187 Morphological Appearance and Expression of Spermatogonial Stem Cell Markers in White Rhinoceros Testicular Tissue

M. C. Gomez [A] , Y. Cates [B] , D. B. Stansfield [C] , C. Young [A] , R. Klee [C] and B. Durrant [A]
+ Author Affiliations

Reproduction, Fertility and Development 30(1) 233-234

Published: 4 December 2017

Abstract

Spermatogonial stem cells (SSC) have been isolated from testicular tissue (TT) of several mammalian species and differentiated into mature spermatozoa following transplantation or *in vitro* culture. The northern white rhinoceros (NWR; *Ceratotherium simum cottoni*) is critically endangered. Thus, frozen NWR TT, cryopreserved and stored at the San Diego Zoo's Frozen Zoo®, potentially contain SSC that could be a source of spermatozoa. The method used for cryopreserving TT may affect the integrity and number of SSC. Therefore, identifying alterations in the seminiferous tubules (ST) of frozen-ndash;thawed-NWR TT will provide insight into the condition of the SSC. Therefore, our aims were to (1) determine the effect of freezing rhinoceros TT on the structure of epithelium, and (2) identify SSC (GFRα1, GPR125) and pluripotent (SSEA-4 and Oct-4) markers. Testicular tissue of an adult NWR and a stillborn southern white rhinoceros (SWR) were frozen by equilibration of TT for 30 min at 4.0°C in PBS and 1.5 M dimethyl sulfoxide (DMSO), cooled at 2.0°C/min to −4.0°C, 0.3°C/min to −40°C, and plunged into liquid nitrogen. Tissues were thawed at 37°C in a water bath and DMSO removed in a 4-step dilution. Tissue was then fixed, dehydrated, and paraffin embedded. For morphological evaluations, frozen-ndash;thawed tissue was sectioned and stained with hematoxylin and eosin (H&E). The TT from both rhinoceros collected immediately after death (fresh) and stained with H&E were used as a control for cryopreservation. Localization of SSC and pluripotent markers in ST of frozen-ndash;thawed TT was detected by immunohistochemistry. Morphologically, fresh-NWR TT was severely altered, displaying large epithelium gaps and partial (62.2%) or total detachment (37.7%) from, and slight damage (35.5%) to, the basement membrane. The number of pyknotic nuclei per ST was moderate (15.6 ± 7.2%). Many of these changes could have resulted from autolysis and handling before tissue preparation. In contrast, histological appearance of fresh-SWR was good, with 98.3% of the tubules intact, and a small proportion of pyknotic cells (0.8 ± 1.5%). Seminferous tubule (n = 30/male) length and width (μm; ± SEM) differed between NWR (635.2 ± 34.4 × 214.6 ± 10.8) and SWR (277.7 ± 13.8 × 73.2 ± 2.4; $P < 0.05$). Damages after cryopreservation compared with fresh tissue comprised (1) epithelium detachment, NWR = 100% ($P < 0.0001$), and SWR = 43.3% ($P < 0.001$); (2) basement membrane alteration, only in NWR (93.0%; $P < 0.001$); and (3) decreased length and width in the ST, NWR = 409.4 ± 18.1 × 173.4 ± 8.2 ($P < 0.05$), and SWR = 195.2 ± 8.3 × 61.6 ± 2.8 ($P < 0.05$), with loss of lumen in both males. Immunohistochemistry revealed that NWR expressed GFRα1 and GPR125 at various stages of spermatogenqaesis, whereas Oct-4 was detected in few cells. In contrast to

NWR, Oct-4 expression in SWR was located at the basement membrane; SSEA-4 was not detected in either male. In conclusion, freezing-induced morphological alterations in rhinoceros ST and positive expression of markers for SSC and pluripotency suggest the presence of SSC. Further studies are required to evaluate the viability of rhinoceros SSC.