Animal Reproduction Science 167 (2016) 22–30

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Factors impacting the success of post-mortem sperm rescue in the rhinoceros

T.L. Roth[a,*], M.A. Stoops[a], T.R. Robeck[b], J.K. O'Brien[b]

[a] Center for Conservation and Research of Endangered Wildlife (CREW), Cincinnati Zoo & Botanical Garden, Cincinnati, OH 45220, USA
[b] SeaWorld and Busch Gardens Reproductive Research Center, SeaWorld Parks and Entertainment, 2595 Ingraham St., San Diego, CA 92109, USA

ARTICLE INFO

Article history:
Received 16 October 2015
Received in revised form 13 January 2016
Accepted 31 January 2016
Available online 2 February 2016

Keywords:
Sperm banking
White rhinoceros
Black rhinoceros
Indian rhinoceros
Sumatran rhinoceros
Genome resource banks
Assisted reproduction

ABSTRACT

The goal of this study was to identify factors that influenced the ability to successfully rescue sperm post-mortem from rhinoceroses maintained in North American zoos. Factors considered included procedural technicalities, individual rhinoceros characteristics and timing. 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%). Of the males in which sperm recovery was attempted ($n = 21$), 62% yielded quality samples considered adequate for cryopreservation (≥30% motility with ≥2.0 forward progressive status). 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 1–51 h post-mortem from rhinoceroses 8 to 36 years old. 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 rhinoceros yielded significantly more sperm on average (40×10^9) than the African black rhinoceros (3.6×10^9; $P < 0.01$) and the African white rhinoceros (3.2×10^9; $P < 0.05$). Across all species and samples assessed ($n = 11$), mean post-thaw sperm motility (41%), was only 15% less than pre-freeze motility (56%) and only decreased to 22% during the 6 h post-thaw assessment period. Rhinoceros sperm rescue post-mortem is relatively successful across a wide range of variables, especially when tissues are removed and cooled promptly after death, and should be considered standard practice among zoos.

1. Introduction

Given the increasing anthropogenic pressures on wildlife around the globe coupled with the challenges of climate change, there is a growing realization that many species may be driven to extinction by the end of the 21st century (Barnosky et al., 2011). Faced with this reality, conservation scientists are acknowledging the need to focus more attention on cryopreserving genetic resources from extant species while the opportunity still exists (Wildt, 2000; Harnal et al., 2002; Fickel et al., 2007). Relatively small-scale, yet still significant efforts to bank wildlife materials including sperm, embryos and cell lines have been ongoing in several zoo-associated labs for over two decades. Therefore, it seems an opportune time to evaluate these somewhat limited, opportunistic efforts in an attempt to capitalize on what we can learn from existing results and improve success and efficiency in the future.

* Corresponding author.
E-mail address: terri.roth@cincinnatizoo.org (T.L. Roth).

http://dx.doi.org/10.1016/j.anireprosci.2016.01.019
0378-4320/

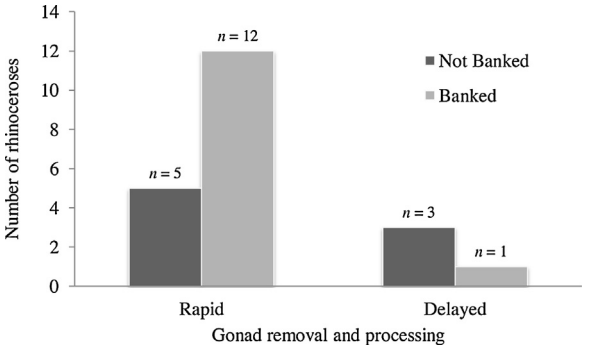

Fig. 1. Number of successful (banked) versus failed (not banked) rhinoceros sperm rescue attempts following rapid (<4 h post-mortem) tissue removal and cooling or delayed (>4 h post-mortem) tissue removal and cooling.

