

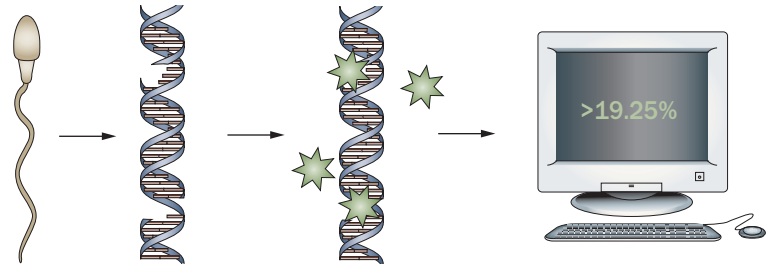
MALE FACTOR INFERTILITY

TUNEL evaluation of sperm DNA damage

A study from the Cleveland Clinic reports standardized results of the TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling) assay for the assessment of sperm DNA damage. This assay measures the presence of free 3' hydroxyl groups indicative of DNA strand fragmentation. Sperm DNA damage is a marker of outcome for both spontaneous pregnancy and assisted reproduction techniques, and as such is an important clinical parameter for men undergoing fertility evaluation. To date, however, threshold values for TUNEL are not as widely established as those for an alternative test of DNA damage, the more-technically demanding sperm chromatin structure assay. The present study sought to address this deficiency.

The researchers enrolled 194 men who were seeking fertility care for a range of etiologies, and 25 controls with normal results of semen analysis and either proven or unproven fertility. Sperm DNA fragmentation was evaluated using the TUNEL assay (Apo-Direct kit; Pharmingen, San Diego, CA), followed by flow cytometry analysis.

A cut-off value of 19.25% fragmentation maximized the sensitivity and specificity of the assay (65% and 100%, respectively), with all controls having levels of DNA



damage below this point. High specificity is important in such a test, as a key aim is to avoid false-positive results in healthy men and to definitively identify men who contribute to the fertility problem of a couple. Overall, patients had higher levels of DNA damage than did controls (29.5% versus 11.9%; $P < 0.001$). Variability between and within both the observer and the assay was low—generally less than 10%.

“This test can be used effectively to establish the DNA integrity of the sperm,” says Ashok Agarwal, Director of the Andrology Laboratory at the Cleveland Clinic, who led the research. “It can be offered to a group of select patients with idiopathic infertility or when severe oxidative stress might be adversely affecting semen quality.” These reference values can be used and adapted by other centers, and the TUNEL test can be

offered by any facility with access to flow cytometry, before other, more-expensive, assisted reproductive techniques are considered.

The TUNEL assay works on the premise that an increasing level of DNA fragmentation is itself pathologic, and does not offer information on the specific genes that might be affected by this process. In future, fine-tuning of the test might help to better identify and characterize the DNA damage that it measures. Additional steps to decondense the highly compacted sperm DNA, and to distinguish viable from nonviable sperm cells, might help in this regard.

Nick Groves-Kirkby

Original article Sharma, R. K. *et al.* TUNEL as a test for sperm DNA damage in the evaluation of male infertility. *Urology* doi:10.1016/j.urolgy.2010.04.036