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IS THERE AN OPTIMUM METHOD OF CENTRIFUGATION OF SEMEN FOR SPERM SEPARATION AS DETERMINED BY THE FORMATION OF REACTIVE OXYGEN SPECIES (ROS)

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The objective of this study was to determine the correlation between centrifugation parameters (time and g-force) and ROS production in order to establish an optimal centrifugation speed and time for sperm preparation techniques. Centrifugation has been shown to increase ROS levels in semen. The formation of high levels of ROS is associated with sperm membrane injury through spontaneous lipid peroxidation resulting in the alteration of normal sperm function. The semen of 38 men was evaluated for the formation of ROS before centrifugation, after centrifugation at 200 X g for 2 or 10 minutes and 500 X g for 2 or 10 minutes. All samples tested were negative for the presence of white blood cells (WBC's) by Endtz test. The formation of ROS was measured by the chemiluminescence method using a Berthold luminometer. The ROS formation was regarded as positive when the value was $\geq 10 \times 10^4$ counted photons per minute (cpm). The sperm concentration in each sample was adjusted to $15-20 \times 10^6$ /mL. Eight of 38 subjects (21%) exhibited high levels of ROS in their semen before centrifugation. All 8 showed further increase in ROS formation regardless of g-force or time; the increase was significantly less when centrifugation lasted only 2 minutes as opposed to 10 minutes ($p < 0.0001$). Thirty other semen samples were subjected to centrifugation for 2 and 10 minutes at 200 X g and 500 X g. Twenty-five were ROS negative regardless of g-force or time. Five demonstrated an increase in ROS formation when centrifuged for 10 minutes at 200 X g and 500 X g. Our results indicate that the time of centrifugation is more important than g-force in increasing ROS formation. Based on these results, a shorter duration of centrifugation in the preparation of sperm for assisted reproductive techniques is recommended.