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Editorial Commentary

Flow Cytometry TUNEL Standardization for Assaying Sperm DNA Fragmentation

We read with great interest Muratori's paper (Muratori et al, 2010), because we believe that terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) assay standardization is a matter of great concern and that there are certain steps that must be followed to achieve accurate results.

We agree with the authors of the paper but feel the need to stress several important considerations. As the authors mention in their introduction, the labeling of DNA breaks should be carried out by nuclear staining with propidium iodide (PI; Muratori et al, 2008). We have noted in oligozoospermia samples a clear increase in the percentage of PI-negative events with forward scatter/side scatter similar to sperm, yielding lower results. The upper sperm count limit that could be considered a threshold for this phenomenon is difficult to establish; thus, TUNEL/PI should be applied to all samples, and reference values should be recalculated for future clinical use.

The authors propose 2 methods to quantify the DNA fragmentation percentage within a sperm sample: a threshold-setting method (Muratori et al, 2000) and a blank subtraction method. The results differed substantially between these different methods. The authors chose the first one, stating that it correlated well with semen quality; in our hands, however, no association was found between DNA fragmentation and motility, morphology, and sperm count (Pearson correlation; n = 210). We believe that our data do not invalidate the use of this method because new assays might give different information than previously determined parameters.

As the authors report, fixed samples should not be stored at 4°C or at −20°C because it affects reproducibility. In our experience, the results obtained using fresh compared with stored samples varied unpredictably by ~50% (from −47% to +52%). Other authors suggest storing the samples (4°C, −20°C) until testing (Stronati et al, 2006; Ramos et al, 2008), but we do not agree with them. This point is essential for assay standardization. When the procedure is followed rigorously, the results are precise: our intra-assay coefficient of variation is 3% (aliquot processed immediately after fixation), which is in agreement with data reported by Muratori et al (2010). Finally, we congratulate Muratori's team because their comprehensive studies have helped us improve the analysis of sperm DNA fragmentation.

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References

Muratori M, Piomboni P, Baldi E, Filimberti E, Peccioli P, Moretti E, Gambera L, Baccetti B, Biagiotti R, Maggi M. Functional and ultrastructural features of DNA fragmented human sperm. *J Androl*. 2000;2:903–912.

Muratori M, Forti G, Baldi E. Comparing flow cytometry and fluorescence microscopy for analyzing human sperm DNA fragmentation by TUNEL labeling. *Cytometry A*. 2008;73:785–787.

Muratori M, Tamburrino L, Tocci V, Constantino A, Marchiani S, Giachini C, Laface I, Krausz C, Meriggiola MC, Forti G, Baldi E. Small variations in crucial steps of TUNEL assay coupled to flow cytometry greatly affect measures of sperm DNA fragmentation. *J Androl*. 2010;31:336–345.

Ramos L, van der Heijden GW, Derijck A, Berden JH, Kremer JA, van der Vlag J, de Boer P. Incomplete nuclear transformation of human spermatozoa in oligo-astheno-teratospermia: characterization by indirect immunofluorescence of chromatin and thiol status. *Human Reprod*. 2008;23:259–270.

Stronati A, Manicardi GC, Cecati M, Bordicchia M, Ferrante L, Spanò M, Toft G, Bonde JP, Jönsson BA, Rignell-Hydbom A, Rylander L, Giwercman A, Pedersen HS, Bonefeld-Jørgensen EC, Ludwicki JK, Lesovoy V, Sakkas D, Bizzaro D. Relationships between sperm DNA fragmentation, sperm apoptotic markers and serum levels of CB-153 and pp-DDE in European and Inuit populations. *Reproduction*. 2006;132:949–958.

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