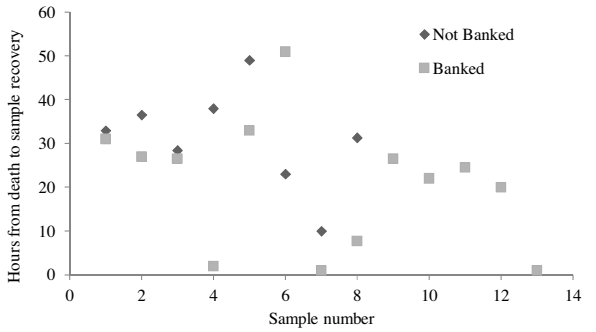

Fig. 2. Scatter plot demonstrating no strong relationship between the interval from death to sperm recovery (up to 51 h post-mortem) and the ability to harvest quality samples ($n = 13$ successful/banked and $n = 8$ unsuccessful/not banked).

Arguably, the rhinoceros is one taxon that stands out as a primary candidate for concerted genetic banking efforts. First, all rhinoceros species are currently or have historically come very close to extinction (Milliken et al., 2009). Furthermore, even those species that have made an impressive recovery are now severely threatened by the recent and rapid escalation in poaching activities (Milliken, 2014). In addition, rhinoceroses give birth to singletons after a lengthy gestation (16 months), rendering populations incapable of rebounding quickly when numbers drop to perilously low levels. Although zoos accredited by the Association of Zoos and Aquariums (AZA) currently maintain three rhinoceros species within their facilities (the African white rhino, *Ceratotherium simum*; the African black rhino, *Diceros bicornis*; and the Indian rhino, *Rhinoceros unicornis*), and all three breed successfully in captivity, within each exists a substantial number of individuals that have never reproduced (Guldenschuh and von Houwald, 2009; Christman, 2011a,b). Unless their genetic potential is preserved for future use, each individual's unique genetic milieu will be lost at death.

Although sperm and embryo banking is often touted as a means of ensuring the future for endangered species, the reality is that the material banked today must be functionally competent when thawed in the future, and there must be proven protocols available for producing pregnancies in the sperm and embryo recipients, or the limited, valuable banked resources are likely to be exhausted to no avail. In the case of the rhino, proof already exists that cryopreserved semen is fully functional post-thaw after many years in liquid nitrogen, and protocols have been established for producing offspring following artificial insemination with frozen–thawed sperm in both Indian rhinoceroses and white rhinoceroses (Stoops et al., 2007; Hermes et al., 2009; respectively). Therefore, sperm banking in the rhinoceros is based on an existing solid scientific foundation and is likely to yield fully functional samples capable of producing live young in the future. However, inherent in efforts to recover quality sperm samples for cryopreservation post-mortem are logistical and biological challenges that need to be identified so that methodologies for overcoming these obstacles can be employed to increase the odds of success.

Several challenges inherent to post-mortem sperm rescue have been investigated in other species and provide a precedent for this study. In Iberian red deer, both season and time of rescue post-mortem impacted sperm recovery efforts (Martinez-Pastor et al., 2005). Similarly, time of rescue relative to time of death significantly impacted sperm quality in the Spanish ibex (Fernández-Santos et al., 2011). In many species including the horse, ram, boar, deer, dog and mouse, tissue storage and/or transport at a cooler environmental temperature facilitated the harvest of quality sperm many hours post-mortem (Kikuchi et al., 1998; An et al., 1999; Yu and Leibo, 2002; Soler et al., 2005; Lone et al., 2011; Monteiro et al., 2013). Despite the difficulties, sperm collected and cryopreserved post-mortem and later used for artificial insemination has already produced offspring in several nondomestic species including the chimpanzee (Kusunoki et al., 2001), marmoset (Morrell et al., 1998), eland (Bartels et al., 2001) and Spanish ibex (Santiago-Moreno et al., 2006), proving the fertility of such samples.

The overall goal of this study was to retrospectively analyze data collected over a 16 year period to identify factors associated with the ability to rescue sperm from rhinoceroses post-mortem. Our three hypotheses were that the following factors impact our ability to rescue quality sperm samples post-mortem from the rhinoceros: (1) technical and temporal differences in post-mortem reproductive tissue removal, processing, packaging and shipping; (2) variation in rhinoceros characteristics (age, type and length of illness, method of death); and (3) environmental factors (temperature, season).

2. Materials and methods

2.1. Animals

A total of 23 mature male rhinoceroses representing 4 species and maintained at 13 different AZA-accredited 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. The range in ages, mean and median age for each species were (8–32 year; 20.9 year; 21