New Multi-Parametric Flow Cytometry Analysis Unveils SCSA Limitations, Enhancing Sperm DNA Fragmentation Assessment.

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Introduction: Chances of natural conception or intra-uterine-insemination (IUI) success are significantly reduced when a sperm DNA fragmentation index (DFI) superior to 30% is observed. But this notion and the usefulness of the test remains controversial. The present set of experiments inspects the clinical performance of the test and provides explanations for the discrepancy in results.

Method: 1) DFI scores from an ejaculate and serial dilutions of it were compared to identify any interference factors in the test. 2) In another set of experiments, sperm cells were either single labelled with Acridin (classic method) or double labelled with Acridin and Hoechst. DFI scores were then compared to verify if Hoechst addition creates any interference. 3) In a third set of experiments, the new multiparametric method (MPM) was set by a) double staining sperm with Hoechst and Acridin, and b) characterizing the constituents using a gating combination of forward/side scattering and Hoechst fluorescence. The MPM allowed to discriminate between debris, spermatozoa, and leucocytes without involving Acridin in the gating strategy. Then, the different constituents could be independently plotted into the classical analysis template for DFI calculation using their Acridin fluorescence. DFIs were measured from three spermatozoa-free samples (blanks) and twenty-two samples from different men using the classical method and the MPM. Results from the two methods were compared.

Results: 1) Serial dilutions yielded different results compared to the undiluted sample, thus suggesting an interference in the test. 2) Hoechst-Acridin double stained samples had statistically identical DFIs (Paired T-test P value >0,76) compared to the Acridin single stains, thereby ruling out Hoechst interference in the classical test. 3) MPM did not detect any spermatozoa in the blank sample, whereas 5.6% ± 1.4 of the events from the blank were misidentified as spermatozoa with the classical method. The misidentified spermatozoa average DFI was 71.2% ± 16.6. The number of events identified as spermatozoa and the average DFI were both significantly lower with the MPM when compared to the classical method (P<0,001). Also, an average of 32.7% ± 8.7 of events identified as spermatozoa by the classical method were not spermatozoa but mostly debris according to the MPM. Finally, a weak correlation between results obtained by the two methods (r= 0,21) was obtained. However, patients with abnormally high DNA fragmentation levels according to the classical method were not necessarily considered abnormal by the MPM and vice versa.

Conclusion: The classical method does not allow to discriminate spermatozoa from other sperm constituents. This flaw is likely to have a major impact on DFI results, their clinical value, and the choice of treatment. The present work suggests 1) that sperm DNA fragmentation tests as performed presently don't meet the clinical laboratory standards for diagnostic testing 2) a better way to perform the test and 3) the necessity to establish new clinical reference values.

It raises the possibility that 1) debris prevalence in oligozoospermia samples compared to normal ones has skewed the results and led the scientific community to conclude to a correlation between oligozoospermia and poor DNA integrity, 2) testicular spermatozoa appear like having better DNA integrity than ejaculated ones only because less debris are present during the analyse, 3) that spermatozoa selection techniques improve DFI scores by removing debris rather than selecting optimal sperm. Further research is required to verify those hypotheses.