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The Sperm Chromatin Dispersion Test: A Simple Method for the Determination of Sperm DNA Fragmentation

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Sperm DNA fragmentation is being increasingly recognized as an important cause of infertility. We herein describe the Sperm Chromatin Dispersion (SCD) test, a novel assay for sperm DNA fragmentation in semen. The SCD test is based on the principle that sperm with fragmented DNA fail to produce the characteristic halo of dispersed DNA loops that is observed in sperm with non-fragmented DNA, following acid denaturation and removal of nuclear proteins. This was confirmed by the analysis of DNA fragmentation using the specific DNA Breakage Detection-Fluorescence In Situ Hybridization (DBD-FISH) assay, which allows the detection of DNA breaks in lysed sperm nuclei. Sperm suspensions either prepared from semen or isolated from semen by gradient centrifugation were embedded in an agarose microgel on slides and treated with 0.08 N HCl and lysing solutions containing 0.8 M dithiothreitol (DTT), 1% sodium dodecyl sulfate (SDS), and 2 M NaCl. Then, the slides were sequentially stained with DAPI (4',6-diamidino-2-phenylindole) and/or the Diff-Quik reagent, and the percentages of sperm with nondispersed and dispersed chromatin loops were monitored by fluorescence and brightfield microscopy, respectively. The results indicate that all sperm with nondispersed chromatin displayed DNA fragmentation, as measured by DBD-FISH. Conversely, all sperm with dispersed chromatin had very low to undetectable DBD-FISH labeling. SCD test values were significantly higher in patients being screened for infertility than in normozoospermic sperm donors who had participated in a donor insemination program. The coefficient of variation obtained using 2 different observers, either by digital image analysis (DIA) or by brightfield microscopy scoring, was less than 3%. In conclusion, the SCD test is a simple, accurate, highly reproducible, and inexpensive method for the analysis of sperm DNA fragmentation in semen and processed sperm. Therefore, the SCD test could potentially be used as a routine test for the screening of sperm DNA fragmentation in the andrology laboratory.

Key words: Structure, human sperm, decondensation

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