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Semen Characteristics After Overnight Shipping: Preservation of Sperm Concentrations, HspA2 Ratios, CK Activity, Cytoplasmic Retention, Chromatin Maturity, DNA Integrity, and Sperm Shape

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We tested several approaches that can be used to preserve sperm attributes and the objective biochemical markers of sperm maturity and function for assessment in a remote centralized laboratory after overnight shipping of semen samples. Addition of phenyl-methyl-sulfonyl-fluoride (PMSF) to a final concentration of 20 µg/mL semen at 4°C has preserved sperm concentrations and HspA2 isoform ratios, even at room temperature, simulating a shipping delay in moderate ambient temperatures. Regarding the attributes of individual spermatozoa, the patterns of CK-immunocytochemistry (demonstrates cytoplasmic retention in diminished-maturity spermatozoa); aniline blue staining pattern (tests chromatin maturity); sperm shape assessed by both Kruger strict morphology and computer assisted morphometry; and sperm DNA integrity, as tested by DNA nick translation, all remained unchanged. Thus, the PMSF-4°C conditions preserved sperm concentrations and the cytoplasmic and nuclear biomarkers of sperm cellular maturity and function for next-day analysis. This shipping method will facilitate the early detection of subtle changes in semen quality that can affect sperm function, even when there has been no decline in sperm concentrations to signal possible toxic effects. Furthermore, sample preservation will enable investigators to evaluate semen for toxicology studies and for diagnosis of male infertility from remote locations. Home collection of semen should enhance study participation, and semen assessment in centralized laboratories will address concerns regarding interlaboratory variations and quality control.

Key words: Reproductive toxicity, male infertility, cytoplasmic and nuclear biomarkers