

Apoptosis Signal Transduction and the Maturity Status of Human Spermatozoa

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INTRODUCTION

Numerous studies have shown the presence of DNA strand breaks in human ejaculated spermatozoa that might be a result of impaired spermatogenesis or degradation due to apoptosis (referred to as programmed cell death).¹ Although the pathways of apoptosis used in spermatogenesis are poorly understood,²⁻⁴ differences in selected molecular markers involved in apoptosis, between males with normal and abnormal sperm parameters, are thought to be indicative of an abortive apoptosis.⁵⁻⁷ The fertilization capacity of human sperm correlates with their maturity status. It was our aim to investigate the main elements of the apoptosis signaling cascade in mature and immature sperm: Caspase activity (aCP), disruption of mitochondrial membrane potential ($\Delta\psi_m$) and DNA fragmentation (TU+).

METHODS

Semen samples from healthy donors were pooled to create 18 pools. The liquefied semen was loaded onto a 47% and 90% discontinuous isolate gradient (Irvine Scientific, Santa Ana, CA) and centrifuged at $500 \times g$ for 20 min at room temperature. The resulting interface between the 47% and 90% layers (immature spermatozoa) and the 90% pellet (mature spermatozoa) were aspirated, and transferred to separate test tubes. The pellets for both fractions were resuspended in Biggers-Whitten-Whittingham medium (BWW) and centrifuged at $500 \times g$ for 7 minutes.

Active caspases 8, 9, and 3 were detected in living spermatozoa by carboxy-fluorescein-labeled caspase inhibitors. The detection of activated caspases by the inhibitor was performed according to the manufacturer's instruction manual in the flu-

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orescein caspase activity kit (CaspaTag™, S730x, Intergen Co., Oxford, England) with controls. Mitosensor™, a lipophilic cationic dye (5,5', 6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimid-azolyl carbocyanine chloride, C25H27Cl3N), was used to detect disrupted or intact transmembrane potential of mitochondria in vital spermatozoa (Apo Alert™ Mitochondrial Membrane Sensor kit, Ct No. K2017-1, Clontech, CA, USA).

Spermatozoa with intact mitochondria (IM) excite an intense red fluorescence as a result of forming aggregates. Green fluorescence of their monomers indicates disrupted $\Delta\Psi_m$. The proportion of cells with DNA fragmentation (TU+) was measured in the same fractions using the TUNEL assay (APO-DIRECT™, Flow Cytometry Kit for Apoptosis, Cat. No. APT110, Chemicon, USA). The assay was used according to the manufacturer's instructions. All fluorescence signals of labeled spermatozoa were analyzed by the flow cytometer, FACscan (Becton Dickinson).

RESULTS

Compared to the immature fraction, the mature subset had a lower level of aCP 9 (31.3 ± 12.5 vs. 53.1 ± 14.7 , [% \pm SD], $P < 0.01$), CP8 (29.4 ± 13.7 vs. 53.6 ± 18.1 , $P < 0.01$) and CP3 (30.2 ± 14.4 vs. 53.6 ± 17.4 , $P < 0.01$); a higher proportion of cells with intact $\Delta\Psi_m$ (56.1 ± 22.2 vs. 40.7 ± 15.2 , $P < 0.05$); and a lower proportion with TU+ (31.6 ± 15.6 vs. 39.7 ± 16.7 , $P < 0.01$).

There was a significant positive correlation of activity between, aCP 3 with aCP 8 and aCP 9. Intact $\Delta\Psi_m$ was inversely correlated with all aCP in mature cells, and with aCP 8 and aCP 3 in immature cells ($P < 0.01$ for each aCP, respectively). The aCP 9 of an immature fraction followed the same trend ($P = 0.051$). The TU+ cells showed no significant correlation with aCP or $\Delta\Psi_m$.

CONCLUSIONS

Incomplete maturation of human ejaculated spermatozoa is associated with an increase of caspase 3, 8, and 9 activity in the spermatozoa. This activity is also associated with the disruption of mitochondrial membrane potential in the immature fraction. The activated apoptotic process does not immediately affect the levels of DNA fragmentation. Reaching maturity may implicate a deactivation of the apoptosis-signaling cascade in human sperm. However, the exact mechanisms of caspase activation and disruption of the mitochondrial membrane potential in ejaculated sperm are yet to be elucidated and further research in this direction is needed.

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