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suggesting that both might be affected by the same deleterious factors. Although PMI, HOSPMI and MIT values decreased approximately 40% after freezing, we feel that such sperm samples could be used for in vitro embryo production, if not by IVF, by ICSI. Of course, additional studies are needed to validate our suggestion.

209 FUNCTIONAL ASSESSMENT OF WHITE RHINOCEROS *CERATHOTERIUM SIMUM* EPIDIDYMAL SPERMATOZOA BEFORE AND AFTER CRYOPRESERVATION

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Biological Resource Banks represent a potentially valuable tool for species conservation. It is, however, necessary to understand the species-specific cryopreservation process and its consequences for spermatozoa to aid in the development of assisted reproduction as a future conservation tool. The aim of this study was to assess the in vitro functionality of white rhinoceros *Ceratotherium simum* epididymal spermatozoa both before and after cryopreservation. Testes from a harvested white rhino bull were removed and transported at 5°C to the laboratory within 4 h. The cauda epididymis was dissected out and flushed with 2 mL of Tris-citrate egg yolk extender (fraction A, Biladyl, Minitüb, Germany). A 0.1 mL aliquot was removed for analysis and the balance (9 mL; 2 mL fraction A + 7 mL sperm sample) mixed with an additional 27.2 mL of Tris-citrate egg yolk with glycerol (fraction B, Bidadyl). The extended sample was allowed to cool to 4°C over a 6-h period before an additional 29.2 mL of cooled fraction B were added (final sperm concentration = 150×10^6 mL^{-1}). Sperm samples were loaded into 0.25-mL straws and frozen over LN_2 vapor (4 cm for 20 min) for later assessment. Sperm straws were thawed by placing the straws in water at 37°C for 30 s. Pre-freeze and post-thaw evaluations were carried out in the same manner. Media used included: HEPES for washing (20 mM HEPES, 355 mM sucrose, 10 mM glucose, 2.5 mM KOH) and HEPES saline (197 mM NaCl, instead of sucrose). An aliquot was diluted with HEPES (washing) and centrifuged for 5 min at 600 × g; the pellet was resuspended in HEPES saline. Sperm motility (total motility %, TM; and progressive motility %, PM) was assessed using phase contrast microscopy (×200; 37°C). Sperm plasma membrane status was assessed using the fluorescent dye, propidium iodide (50 ng mL^{-1} in HEPES saline; 10 min, RT). Percentage of cells with plasma membranes intact (unstained; PMI) was recorded. Mitochondrial status was assessed with the fluorescent dye, JC-1 (7.5 µM in HEPES saline; 30 min, 37°C). The % of cells with an orange-stained midpiece was recorded (active mitochondria; MIT). Resilience to hypoosmotic shock (HOS test) was assessed by diluting a sample in 100 mOsm/kg HEPES saline (1 : 20; 15 min, RT). An aliquot was stained with PI to assess plasma membrane status (HOSPMI), and the rest was fixed with formaldehyde, and % coiled tails (positive endosmosis; HOST) was estimated using phase contrast microscopy (×400). Evaluations of PMI, MIT and HOSPMI were performed using fluorescence microscopy (×400, 450–490 nm excitation filter). The results indicated that quality was good pre-freezing (TM: 60%; PMI: 86%; MIT: 100%), except for a PM value of 15%. After thawing, although there was a drop in TM (30%), there was no decrease in PM (20%). Our in vitro functional assessment indicated a loss of quality between the pre-freeze and post-thaw evaluations, but PMI and MIT maintained their pre-thaw levels (60% and 72%, respectively). The HOS test, which indicates plasma membrane integrity, decreased from the pre-freeze level (91%) to a post-thaw value of 70%. HOSTPMI was 72% pre-freeze, and decreased to 54% post-thaw. In conclusion, epididymal spermatozoa from the white rhino may retain its functionality after cryopreservation in a commerically available cryo-extender (Bidadyl). The use of assisted reproduction techniques could someday play a role in the management and conservation of the white rhinoceros and related species.

210 INCREASE IN OVULATION RATE AFTER IMMUNIZATION OF MALPURA EWES AGAINST A SYNTHETIC PEPTIDE SEQUENCE OF THE α-SUBUNIT OF BOVINE INHIBIN

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Unlike many other breeds of sheep (e.g. Boroola, Romney or Merino) which have high fecundity, the Malpura ewe, an Indian breed of sheep, is marked by an ovulation rate of one and a low incidence of twinning. Active immunization against a number of inhibin-based synthetic peptides has been reported to increase ovulation rates in these high fecundity breeds of sheep. The objective of the present study was to explore the possibility of increasing ovulation rates in Malpura ewes by active immunization against a synthetic peptide replica of the N-terminal sequence of the bovine inhibin. Adult Malpura ewes ($n = 5$) were actively immunized against a synthetic peptide that corresponded to the N-terminus of the α-subunit of bovine inhibin [bI$_\alpha$(1–29)Tyr^{30}]. The peptide was conjugated to ovalbumin, with a peptide-to-ovalbumin ratio of around 20 moles mole^{-1}, to increase its antigenicity. Control ewes ($n = 5$) were immunized against ovalbumin. On the day of primary immunization, 400 µg of peptide-ovalbumin conjugate or ovalbumin were dissolved in 1 mL of isotonic saline, emulsified with an equal volume of Freund's complete adjuvant and injected at four sites in each ewe. Following this, boosters 1, 2 and 3 were given on Days 28, 56 and 84, respectively, of the experiment (Day 0 = day of primary immunization); boosters were 200 µg of peptide-ovalbumin conjugate or ovalbumin dissolved in 1 mL of isotonic saline and emulsified with an equal volume of Freund's incomplete adjuvant. Estrus was synchronized by a double injection schedule of $PGF_{2\alpha}$ (7.5 mg Lutalyse, once each on Days 35 and 45). The animals were subsequently allowed to undergo normal cyclicity until the end of the experiment. Ovulation rate was determined by counting the number of corpora lutea observed during laparoscopic examinations approximately 5 days after estrus during three estrous cyles following treatment. The ovulation rate between control and immunized groups was compared by repeated measures ANOVA. Immunization of the Malpura ewes against the synthetic peptide sequence of the α-subunit of bovine inhibin [bI$_\alpha$(1–29)Tyr^{30}] increased ovulation rate over 5-fold

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