116 FACTORS IMPACTING THE SUCCESS OF POSTMORTEM SPERM RECOVERY AND CRYOPRESERVATION IN THE RHINOCEROS

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Abstract.

Given the increasing anthropogenic pressures on wildlife around the globe coupled with the challenges of climate change, cryopreservation of genetic resources from extant species should be prioritized while the opportunity still exists. Arguably, the rhinoceros stands out as a primary candidate for concerted gene banking efforts given its historical brush with extinction and today's escalating poaching crisis. The goal of this study was to identify factors that influenced the ability to successfully recover and cryopreserve sperm postmortem from rhinos maintained in North American zoos. Factors considered included procedural technicalities, individual rhino characteristics, and timing. A total of 23 mature male rhinos ranging in age from 8 to 43 years, representing 4 species and maintained at 13 different zoos, were opportunistically included in this study over a 16-year period (1998–2014). The majority of the males were African black rhinoceros (n = 14), followed by Indian rhinoceros (n = 5), African white rhinoceros (n = 3), and a single Sumatran rhinoceros. All zoos received a protocol requesting that reproductive tissues (testes, epididymides, and vas deferens) be removed from the rhino as soon as possible after death, kept moist, cooled slowly, and shipped cool (5°C) overnight to the lab for processing. Samples of adequate quality (≥30% motility with ≥2.0 forward progressive status) were cryopreserved according to a previously published protocol (O'Brien and Roth 2000 J. Reprod. Fertil. 118, 263–271). Gross testicular pathology was noted in 17.4% of males (4/23) but did not impact sperm recovery except in one case of azoospermia (4.3%). Sixty-two percent of the males (13/21) in which sperm recovery was attempted yielded quality samples adequate for cryopreservation (black rhino, n = 7; white rhino, n = 3; Indian rhino, n = 2; Sumatran rhino, n = 1). A high percentage of males (70.6%; 12/17) from which reproductive tissue was removed and cooled ≤4 h after death yielded quality sperm samples, whereas only 25% (1/4) of males from which tissue was removed >4 h after death yielded quality samples. Quality samples were recovered up to 51 h postmortem from rhinos ranging in age from 8 to 35 years. Neither type of illness (prolonged or acute) or method of death (euthanasia or natural) affected the ability to harvest quality samples (P > 0.05). The Indian rhino yielded significantly more sperm on average (40×10^9) than the African black rhino (3.6×10^9; P < 0.01) and the African white rhino (3.2×10^9; P < 0.05). Mean pre- and post-thaw percent sperm motility for black (n = 6; 53 and 38%), white (n = 2; 80 and 63%), Indian (n = 2; 50 and 45%), and Sumatran (n = 1; 50 and 38%) rhino samples assessed indicated a reduction of just 5 to 17% post-thaw. In conclusion, rhino sperm recovery postmortem is relatively successful across a wide range of variables, especially when tissues are removed and cooled promptly after death, and should become standard practice in zoos.