Superparamagnetic Extraction Of Ejaculated Human Spermatozoa With Early Apoptotic Changes
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**Introduction and Objective:** Externalization of phosphatidylserine (EPS) on the outer leaflet of the plasma membrane is one of the earliest events of apoptosis. There is an inverse correlation between the proportion of EPS+ sperm and the semen quality. The aim of our study was to separate the spermatozoa with and without EPS and to evaluate the caspase activity (aCp), mitochondrial membrane potential ($\Delta\psi_m$) and DNA fragmentation in both the groups.

**Design:** A prospective study

**Methods:** Semen samples from healthy donors were pooled to create 18 pools of semen. After separation by 2-layer density gradient centrifugation, the mature and the immature fractions were collected as the 90% pellet and at the interface between the 47% and 90% layers, respectively. Each of these fractions were further divided into sub fractions of spermatozoa depending on EPS, using Annexin V labeled superparamagnetic beads, that bind specifically to EPS. The passage through a magnetic field separated out the EPS+ sperm from the EPS- ones. Using the carboxyfluorescein derivatives the levels of aCp 9, 8, & 3 were estimated in both the fractions. Sperm with intact $\Delta\psi_m$ were estimated in all the subsets using a lipophilic cationic dye. Spermatozoa with intact $\Delta\psi_m$ excite an intense red fluorescence while those with disrupted $\Delta\psi_m$ excite green. The proportion of cells with DNA fragmentation (TU+) was measured in the same fractions using the TUNEL assay. All fluorescence signals were analyzed using flow cytometry.

**Results:** The EPS+ sperm had significantly higher levels of aCp, disrupted $\Delta\psi_m$ and TU+ than the EPS- ones ($p < 0.01$).

**Conclusions:** The EPS negative spermatozoa isolated by superparamagnetic separation have significantly lower levels of aCp, disrupted $\Delta\psi_m$ and TU+ than the EPS+ ones. We speculate that the removal of EPS+ spermatozoa may improve the outcome of assisted reproduction failures, attributed to high levels of sperm DNA damage.
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