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Changes in motion characteristics, plasma membrane integrity, and acrosome morphology during cryopreservation of buffalo spermatozoa

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Motion characteristics, plasma membrane integrity, and acrosome morphology of buffalo spermatozoa after different stages of cryopreservation (ie, dilution, cooling to 4 degrees C, equilibration at 4 degrees C, and freezing and thawing) were examined. Semen ejaculates from 4 buffalo bulls were pooled (n = 5), diluted in tris-citric acid extender, cooled to 4 degrees C over 2 hours, equilibrated at 4 degrees C for 4 hours, dispensed into 0.5-mL straws, and frozen in a programmable cell freezer before plunging into liquid nitrogen. Frozen semen was thawed at 37 degrees C for 15 seconds. After completion of each stage, sperm motion characteristics, plasma membrane integrity, and acrosomal morphology were determined using computer-assisted semen analysis, hypo-osmotic swelling assay, and phase-contrast microscopy, respectively. Data were presented as mean +/- standard error of the mean. Visual and computerized motility did not differ due to dilution, cooling, or equilibration (77.3% +/- 2.3% and 90.5% +/- 1.2%, respectively), but was reduced (P < .05) after freezing and thawing (53.0% +/- 4.6% and 48.6% +/- 6.5%, respectively). Linear motility of spermatozoa was lower (P < .05) after dilution or equilibration (56.2% +/- 2.4%) than after cooling or freezing and thawing (79.6% +/- 1.4%). Sperm curvilinear velocity was reduced (P < .05) from 112.4 +/- 5.3 microm/sec after dilution to 96.0 +/- 5.8 microm/s after cooling, and from 87.6 +/- 4.1 microm/s after equilibration to 69.4 +/- 2.0 microm/s after freezing and thawing. Sperm lateral head displacement differed (P < .05) after each stage (ie, dilution, 3.9 +/- 0.2 microm; cooling, 2.3 +/- 0.2 microm; equilibration, 3.1 +/- 0.3 microm; and freezing and thawing, 1.7 +/- 0.2 microm). Spermatozoa with intact plasma membranes were 80.2% +/- 3.9% after dilution, reduced (P < .05) to 60.4% +/- 5.6% after equilibration, and then to 32.6% +/- 3.8% after freezing and thawing. The percentage of spermatozoa with normal acrosomes remained higher after dilution, cooling, or equilibration (73.2% +/- 2.4%) than after freezing and thawing (61.8% +/- 2.4%; P < .05). In conclusion, the maximal damage to the motility apparatus, plasma membrane, and acrosomal cap of buffalo spermatozoa occurs during freezing and thawing followed by equilibration.