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JOURNAL ARTICLE

Cryopreservation reduces the ability of equine spermatozoa to attach to oviductal epithelial cells and zonae pellucidae in vitro

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Two bioassays were used to evaluate the interaction of fresh and cryopreserved equine semen with oviductal epithelial cells (OEC) and with the zona pellucida (ZP). Split ejaculates were either stored at room temperature or frozen and thawed. In experiment 1, progressive motility and membrane integrity were evaluated for each treatment. Fluorescent labeled spermatozoa were cocultured with monolayers of OEC for 30 minutes, and the number of sperm attached to OEC was counted by fluorescence microscopy and analysis of digitized images. Motility of spermatozoa attached to OEC was observed at 0.5, 3, 6, 18, 24, and 48 hours after insemination. In experiment 2, progressive motility, membrane integrity, and acrosomal integrity were determined. Differential labeling with the fluorochromes fluorescein isothiocyanate (FITC) or tetramethylrhodamine isothiocyanate (TRITC) was used to distinguish fresh and frozen-thawed spermatozoa. Equal numbers of motile spermatozoa from each treatment were incubated with salt-stored equine oocytes for 4 hours, and the number of spermatozoa firmly bound to the ZP was counted using dual-wavelength epifluorescence microscopy. Fewer ($P < 0.001$) cryopreserved spermatozoa attached to OEC compared to spermatozoa stored at room temperature. The motility of spermatozoa attached to OEC decreased over time within each treatment group ($P < 0.001$), but this decrease was not different between treatments. The mean number of spermatozoa bound per ZP and percentage of acrosome-intact spermatozoa were lower ($P < 0.05$) for frozen-thawed than for fresh spermatozoa. There was no effect of stallion on acrosomal status of frozen-thawed spermatozoa; however, the number of spermatozoa bound per ZP was different between stallions within treatments ($P < 0.05$). These results indicate that the ability of cryopreserved equine spermatozoa to attach to equine OEC or ZP in vitro is reduced compared to fresh extended spermatozoa due to changes other than a reduction in post-thaw motility or membrane integrity. The decreased ability of frozen-thawed spermatozoa to attach to OEC or to ZP could explain, in part, the reduced fertility of cryopreserved compared to fresh spermatozoa in the horse.