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Identification of sperm head subpopulations with defined pleiomorphic characteristics in ejaculates of captive Goeldi's monkeys (*Callimico goeldii*)

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ABSTRACT

The aim of this study was to evaluate the incidence of pleiomorphisms and its influence on the distribution of sperm morphometric subpopulations in ejaculates from the vulnerable Goeldi's monkey (*Callimico goeldii*) by using a combination of computerized analysis system and Principal Component Analysis (PCA) methods. Each sperm head was measured for four primary spermatozoal head dimensional parameters (area [A (μm^2)], perimeter [P (μm)], length [L (μm)] and width [W (μm)]) and three head shape derived parameters (ellipticity [(L/W)], elongation [$(L-W)/(L+W)$] and rugosity [$(4\pi A/P^2)$]). Six separate subpopulations (SPs) were identified: SP1, constituted by very large, narrow and very elliptical spermatozoa ($A = 16.85 \pm 1.56\ \mu m^2$, $W = 2.75 \pm 0.42\ \mu m$ and ellipticity = 2.16 ± 0.24); SP2, characterized by average sized, short, wide and round spermatozoa ($A = 15.00 \pm 1.92\ \mu m^2$, $L = 5.06 \pm 0.49\ \mu m$, $W = 3.51 \pm 0.31\ \mu m$ and ellipticity = 1.44 ± 0.15); SP3, represented by small, wide and slightly round spermatozoa ($A = 14.95 \pm 1.75\ \mu m^2$, $W = 3.47 \pm 0.29\ \mu m$ and ellipticity = 1.48 ± 0.14); SP4 included very small, short and very round spermatozoa ($A = 14.15 \pm 2.38\ \mu m^2$, $L = 4.90 \pm 0.57\ \mu m$ and elongation = 0.18 ± 0.05); SP5 consisted of average sized and slightly elliptical spermatozoa ($A = 15.14 \pm 1.72\ \mu m^2$ and ellipticity = 1.49 ± 0.14); and SP6 included large and round spermatozoa ($A = 16.30 \pm 1.62\ \mu m^2$ and elongation = 0.19 ± 0.04). There were differences in the sperm subpopulation distribution ($P < 0.001$) among the five donors analyzed. In conclusion, the results of the current study confirmed that the use of computer sperm analysis methods combined with PCA cluster analyses are useful methods to identify, classify, and characterize different sperm head morphometric subpopulations in neotropical primates. Broadening our knowledge of *C. goeldii* sperm morphometric abnormalities as well as developing reliable techniques for sperm evaluation may be essential for *ex situ* conservation of this threatened species.

1. Introduction

The occurrence of sperm morphologic abnormalities has been observed in human ejaculates as well as in multiple non-human primate species. Typically, sperm abnormalities occur during spermatogenesis (Abramsson

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et al., 1989; Diemer and Desjardins, 1999). Andrological studies have found that one of the primary sperm abnormalities present in humans and non-human primates are related to sperm head defects (Levek-Motola et al., 2005), which inhibit sperm function (Singer et al., 1980) and consequently fertility potential (Jouannet et al., 1988).

The incidence of pleiomorphisms observed following ejaculation for new world primates is variable, and species-dependent. The study of sperm morphologic abnormalities, such as head, midpiece or tail defects has elucidated important aspects of sperm pathologies (De Jonge, 2012). In neotropical primates used for andrology research such as the marmoset (*Callithrix jacchus*), howler monkey (*Alouatta caraya*) and capuchin monkey (*Cebus apella*), the incidence of sperm pleiomorphisms was variable (from 3.0% to 37.0% primary defects and from 9.0% to 48.0% secondary defects). These studies allowed to obtain relevant biological knowledge which might have a practical use for future research related to endogamy which has adverse effects on sperm fertility potential (Chemes and Rawe, 2003). Moreover, to conduct studies related to sperm morphological abnormalities is important especially in endangered primate species or even in humans (Ober et al., 1999; Charlesworth and Willis, 2009).

