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JOURNAL ARTICLE

Detection of DNA damage in response to cooling injury in equine spermatozoa using single-cell gel electrophoresis

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Single-cell gel electrophoresis (SCGE), or comet assay, has the ability to detect damage at the single cell level and has not been reported for equine sperm. The ability to detect nuclear damage at the single cell level could aid in the advancement of protocols for optimal semen preservation. The goals of these experiments were to adapt this assay for use with equine sperm and to utilize the assay for determining the integrity of equine sperm DNA following treatments with storage at various decreased temperatures (-20 degrees C and 5 degrees C). Results from experiments in which sperm were frozen (-20 degrees C) in the absence of cryoprotectants revealed that significantly more cells with fragmented tails of DNA, or comets, occurred among those exposed to 1, 3, and 5 freeze-thaw cycles (65% +/- 6%, 76% +/- 11%, 92% +/- 6%, respectively) compared with fresh, untreated sperm (19% +/- 16%, $P < .05$). In addition DNA damage was different ($P < .05$) between the three freeze-thaw treatments. Sensitivity of SCGE on equine sperm was further tested with known ratios of frozen-thawed and fresh cells. The amount of detectable DNA damage was positively correlated with the percentage of cryo-damaged cells in each treatment ($r^2 = 0.92$, $P < .05$). Potential damage as a result of cooled storage was also investigated and results revealed that sperm stored for 48 hours (at 5 degrees C) had a higher percentage of comets than that of fresh sperm (63% +/- 13.9% and 28% +/- 15.6%, respectively, $P < .05$). The percentage of viable sperm also decreased linearly over time and was inversely correlated with percent of comets ($r^2 = 0.805$, $P < .001$). Detection of sublethal and/or uncompensable fertility factors in semen, such as DNA fragmentation, could be useful for detecting male differences in semen for cooling or cryopreservation potential and could provide a tool for monitoring and preserving fertility for individual stallions.

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