EVALUATION OF ACROSOMAL STATUS AND SPERM VIABILITY IN FRESH AND CRYOPRESERVED SPECIMENS BY THE USE OF FLUORESCENT PEANUT AGGLUTININ LECTIN AND HYPO-OSMOTIC SWELLING TEST

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Fluorescein-conjugated peanut agglutinin (FITC-PNA) lectin-labeling is an established procedure for assessing the status of the human sperm acrosome. However, unlike the triple-stain technique, FITC-PNA labeling does not provide a simultaneous assessment of cellular viability. This is important to distinguish between 'physiological' and 'degenerative' acrosome loss. In this study, we evaluated whether the hypo-osmotic swelling test (HOST) and staining by Hoechst-33258 can be used as a vital marker in combination with FITC-PNA in fresh and cryopreserved spermatozoa. Spermatozoa were exposed to a hypo-osmotic solution for 60 minutes, and then incubated in a 1µg/mL solution of the fluorescent dye Hoechst-33258 for 10 minutes. Excess stain was removed by washing in phosphate-buffered saline (PBS) solution, and the pellet was resuspended in 100 µL of PBS. Twenty microliters of this solution was subsequently smeared on a microscope slide, and fixed in ice-cold methanol to permeabilize the sperm membranes. The fixed smears were incubated in a 40-µg/mL FITC-PNA solution for 20 minutes. Simultaneous assessment of acrosome and viability scores was done in a fluorescent microscope equipped with appropriate filters and phase contrast illumination. The same slide was examined for FITC-PNA labeling, sperm tail swelling, and for Hoechst-33258 staining by interchanging the filters and phase contrast optics. In fresh specimens, HOST results (80.2% ± 14.2%) were comparable to those obtained using the Hoechst-33258 method (74.8% ± 22.1%; r = 0.95; p <0.0001). However, in cryopreserved specimens these did not correlate (r = 0.22). In conclusion, FITC-PNA labeling in conjunction with HOST provides a simple but effective method for establishing the acrosomal membrane status and viability in fresh human spermatozoa. This technique, however, is not suitable for cryopreserved specimens.