforementioned markers of OS warrants further validation.

Acknowledgements

None.

Footnote

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

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Cite this article as: Esteves SC, Majzoub A, Agarwal A. Technical aspects of sperm DNA fragmentation testing, methods to select sperm with low DNA fragmentation, and usefulness of redox potential measurement in male infertility. *Transl Androl Urol* 2017;6(Suppl 4):S645-S639. doi: 10.21037/tau.2017.05.24