Title: ASSOCIATION OF SPERM DNA FRAGMENTATION MEASURED BY TUNEL WITH SPERM MATURATION MEASURED BY DNA CYTOMETRY

Objective: The terminal uridine deoxynucleotidyl transferase (dUTP) nick end labeling
(TUNEL) measures single and double DNA strand breaks. DNA cytometry using propidium iodide (PI) can provide information on sperm chromatin maturation/condensation status based on the fluorescent intensity of PI. The aim of our study was to evaluate the relationship between sperm DNA fragmentation as measured by TUNEL assay with chromatin maturation/condensation by DNA cytometry.

**Design:** Prospective study

**Materials and Methods:** 117 semen samples were prepared for TUNEL assay using the Apoptosis Detection Kit (APO-Direct, BD Bioscience). PI/RNase were added after the FITC-dUTP reaction for DNA staining according to manufacturer’s instructions. Flowcytometry was used and data acquisition was done for FITC (FL1) as well as PI (FL2-area).

**Results:** The percentage of TUNEL$^{+ve}$ sperm were positively correlated with percentage of immature $(r = 0.36, p = <0.001)$ and subhaploid sperm $(r = 0.53, p = <0.001)$. TUNEL$^{+ve}$ spermatozoa showed a negative correlation with percentage of mature (haploid) sperm $(r = -0.55, p = <0.001)$. % haploid mature sperm were positively correlated with TUNEL$^{-ve}$ percentage $(r = 0.7, p < 0.001)$. There was a significant negative interdependence between percentage of haploid sperm and subhaploid sperm $(r = -0.76, p <0.001)$ and between haploid and % of immature sperm $(r = -0.44, p <0.001)$.

**Conclusions:** Low DNA fragmentation detected by TUNEL assay was associated with sperm maturation/condensation detected by DNA cytometry. We recommend evaluating semen samples by both assays for better understanding of the underlying pathology i.e. sperm immaturity, apoptosis and/or oxidative stress associated with abnormal spermatozoa.

**Support:** None

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