Title: Assessment of sperm motility, viability and apoptosis in human spermatozoa after hydrogen peroxide exposure
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**Objective:** Male infertility has been linked to excessive production of ROS in semen. ROS is involved in sperm cell apoptosis signaling pathway. Apoptosis is distinguished from necrosis, or accidental cell death, by characteristic morphological and biochemical changes. Our objective was to investigate sperm motility, viability, and incidence of apoptosis after exposure to hydrogen peroxide (H$_2$O$_2$).

**Design:** Prospective study.

**Materials and Methods:** Semen samples were collected from 12 semen donors. The neat samples were subjected to double density gradient centrifugation to separate mature and immature sperm subpopulation. Each was resuspended in buffer and divided into 2 aliquots. One aliquot was incubated with 50uL of freshly prepared H$_2$O$_2$ added to 1 mL of sperm suspension at a final concentration of 20uM for 15 min at 37°C, and the other aliquot served as control. The incidence of apoptosis and necrosis was assessed by flowcytometry using the YoPro-1 dye which enters apoptotic cells giving green fluorescence, and propidium iodide (PI), which stains necrotic sperm cells giving red fluorescence.

**Results:** There was no significant difference in sperm motility between stimulated and non-stimulated sperm samples both in mature and immature fractions (table). After exposure to H$_2$O$_2$ the mature sperm fractions showed increases in the mean proportion of apoptotic sperm which did not reach significant level compared to control. On the other hand, there was significant increase in the mean proportion of apoptotic sperm in the exposed immature sperm fractions compared to control. In both the mature and immature fractions, there was an increase in the mean proportion of necrotic sperm compared to control (table).

**Conclusions:** Exposure of human spermatozoa to H$_2$O$_2$ is associated with a decrease in sperm viability. The increase in the proportion of apoptotic sperm reached significance only in the immature fractions. There was no apparent effect on sperm motility. Thus sperm motility may not serve as a marker for early oxidative sperm damage.

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