Available online at www.sciencedirect.com
ScienceDirect
Theriogenology
Theriogenology 66 (2006) 775–784
www.journals.elsevierhealth.com/periodicals/the

Post-mortem semen cryopreservation and characterization in two different endangered gazelle species (*Gazella gazella* and *Gazella dorcas*) and one subspecies (*Gazella gazelle acaiae*)

Joseph Saragusty [a,b], Haim Gacitua [b], Roni King [c], Amir Arav [b,*]

[a] *Department of Animal Sciences, Faculty of Agricultural, Food and Environmental Quality Sciences, Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76000, Israel*
[b] *Institute of Animal Science, Agriculture Research Organization, The Volcani Center, P.O. Box 6, Bet-Dagan 50250, Israel*
[c] *Israel Nature and Parks Authority, 3 Am Ve'Olamo Street, Jerusalem 95463, Israel*

Received 18 August 2005; received in revised form 16 January 2006; accepted 20 January 2006

Abstract

Both *Gazella gazella* and *Gazella dorcas* are endangered species with continually dwindling population size, yet basic knowledge on their spermatozoa is missing. Semen collected post-mortem (PM) from the cauda epididymis of five adult gazelles (three *Gazella gazella gazella*, one *Gazella gazella acaiae* and one *G. dorcas*) was cryopreserved using directional freezing of large volumes (8 mL) with egg-yolk-free extender. Sperm size measurements and SYBR-14/propodium iodide (PI) viability stain validation for use in gazelles were conducted. Post-thaw characterization included motility, viability, acrosome damage evaluation, computerized motility characterization and morphology and sperm motility index (SMI) was calculated.

Extracted sperm motility was 71.67 ± 11.67% (mean ± S.E.M.). Post-thaw motility ranged between 15% and 63%, viability was 57.49 ± 3.24%, intact acrosome was detected in 63.74 ± 2.6% (median 64.8%, upper/lower quartiles 71.79%, 61.82%), and normal morphology ranged between 41% and 63%. Motility characterization showed two sub-groups—highly active and progressively motile spermatozoa with SMI of 62.75 ± 0.38 and low activity and poorly progressive with SMI of 46.16 ± 1.53. Our results indicate that PM preservation of gazelle spermatozoa with satisfactory post-thaw viability is possible and cryobanking is achievable.

Keywords: Cryopreservation; Spermatozoa; Genome resource bank (GRB); Epididymal sperm; Gazelle

1. Introduction

Gazella gazella or Mountain gazelle, and *Gazella dorcas* or Desert gazelle, are two of several closely related species found in the Middle East. Both are listed as vulnerable in the World Conservation Union's (IUCN) red list of threatened species and the Red Book of Vertebrates in Israel [54], with a decline in their world wide population of more than 20% (Desert gazelle) or 30% (Mountain gazelle) and up to 50% decline in the population of the Mountain gazelle in northern Israel in the last decade [31,38,39]. The main causes for the population decline include habitat loss or degradation, over-hunting and predation [38–40,44]. A severe outbreak of foot and mouth disease among the Mountain gazelles in natural reserves in the north of

* Corresponding author. Tel.: +972 8 9484423; fax: +972 8 9475075.
E-mail address: arav@agri.huji.ac.il (A. Arav).

0093-691X/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2006.01.055

Israel resulted in the death of approximately 1500–2000 animals, constituting over 50% of the population there [55,56]. Such an outbreak demonstrates how vulnerable small populations can be.

A tiny population of a highly endangered subspecies of the Mountain gazelle has been described recently and was named Acacia gazelle (*Gazella gazella acaiae*) [43]. This subspecies was found to be closer genetically to the *Gazella gazella cora* found in Saudi Arabia than to the Israeli *Gazella gazella gazella* (Mokady and Geffen, manuscript in preparation). During the last decades, even though the females are reproducing, the population is declining due to low survival rate of the fawns. According to the last survey, conducted in May–June 2005, only 12 individuals were counted. All efforts taken thus far to change this dire demographic trend proved fruitless.

Recognizing these risks and the potential of captive and ex situ breeding, the World Conservation Union (IUCN) has approved in 2002 new guidelines for the conservation and maintenance of existing genetic diversity and viable populations of all taxa [28]. Captive breeding programs for various gazelle species exist for more than 3 decades now. To maintain biodiversity and to limit the negative consequences of small and fragmented populations, such as inbreeding depression [51] and susceptibility to disease outbreaks [55], there is an imperative need for the development of techniques to preserve the gametes of these endangered species. With the advances in reproduction technologies—artificial insemination (AI) and cryopreservation, and with the recognition for the need to protect many species from the danger of extinction, the establishment of genome resource banks (GRB) has been gaining increasing acceptance in recent years [19,20,34,35,46,57,58].

The number of studies carried out on semen preservation in the gazelle species is very limited and, to the best of our knowledge, none have been published on the *G. gazella* semen. To-date, no live offspring has been produced following insemination with cryopreserved semen from gazelle species [15] despite several attempts [25]. The limited research done on the preservation of other gazelle species and related antelopes can be helpful but, in cryobiology, species variations is a well recognized issue [24,36] and the need to develop specific techniques to preserve the gametes of each of the gazelle species is therefore due.

Epididymal sperm collection and preservation is a well documented collection method [6,14,27,29,33,36,41,48,49]. Probably the main advantage of this method is that it enables us to collect sperm post-mortem and, if stored, it can be used to extend the reproductive ''life span'' of that individual. When dealing with endangered species, this may enable us to preserve the spermatozoa of wild and genetically valuable captive males who die in an accident or otherwise. The spermatozoa accumulated in the cauda epididymis is already mature and fertile [12] making it a useful source. Several methods were described as to how to extract the sperm out of the cauda epididymis. These include squeezing the cauda epididymis [33], making cuts in the cauda epididymis [23,33,41], cutting and squeezing [49], extrusion by air pressure [27,32] and flushing the vas deferens [41]. Flushing the vas deference, when compared with the cutting method [41], was showed to be superior, yet it seems to be less suitable for field work.

In directional freezing technique, utilizing the multi-thermal gradient device (MTG®, IMT Ltd., Nes Ziona, Israel), damage to the cells, even when freezing in large volumes, can be minimized [2–4]. Using this technology, several studies have demonstrated the viability and fertility of frozen-thawed semen in a variety of species [4,13,52]. Large volume freezing has numerous additional advantages, making it an attractive alternative to freezing in straws. Risks of contamination, mix ups or loss of straws can all be avoided and storage, handling costs and space can all be reduced. A single insemination dose in a single large tube makes it easy for use in artificial insemination and easy to mark and control. Identifying samples stored in straws exposes them to high temperatures as soon as they are taken out of the liquid nitrogen, incurring further damage to the cells. For a large volume sample taken out of liquid nitrogen to reach a temperature of −100 °C takes over 2 min at room temperature (unpublished observation), giving the handler ample time to identify it and decided if to take it out or return it back to the liquid nitrogen.

The aim of this study was to characterize and evaluate the feasibility of cryopreserving post-mortem epididymal sperm. Achieving this goal may provide us with the means to preserve genetic material from the last few individuals left from *G. gazella acaiae* and hopefully assist in preventing its extinction.

2. Materials and methods

2.1. Semen collection, processing and evaluation

Testicles were obtained post-mortem from each of six sexually mature gazelles that were killed or severely injured (and were later euthanized) in car accidents in