Title: Relationship of Poly (ADP-ribose) polymerase (PARP) homologues to sperm apoptosis

Rajesh Jha, Ph.D, agarwaa@ccf.org, Reda Mahfouz, MD, mahfour@ccf.org, Rakesh
Sharma, Ph.D, sharmar@ccf.org, Uwe Paasch, MD, Uwe.Paasch@medizin.uni-leipzig.de, Sonja Grunewald, MD, sonja.grunewald@medizin.uni.leipzig.de and Ashok Agarwal, Ph.D, agarwaa@ccf.org. 1Reproductive Research Center, Glickman Urological Institute and Department of Obstetrics & Gynecology, Cleveland Clinic, Cleveland, Ohio, United States, 44195 and 2Department of Andrology, Leipzig University, Leipzig, Germany, D04103.

Objective: The role of PARP in human ejaculated spermatozoa is controversial. It is considered to repair the sperm DNA damage. We recently identified PARP homologues in human ejaculated sperm. Our objective was to evaluate the response of sperm cell exposure to different apoptotic inducers (staurosporine or STS and hydrogen peroxide or H$_2$O$_2$) with or without PARP inhibitor (3-aminobenzamide or 3ABA) on PARP homologues in sperm fractions.

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. Each mature and immature sperm fractions was resuspended into HTF media and divided into 5 aliquots (control, H$_2$O$_2$ treated, H$_2$O$_2$+3ABA, STS treated, and STS+3ABA) incubated at 37 °C for 1 hour. The final concentrations were 2.5 µM for STS, 30 µM for H$_2$O$_2$, and 0.3 mM for 3ABA. After incubation the mature and immature aliquots were processed for gel electrophoresis and western blotting. The blots were probed with anti-PARP-1 and rabbit anti-goat HRP conjugated in 1:1000 dilutions.

Results: We detected 3 bands by anti-PARP-1; they were close to 75, 63 and 60 kDa respectively on the immunoblot of mature and immature sperm aliquots. There was an inverse significant correlation ($r = -0.87$) of band intensity of 60 kDa in mature sperm fraction and 75 kDa in immature sperm fraction ($r = -0.70$) with late sperm apoptosis as measured by annexin V/propidium iodide assay. Mature sperm exhibited low protein level of 75 kDa (PARP-1 homologue) in response to H$_2$O$_2$ when compared to control (P<0.04). Other treatment did not affect its level. 75 kDa protein in immature sperm decreased after H$_2$O$_2$ (P<0.028), STS (P<0.001) and 3ABA groups. PARP-9 homologue (63 kDa) showed no differences in mature and immature sperm fractions after various treatments as compared to control. PARP-2 (60 kDa) was detected in the mature sperm fraction in untreated and STS treated groups. Mature sperm fraction showed low level of 60 kDa with STS+3ABA compared to control (P<0.004), while it was not detectable by STS and 3ABA in immature fraction.

Conclusions: Our results suggest the involvement of PARP-2 homologue (60 kDa) in oxidative stress and 75 kDa (PARP-1 homologue) in prevention of immature sperm cell damage. More research is needed to define the role of PARP-9 (63 kDa) in sperm physiology.
Support: None