The new world primate known as Goeldi's monkey (*Callimico goeldii*), is the unique member of the *Callimico* genus and is considered vulnerable by the International Union for Conservation of Nature (IUCN) (Cornejo, 2012) as a consequence of habitat destruction and poaching pressures (Estrada et al., 1999; Miller et al., 2004). Captive population management, using a combination of natural breeding and assisted reproduction, represents one vital component of *ex situ* conservation programs for this threatened species (Savage et al., 2010; Oliveira et al., 2011). Thus, the lack of availability of previous data, difficult management, and poor breeding performance in captivity, make Goeldi's monkey an important species for developing new Assisted Reproductive Technologies (ARTs) that are potentially useful in supporting the management and breeding of other endangered primate species (Morrell and Hodges, 1998). Despite this, to the best of our knowledge, no accurate studies related to sperm morphologic abnormalities in endangered neotropical primates have been conducted, mainly due to *in situ* limitations. An association between computerized and Principal Component Analysis (PCA) techniques could allow classifying the overall spermatozoa population of semen samples into homogeneous separated subpopulations, avoiding the substantial loss of information when traditional statistical procedures are applied to the results (Marti et al., 2011; Valle et al., 2012). Therefore, to investigate the identification and the distribution of sperm morphometric subpopulations on Goeldi's monkey spermatozoa would be of utmost importance as a tool to assess and classify individuals and sperm samples with different morphologic characteristics.

Thus, the objective of this research was (i) to study the main sperm morphological differences in Goeldi's monkey ejaculates, (ii) identify and characterize Goeldi's monkey spermatozoa by using an objective sperm morphometric analysis (iii) and to validate defined sperm subpopulations in Goeldi's monkey.

2. Materials and methods

2.1. Reagents and experiment location

All chemicals used in this study, unless otherwise stated, were of analytical grade and purchased from Sigma–Aldrich Chemical Company (Sigma–Aldrich Brasil Ltd., Sao Paulo, Brazil). The experiment was conducted at the National Primates Center – CENP, Ministry of Health, Brazil, and at the University of São Paulo (USP), Brazil. All procedures were performed in accordance to the Brazilian Animal Protection Law (SISBIO 30086-1) and approved by the Ethical Research Committee of Paulista University (Protocol Number 042/2011 CEP/ICS/UNIP).

2.2. Donors and semen collection

The study was conducted using 15 ejaculates collected from five healthy, reproductively sound Goeldi's monkeys (genetically heterogeneous). Semen samples were collected on a regular basis by electroejaculation using an electroejaculator apparatus (DUBOI®, Campo Grande, Mato Grosso do Sul, Brazil), with slight modifications of a published protocol (Valle et al., 2004) (one collection/week). The entire procedure took approximately 40 min. Males were anesthetized with ketamine hydrochloride (Dopalen®, Vetbrands®, Paulínia, São Paulo, Brazil) and xylazine hydrochloride (Anasedan®, Vetbrands®, Paulínia, São Paulo, Brazil), 20 and 1 mg/kg body weight, respectively, combined together and given as an intramuscular injection. Animals were completely recovered from anesthesia within 60–90 min. To minimize the contamination of semen, the external genitalia was washed just prior to semen collection with liquid soap (MAGMA®, Ananindeua, Pará, Brazil) and the exposed penis was washed with saline. The electroejaculator has a manual control to regulate the intensity and the duration of the electrical stimuli. The output ranged from 0 to 12 V. The rectal probe composed of a resin rod had wires attached to one end of the straight rod and the other end was metal in the form of a cone with a rounded end. The initial stimulus was 2.0 V, with subsequent increases of 0.5 V between each series of stimuli, with a maximum of 7.5 V. Each series of stimuli was composed of 30 stimuli, duration 2–3 s, with an interval of 1–2 s between successive stimuli. All donors were maintained under uniform nutritional and environmental conditions.

2.3. Semen processing and sample staining

Immediately after semen collection (at CENP) into a dry glass tube, the sperm was diluted into 200 μL of *in natura* coconut water extender (coconut water 1:1 (v/v) in ultrapure water, citrate solution (5%), streptomycin, penicillin, 294 mOsm, pH 6.5; Araújo et al., 2009) at 37 °C in a water bath and the sperm quality of each ejaculate was assessed (volume, sperm concentration and sperm motility) by using phase-contrast microscopy. Microscopic slides were prepared by placing the stained sperm sample on the clear end of a frosted slide and dragging the drop across the slide to create a thin-feathered smear (two smears per