

PRESERVATION OF MITOCHONDRIAL INTEGRITY AND IMPROVEMENT OF CRYOSURVIVAL RATES BY MAGNETIC CELL SORTING

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Background and aims: Superparamagnetic annexin V - conjugated microbeads are able to eliminate spermatozoa with externalized phosphatidylserine, a membrane feature of apoptotic cells as well as spermatozoa with deteriorated plasma membrane. Our objective was to evaluate the effects of annexin V - Magnetic Cell Sorting (MACS) in cryopreservation-thawing protocols and on integrity of sperm mitochondrial transmembrane potential and mitochondrial integrity survival rate.

Materials and Methods: Mature spermatozoa of ten healthy donors were prepared by density gradient centrifugation and divided into 2 aliquots afterwards. The first one was subjected to annexin-V MACS followed by cryopreservation and thawing, while the second was cryopreserved-thawed without MACS to serve as control.

Results: Annexin-negative sperm separated by MACS showed significantly higher levels of intact mitochondria (iTMP) following cryopreservation-thawing ($45.4 \pm 8.6\%$) compared to sperm that were not separated ($15.8 \pm 4.6\%$, $p < 0.01$). Similarly, annexin-negative spermatozoa had a superior mitochondrial integrity survival rate than sperm cryopreserved without Magnetic Cell Sorting (56.9 ± 14.0 vs. 20.8 ± 6.6 , $p < 0.01$).

Conclusion: Separating a distinctive population of non-apoptotic spermatozoa with intact membranes may optimize cryopreservation-thawing outcome. Magnetic Cell Sorting using annexin-V microbeads enhances the integrity of transmembrane mitochondrial potential and mitochondrial integrity cryosurvival rates following cryopreservation.