Title: Evaluation of poly (ADP-ribose) polymerase cleavage (cleaved-PARP) in sperm fractions after sperm apoptosis induction
Objective: Sperm cell apoptosis is an energy and protein dependent process that results in sperm DNA damage. PARP cleavage (cPARP) is stimulated by DNA breaks for help in repair process, so it is considered as an early marker for apoptosis. Staurosporine (STS) is a PARP inducer while 3-aminobenzamide (3ABA), is a PARP inhibitor. Sperm DNA damage due to oxidative stress is considered as one of the causes of male infertility. Conflicting reports exist regarding the presence of whole or cPARP in human ejaculated spermatozoa. We evaluated: 1) the presence of cPARP in mature ejaculated spermatozoa and 2) if we can control its level in sperm fraction using apoptosis inducers with or without PARP inhibitor.

Design: Prospective-controlled study.

Materials and Methods: Semen samples were collected from 10 healthy donors. The neat semen samples were divided into mature and immature sperm fractions using double density gradient centrifugation. Mature and immature sperm fractions were resuspended into HTF media and divided in 5 aliquots (control, H₂O₂ treated, H₂O₂+3ABA, STS treated, and STS+3ABA) incubated at 37 °C with 5 % CO₂ for 1 hour. The final concentrations were 2.5 μM for STS, 30 μM for H₂O₂, and 0.3 mM for 3ABA. After incubation, the mature and immature aliquots were evaluated for the early and late apoptosis by 1) annexin V, activated caspase-3, 2) sperm DNA damage by TUNEL and 3) cPARP by FACS.

Results: cPARP was detected in both neat and mature fractions. There were no significant differences between mature versus immature fractions regarding most of molecular apoptosis markers for treated and untreated sperm fractions for cPARP modulation.

Conclusions: Cleaved PARP is present in neat as well as in washed ejaculated human spermatozoa. Its level is related to the extent of sperm DNA damage. PARP inhibitors can not prevent/repair oxidative or chemical-induced sperm DNA damage.

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