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Variability and Laboratory Factors Affecting the Sperm Chromatin Structure Assay in Human Semen

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During the past decade, the sperm chromatin structure assay (SCSA) has become an important tool for assessing semen quality in the human andrology laboratory. The SCSA uses the metachromatic properties of the fluorescent dye acridine orange (AO) in combination with flow cytometry to determine the sperm DNA susceptibility to denaturation in situ. The objective of this study was to evaluate laboratory factors affecting the SCSA and the variation between replicates. Semen ejaculates from 3 healthy volunteers were analyzed using the SCSA protocol as described by Evenson and Jost (2000), determining the X-mean, Y-mean, DNA fragmentation index (DFI), standard deviation of DFI (SD-DFI), and high DNA stainability (HDS). In experiment 1, the effects of thawing time, time of day, day, laboratory technician, donor, and incubation period before analysis were investigated. In experiment 2, the effects of sheath fluid, AO equilibration buffer, day, laboratory technician, donor, and incubation period before analysis were investigated. A significant difference was found between the 3 donors with respect to the X-mean, Y-mean, DFI, SD-DFI, and HDS. It was shown that incubation of the semen samples on ice postthaw had a significant effect on the X-mean, Y-mean, DFI, and SD-DFI. The laboratory technician conducting the analysis accounted for up to 15.4% for the variation of the SCSA measurements. The time of day affected the variation for the Y-mean (23.5% of the total variation of the Y-mean), and the day affected the variation for the X-mean (82.8% of the total variation of the X-mean). Incubation on ice for 5 to 25 minutes postthaw had a significant effect on the DFI and SD-DFI in both experiments. This study shows that several protocol steps in the SCSA affect the results obtained from the assay. Precise protocol description and standardization of the SCSA are therefore essential to achieve high agreement within and between different laboratories.

Key words: Flow cytometry, DNA integrity, spermatozoa, acridine orange

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