Differential production of reactive oxygen species by subsets of human spermatozoa

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Objectives: Reactive oxygen species (ROS) play an important role in the pathogenesis of male infertility. Immature spermatozoa, as well as leukocytes in semen, have been shown to produce high levels of ROS. The purpose of this study was to evaluate differences in ROS production by subsets of human spermatozoa at different stages of maturation obtained by fractionation of ejaculated spermatozoa by density gradient centrifugations.

Design: A prospective controlled analysis of the ROS production and sperm morphology in patients undergoing evaluation for infertility.

Materials and Methods: Endtz test negative semen samples from 16 males being evaluated for infertility (which includes both males with normal and abnormal semen parameters) and 17 normal healthy donors were fractionated into four different subsets by a three-layer discontinuous Isolate density gradient (47%, 70%, and 90%). Fraction 1 was enriched in immature germ cells and immature sperm, fractions 2 and 3 contained, mostly, immature sperm with cytoplasmic droplets, and fraction 4 contained, for the most part, morphologically normal sperm, as determined by histochemical analysis. Each fraction was evaluated for semen characteristics by WHO criteria, ROS production by chemiluminescence method, and sperm morphology according to WHO and Kruger's strict criteria.

Results: ROS production was significantly different in all four fractions from both patients and donors (p <0.01). ROS production was highest in fraction 2 and lowest in fraction 4 (p< 0.01). Sperm recovery in fraction 4 was significantly lower in patients than in donors (21.2 ± 8 vs. 29.3 ± 12; p = 0.032) and significantly higher in fraction 2 (28.0 ± 10 vs. 20.2 ± 10; p = 0.035). Motility recovery in each fraction was inversely related to ROS levels (p <0.02).

Conclusions: These results indicate that there appears to be an association between defective sperm maturation and increased ROS production. Therefore, it is possible that this increase in ROS production by sperm may be related to incomplete extrusion of the cytoplasmic droplet during spermiogenesis and that defective spermiogenesis may play an important role in the pathogenesis of male infertility.

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