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JOURNAL ARTICLE

The effect of reactive oxygen species on equine sperm motility, viability, acrosomal integrity, mitochondrial membrane potential, and membrane lipid peroxidation

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The objective of this study was to examine the influence of reactive oxygen species (ROS), generated through the use of the xanthine (X)-xanthine oxidase (XO) system, on equine sperm motility, viability, acrosomal integrity, mitochondrial membrane potential, and membrane lipid peroxidation. Equine spermatozoa were separated from seminal plasma on a discontinuous Percoll gradient, and spermatozoa were incubated with 0.6 mM X and 0.05 U/mL XO for 30 minutes. Catalase (150 U/mL), superoxide dismutase (SOD, 150 U/mL), or glutathione (GSH, 1.5 mM) were evaluated for their ability to preserve sperm function in the presence of the induced oxidative stress. At the end of the 30-minute incubation, sperm motility was determined by computer-assisted semen analysis. Viability and acrosomal integrity were determined by Hoechst-Pisum sativum staining, and mitochondrial membrane potential was determined by staining with JC-1. Incubation with the X-XO system led to a significant ($P < .01$) increase in hydrogen peroxide production and an associated decrease ($P < .01$) in motility parameters. Total motility was significantly ($P < .01$) lower in the presence of X-XO compared with the case of the control (29%+/-9% vs 73%+/-1%, respectively). Catalase, but not SOD, prevented a decline in motility secondary to oxidative stress (71%+/-4% vs 30%+/-3%, respectively). The addition of glutathione had an intermediate effect in preserving sperm motility at the end of the 30-minute incubation (53%+/-3%). No influence of X-XO could be determined on viability, acrosomal integrity, or mitochondrial membrane potential. In order to promote lipid peroxidation, samples were incubated with ferrous sulfate (0.64 mM) and sodium ascorbate (20 mM) for 2 hours after the X-XO incubation. No increase in membrane lipid peroxidation was detected. This study indicates that hydrogen peroxide is the major ROS responsible for damage to equine spermatozoa. The decrease in sperm motility associated with ROS occurs in the absence of any detectable decrease in viability, acrosomal integrity, or mitochondrial membrane potential or of any detectable increase in lipid peroxidation.

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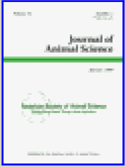

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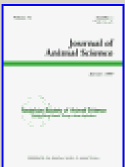

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