
COMPARISON OF CONVENTIONAL EQUIAXED AND DIRECTIONAL FREEZING IN RHINOCEROS (*Ceratotherium simum*, *Diceros bicornis*)

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Abstract

To increase the quality of cryopreserved sperm for potential use in artificial inseminations in rhinoceros, the conventional equiaxed and the new multi-thermal gradient directional freezing methods were compared. Fourteen sexually mature white and two black rhinoceros bulls (*Ceratotherium simum*, *Diceros bicornis*) were examined with ultrasound and electroejaculated. Semen samples were immediately extended 1:1 (v:v) with an egg-yolk, DMSO, lactose-based extender and aliquoted into 0.5-ml straws for conventional freezing, and 2-ml and 10-ml glass hollow tubes for directional freezing. In addition to functional assays, the assessment of basic spermatid parameters were evaluated prior to and after both freezing methods. Directional freezing improved the sperm viability by 7% ($P<0.005$), motility by 8% ($P<0.005$), and normal morphology by 26.3% ($P<0.005$) as compared to conventional freezing. Using the previously described egg-yolk extender, xanthenic acid, cytochalasin D, potassium and EDTA as extender additives were tested individually and collectively, in an attempt to improve the post-thaw sperm quality of both freezing methods. Whereas post-thaw quality with directional freezing was not improved or decreased ($P>0.05$) by any of the extender additives; conventional freezing proved more sensitive to the extender composition. All additives combined had a dramatic negative effect on post-thaw sperm normal morphology ($P<0.005$) and the additive cytochalasin D improved sperm motility by 17% ($P<0.05$) compared to the other additives after conventional freezing. In this first direct comparison of these two freezing technologies, directional freezing proved to facilitate higher gamete survival after cryopreservation and was independent and not reactive to variations in the cryomedia. These results suggest that directional freezing could be a valuable technology for other species with cryosensitive spermatozoan as well.