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SCIENCE DIRECT
Theriogenology
Theriogenology 63 (2005) 246–259
www.journals.elsevierhealth.com/periodicals/the

Flow cytometric sorting of non-human primate sperm nuclei

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Received 9 March 2004; accepted 21 April 2004

Abstract

Pre-determination of the sex of offspring has implications for management and conservation of captive wildlife species, particularly those with single sex-dominated social structures. Our goal is to adapt flow cytometry technology to sort spermatozoa of non-human primate species for use with assisted reproductive technologies. The objectives of this study were to: (i) determine the difference in DNA content between X- and Y-bearing spermatozoa (ii) sort sperm nuclei into X- and Y-enriched samples; and (iii) assess the accuracy of sorting. Spermatozoa were collected from two common marmosets (*Callithrix jacchus*), seven hamadryas baboons (*Papio hamadryas*) and two common chimpanzees (*Pan troglodytes*). Human spermatozoa from one male were used as a control. Sperm nuclei were stained (Hoechst 33342), incubated and analyzed using a high-speed cell sorter. Flow cytometric reanalysis of sorted samples (sort reanalysis, 10,000 events/sample) and fluorescence in situ hybridization (FISH; 500 sperm nuclei/sample) were used to evaluate accuracy of sorting. Based on fluorescence intensity of X- and Y-bearing sperm nuclei, the difference in DNA content between X and Y populations was 4.09 ± 0.03, 4.20 ± 0.03, 3.30 ± 0.01, and 2.97 ± 0.05%, for marmoset, baboon, chimpanzee and human, respectively. Sort reanalysis and FISH results were similar; combined data revealed high levels of purity for X- and Y-enriched samples (94 ± 0.9 and 93 ± 0.8%, 94 ± 0.7 and 94 ± 0.5%, 91 ± 0.9 and 97 ± 0.6%, 94 ± 0.6 and 94 ± 0.9%, for marmoset, baboon, chimpanzee and human,

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doi:10.1016/j.theriogenology.2004.04.013

respectively). These data indicate the potential for high-purity sorting of spermatozoa from non-human primates.

Keywords: Sperm sorting; Sperm sexing; Sex pre-determination; Flow cytometry; Primates

1. Introduction

In conjunction with assisted reproductive technology, sex pre-selection of offspring through the use of sexed spermatozoa [1] has great potential as a captive population management strategy for wildlife species, particularly those with single-sex dominated social structures [2]. Birth of normal offspring using spermatozoa sorted by flow cytometry has been achieved in numerous domestic species [3–5], humans [6–8] and in one wildlife species, the elk (*Cervus elaphus nelsoni*, [9]). Although sperm sorting methods have been developed for one primate, the human [8,10], there exists great variation in gamete physiology among primates, and species-specific protocols for sperm staining, sorting and preservation will be required.

Reanalysis of sorted samples (sort reanalysis [11]) using the flow cytometer is the standard method for evaluation of accuracy of sorting and can be performed in a short interval (30 min) and at low additional cost. However, sort reanalysis results may not be consistently accurate for species where the DNA content difference between X and Y chromosome-bearing spermatozoa is <3.0% [11]. Sort reanalysis results can be validated by single sperm analysis using fluorescence in situ hybridization (FISH, [11]) or the polymerase chain reaction (PCR, [12]) using probes/primers specific to the X and/or Y chromosome(s). These techniques have been used in humans to assess accuracy of sorting [8,10] and in a western lowland gorilla (*Gorilla gorilla gorilla*) to determine fetal sex [13]. The feasibility of using FISH probes labeling specific loci on human chromosomes for identification of chromosomes in spermatozoa of other primates has not been investigated.

The goal of this research was to establish basic flow cytometry parameters and methodologies for sorting sperm nuclei in several non-human primate species. Specific objectives were to: (i) determine the difference in DNA content between X- and Y-bearing sperm nuclei (ii) sort sperm nuclei into X- and Y-enriched samples; and (iii) assess the accuracy of sorting using sort reanalysis and FISH.

2. Materials and methods

Procedures described herein were approved by The University of Sydney's Animal Ethics Committee.

2.1. Study design

Four primate species representing new world monkeys (common marmoset, *Callithrix jacchus*, $n = 2$), old world monkeys (hamadryas baboon, *Papio hamadryas*, $n = 7$), great