Animal Reproduction

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SUPPORTING BIOTECHNOLOGIES: CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSTIC IMAGING, MOLECULAR BIOLOGY AND "OMICS"

Effect of three cryoprotectant solutions on the cryopreservation of spermatozoa recovered from the epididymis of Cervus elaphus post-mortem

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The recovery of spermatozoa from the epididymis of animals that die suddenly is an alternative to enhance germplasm banks and allow future use for the propagation of wild animals. In this context, the present study evaluated the protective potential of three cryoprotectant media for the freezing of epididymal spermatozoa from Cervus elaphus (Red Deer): 1. Botubov® (20% egg yolk); 2. Criocell 1, based on trehalose, fructose, amino acids, soy lecithin, 7% glycerol, and 10% egg yolk; 3. Criocell 2, same base as Criocell 1, but with 5% egg yolk. Testicles of an adult Red Deer from the Brasília Zoo (DF, Brazil) were transported at 5°C to the Embrapa Cerrados Laboratory. The material was stored for an additional two days at 5°C to simulate prolonged transport. Subsequently, spermatozoa were collected by incising the tail of the epididymis, and sperm motility, vigor (scale 0–5), and concentration were evaluated. The spermatozoa were diluted in the three cryopreservation solutions and frozen in 0.5 ml straw with 20x106 cells using an automated freezing curve (TK-3000 SE machine). Straws were thawed in water bath at 37°C for 30 seconds and subjective motility, membrane integrity (Propidium Iodide and Carboxyfluorescein Diacetate), acrosome integrity (fluorescein isothiocyanate-conjugated peanut agglutinin) and DNA integrity (Tunel) were assessed to evaluate the impact of cryopreservation. The data obtained of thawing of 3 straws from each treatment were evaluated by Kruskal-Wallis test and Dunn test at a significance level of 5% ($p<0.05$). Initial fresh sperm motility was 40% with a vigor score of 2. After dilution in the different media, total motility improved; Botubov reached 50% motility, Criocell 1 reached 55% and Criocell 2 reached 60%. In all treatments, vigor increased from 2 to 3. There was no significant difference ($P>0.05$) in post-thaw sperm motility among the three cryoprotectant solutions (45.00±5.00%, 31.66±2.88% and 45.00±5.00% for Botubov, Criocell 1, and Criocell 2, respectively). Membrane integrity was also similar across treatments (29.16±2.46%, 24.5±5.26% and 27±4.33%). However, treatments with Botubov and Criocell 2 provided better protection of the acrosome (40.17±2.98% and 32.42±1.88%, respectively; $P<0.05$) compared to Criocell 1 (25.67±3.18%). None of the treatments were damaging to DNA, with over 97% of spermatozoa showing intact DNA. These results demonstrate that storing testicles at 5°C reduces sperm metabolism and maintains viability for up to two days, which is sufficient for long distance transportation to laboratories. Additionally, Botubov and Criocell 2 (with reduced egg yolk content at 5%) better protected epididymal spermatozoa from this species during cryopreservation. Considering the specific characteristics of each species, we inferred that epididymal spermatozoa from Red Deer adapt better to Botubov and Criocell 2 during the cryopreservation process.

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