INDUCTION OF TYPE I AND TYPE II APOPTOSIS IN HUMAN SPERMATOZOA

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Apoptosis is transduced by receptor (type I) or mitochondria mediated (type II) pathways. In spermatozoa CD95 and caspase (CP) 8 activation (type I) as well as disruption of the mitochondrial membrane potential (MMP), activation of CP9 (type II) and shared effector CP3 have been reported.

The aim of our study was to examine the transduction of these pathways in human spermatozoa using specific inducers of type I and II apoptosis.

Semen samples from 15 healthy donors were aliquoted and incubated with either type I (inducing CD95 antibody) or type II (Betulinic acid) inducers of apoptosis. Samples were incubated with 2 µg/mL CD95 antibody and 60 µg/mL Betulinic acid for 1 h and 10 min respectively. Incubation in phosphate buffer saline served as control. The levels of active CP3, 8 and 9 were estimated using carboxyfluorescein derivatives and analyzed by flow cytometry. In addition to sperm motility, we examined disruption of MMP using a lipophilic cationic dye.

CD95 treatment did not result in type I apoptosis as no significant increase was seen in activation of CP8 and CP3. In contrast, type II apoptosis was induced by Betulinic acid as observed by a significant increase in CP9 and 3 activation, disruption of MMP and loss of spermatozoal motility.

Our results indicate that the presence of CD95 may not have a pathophysiological function in mediating type I apoptosis. On the other hand, mitochondrial associated apoptosis plays an important role in human spermatozoa.