

MITOCHONDRIAL MEMBRANE POTENTIAL ($\Delta\psi_m$) IN DIFFERENT SUBSETS OF FRESH AND CRYOPRESERVED HUMAN SPERMATOOZA

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$\Delta\psi_m$ is an important indicator of the functional integrity of spermatozoa. Alterations in mitochondrial membrane potential may occur during early stages of apoptosis. Also, freeze-thaw procedures may induce sublethal damage to sperm structure and functional characteristics. The objective of our study was to examine the $\Delta\psi_m$ in mature and immature subsets of fresh and cryopreserved ejaculated human spermatozoa. Semen samples from healthy donors were pooled together to create 10 pools of cryopreserved (CRP) and 8 pools of fresh semen. After 2-layer density gradient centrifugation, the mature and immature fractions were separated. The proportion of cells with intact $\Delta\psi_m$ was measured in all of the subpopulations using a lipophilic cationic dye that fluoresces red in intact $\Delta\psi_m$ and green with disrupted $\Delta\psi_m$. The results were analyzed using bi-color flow cytometry. Our results indicate that the mature subset of ejaculated spermatozoa have significantly higher levels of intact $\Delta\psi_m$ as compared to the immature subset, thereby providing an estimate of higher and intact metabolic function in the former. Cryopreservation was found to affect the $\Delta\psi_m$ of the immature spermatozoa but not mature subset. It is possible that the immature fraction of spermatozoa have a limited capacity to endure freezing and thawing procedures as compared to the mature fraction. The disruption of mitochondrial membrane may play an important role in mediating the damage associated with cryopreservation.

	Mature fraction*		Immature fraction*	P†		
Intact $\Delta\psi_m$ (%)	56.1 ± 22.2		40.7 ± 15.2	< 0.05		
	Mature fraction*			Immature fraction*		
	Fresh	CRP	P††	Fresh	CRP	P††
Intact $\Delta\psi_m$	58.3 ± 24.8	50.7 ± 23.7	> 0.05	53.1 ± 12.0	37.4 ± 15.9	< 0.05

*Values expressed as mean ± SD; P < 0.05 was significant by †Wilcoxon matched pair test and ††Mann-Whitney U test.