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Evaluation of Chromosome Breakage and DNA Integrity in Sperm: An Investigation of Remote Semen Collection Conditions

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Collection of ejaculated semen at a remote site (outside of the laboratory) would facilitate participation rates and geographic diversity in reproductive epidemiology studies. Our study addressed concerns that remote collection and overnight mail return might induce chromosome/DNA damage. We collected semen from 10 healthy men. Part of each sample was snap frozen in liquid nitrogen and the rest held at 22 ± 1°C for 24 hours in a transport container (simulating ambient temperature during overnight return) then snap frozen. DNA breakage and fragmentation were measured using tandem-label sperm-fluorescence in situ hybridization (FISH), terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL), and neutral comet assay. Tandem-label sperm-FISH and TUNEL detected no statistically significant difference between sperm fresh frozen (FF) and those frozen after 24 hours (F24). The mean frequency of chromosome breakage per 10 000 cells scored in sperm-FISH for FF and F24 was 10.5 ± 1.3 breaks and 11.2 ± 1.1 breaks, respectively (P = .69, Student's t test). The mean frequency of TUNEL-positive cells per 2000 cells scored in FF and F24 was 136 ± 29 and 213 ± 28 cells, respectively, which approached but did not reach statistical significance (P = 0.07, Student's t test). The neutral comet assay detected a statistically significant difference in DNA strand breakage between the 2 groups (percentage of DNA in the tail P = 0.037; tail moment P = 0.006; and tail length P = 0.033, all Student's t test). The mean frequency of damage denoted by tail length in µm per 100 cells scored in FF and F24 was 175.0 ± 15.5 and 152.2 ± 17.6 µm, respectively. Tandem-label sperm-FISH, TUNEL, and neutral comet assay are useful analytical techniques for laboratory-based studies of human sperm genomic integrity; however, for field studies incorporating the nonrefrigerated return of semen after 24 hours, only chromosome breakage at a level that can be detected using tandem-label sperm-FISH was unaffected. TUNEL and neutral comet assay need further study before they are used in specimens collected at remote sites and transported to a central laboratory.

Key words: Snap-freezing, sperm-FISH, TUNEL, neutral comet assay

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