

## Impact of Apoptosis on Sperm Morphology and the Sperm Deformity Index

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**Introduction:** Sperm apoptosis has been implicated as a cause of male infertility.

Externalization of phosphatidylserine (PS) to the sperm outer membrane leaflet is considered to mark terminal apoptosis. Activated caspase-3 levels and integrity of the mitochondrial membrane potential (MMP) are other markers of apoptosis in sperm. The relationship between apoptosis and the sperm morphology has not been well characterized. Sperm deformity index (SDI) is a novel quantitative expression of sperm morphological quality that has been shown to have an enhanced predictive power and reproducibility compared to assessing the percentage of sperm with normal morphology. The aim of this study was to investigate the relationship between apoptosis and the sperm morphology profile including the SDI score in a large group of healthy men.

**Material and methods:** Semen specimens collected from 50 healthy donors (sperm count  $\geq 20 \times 10^6$  spermatozoa/mL and at least 50% progressive sperm motility) were prepared by density gradient centrifugation. Magnetic cell sorting (MACS) after incubating the prepared sperm with paramagnetic annexin V-conjugated microbeads eliminated spermatozoa with externalized PS. The procedure delivers 2 sperm fractions: annexin-negative (non-apoptotic) and annexin-positive (apoptotic).

Activated caspase-3 levels and MMP integrity were assessed as markers of apoptosis in the annexin-negative and positive aliquots following MACS. Sperm morphology was assessed using the Tygerberg's strict criteria. The SDI score was calculated by dividing the total number of deformities observed by the number of sperm randomly selected and evaluated, irrespective of their morphological normality. Results are expressed as mean  $\pm$  standard deviation. Pair-wise comparisons were made using Student's t-test and correlation coefficients were calculated by Spearman's rank test.

**Results:** The annexin-negative fraction separated by MACS had significantly lower mean SDI score compared to the annexin-positive fraction (mean SDI score  $1.39 \pm 0.1$  and  $1.77 \pm 0.27$ , respectively,  $P < 0.0001$ ). Also, the annexin-negative fraction had significantly higher mean percentage of sperm with normal morphology compared to the annexin-positive fraction (means  $19 \pm 9.2\%$  and  $13 \pm 6.4\%$ , respectively,  $P < 0.0001$ ). SDI scores showed significant positive correlation with caspase-3 activation ( $r = 0.75$ ,  $P < 0.0001$ ) and significant negative correlation with MMP integrity ( $r = -0.77$ ,  $P < 0.0001$ ). The proportion of sperm with normal morphology had a significant negative correlation with caspase-3 activation ( $r = -0.37$ ,  $P = 0.0002$ ) and significant positive correlation with MMP integrity ( $r = 0.42$ ,  $P < 0.0001$ ). Based on the value of  $r$  statistics the interdependence between the SDI score and apoptotic markers was stronger than that between normal sperm morphology and apoptotic markers.

**Conclusions:** Apoptosis shows significantly positive correlation with sperm morphology using the SDI scores. Non-apoptotic spermatozoa show lower incidence of morphological anomalies.