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Reactive Oxygen Species and Cryopreservation Promote DNA Fragmentation in Equine Spermatozoa

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The objective of this study was to examine the effect of reactive oxygen species (ROS) and cryopreservation on DNA fragmentation of equine spermatozoa. In experiment 1, equine spermatozoa were incubated (1 hour, 38°C) according to the following treatments: 1) sperm alone; 2) sperm + xanthine (X, 0.3 mM)-xanthine oxidase (XO, 0.025 U/mL); 3) sperm + X (0.6 mM)-XO (0.05 U/mL); and 4) sperm + X (1 mM)-XO (0.1 U/mL). In experiment 2, spermatozoa were incubated (1 hour, 38°C) with X (1 mM)-XO (0.1 U/mL) and either catalase (200 U/mL), superoxide dismutase (SOD, 200 U/mL), or reduced glutathione (GSH, 10 mM). Following incubation, DNA fragmentation was determined by the single cell gel electrophoresis (comet) assay. In experiment 3, equine spermatozoa were cryopreserved, and DNA fragmentation was determined in fresh, processed, and postthaw sperm samples. In experiment 1, incubation of equine spermatozoa in the presence of ROS, generated by the X-XO system, increased DNA fragmentation ($P < .005$). In Experiment 2, the increase in DNA fragmentation associated with X-XO treatment was counteracted by the addition of catalase and GSH but not by SOD, suggesting that hydrogen peroxide and not superoxide appears to be the ROS responsible for such damage. In experiment 3, cryopreservation of equine spermatozoa was associated with an increase ($P < .01$) in DNA fragmentation when compared with fresh or processed samples. This study indicates that ROS and cryopreservation promote DNA fragmentation in equine spermatozoa; the involvement of ROS in cryopreservation-induced DNA damage remains to be determined.

Key words: DNA damage, oxidative stress, antioxidants, horse, sperm

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