INDUCTION OF EXTERNALIZATION OF PHOSPHATIDYL SERINE (EPS) IN EJACULATED HUMAN SPERMATOZOA BUT NOT CASPASE ACTIVATION USING TWO MODELS OF OXIDATIVE STRESS
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Reactive oxygen species (ROS) are involved in the pathophysiology of male infertility. High levels of ROS results in oxidative stress. Both abnormal spermatozoa and / or activated leukocytes (neutrophils) are main sources of ROS. Hypochlorous acid (HOCl) is produced by activated leukocytes from hydrogen peroxide (H₂O₂). ROS are known to induce EPS on the outer leaflet of the plasma membrane of spermatozoa. This is one of the earliest events of late apoptosis. Caspase activation (Caspase 8, 9, 1 and 3), a final common pathway of apoptosis is found in spermatozoa and is also associated with EPS. Hence it may be responsible for transduction of apoptotic signals. Data on ROS dependent caspase activation are conflicting. The aim of our study was to test the hypothesis if caspase activation can be induced by two ROS inducing models using HOCl or H₂O₂. Semen samples from 25 healthy donors were subjected to incubation with HOCl (10⁻³ mol/L, n = 15), H₂O₂ (0, 50, 100 and 200 µmol/L, n = 10) or PBS (control). Using carboxyfluorescein derivatives, the levels of active caspase (aCP) 1, 3, 8 and 9 were estimated after one hour of HOCl incubation. aCP3 was measured at 0, 15, 30, 60 and 120 min after H₂O₂ incubation. All aCP’s were analyzed via flow cytometry. HOCl did not result in a significant increase in aCP 1, 3, 8 or 9. Similarly we found no significant increase in aCP3 after H₂O₂ treatment. Our results show spermatozoa exposed to oxidative stress do not activate caspase 1, 3, 8 or 9 as demonstrated by the two ROS models. We propose that oxidative stress may therefore occur by caspase independent pathways.