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Sperm head morphometry in ejaculates of adult marmosets (*Callithrix jacchus*): A model for studying sperm subpopulations and among-donor variations

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Abstract

In humans and other mammals, sperm morphology has been considered one of the most important predictive parameters of fertility. The objective was to determine the presence and distribution of sperm head morphometric subpopulations in a nonhuman primate model (*Callithrix jacchus*), using an objective computer analysis system and principal component analysis (PCA) methods to establish the relationship between the subpopulation distribution observed and among-donor variation. The PCA method revealed a stable number of principal components in all donors studied, that represented more than 85% of the cumulative variance in all cases. After cluster analysis, a variable number (from three to seven) sperm morphometric subpopulations were identified with defined sperm dimensions and shapes. There were differences in the distribution of the sperm morphometric subpopulations ($P < 0.001$) in all ejaculates among the four donors analyzed. In conclusion, in this study, computerized sperm analysis methods combined with PCA cluster analyses were useful to identify, classify, and characterize various head sperm morphometric subpopulations in nonhuman primates, yielding considerable biological information. In addition, because all individuals were kept in the same conditions, differences in the distribution of these subpopulations were not attributed to external or management factors. Finally, the substantial information derived from subpopulation analyses provided new and relevant biological knowledge which may have a practical use for future studies in human and nonhuman primate ejaculates, including identifying individuals more suitable for assisted reproductive technologies.

Keywords: Sperm morphometry; Principal component analysis; Sperm subpopulations; Cluster distribution; Nonhuman primate; *Callithrix jacchus*

1. Introduction

The use of nonhuman primate individuals for reproductive research has been of crucial importance for several decades. The new world primate known as the common marmoset (*Callithrix jacchus*), a member of the *Callitrichidae* family, has been successfully used as a human and nonhuman primate model for andrological research and many other fields in reproductive sciences [1–3]. This species is a good model for andrology, because of various biological factors, such as a similar testicular epithelium, spermatogenic organization, and

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spermatogenenic process, making this species a model for human reproduction [2,4]. All these physiological factors, together with the relative availability of previous data, economical and easy maintenance, and finally, good breeding performance in captivity, make it an important model for development of assisted reproductive technologies, which are potentially useful in supporting the management and breeding of other endangered primate species [5].

Because sperm abnormalities are regarded as indicators for reduced fertility in both human and nonhuman primates [6], it is necessary to develop methods based on nonsubjective techniques for measuring sperm characteristics. The introduction of automated sperm morphometry analysis may solve the problem of subjective evaluations on sperm morphology, because with this technology, semen research has gained objectivity and sensitivity [7]. However, although it is possible to minimize inter- and intraobserver variability with this technique, the classical approach considering the whole ejaculate as a homogeneous population with a normal distribution to assess sperm quality or biophysiological factors is considered erroneous [8]. In that regard, the existence of well-defined sperm subpopulations within mammalian ejaculates is now widely accepted by the scientific community [9,10]. Thus, there is a substantial loss of information when traditional statistical procedures are applied to the results, because the real distribution of sperm morphometry is not uniform and normal, but rather structured in separate subpopulations [11]. An association between computerized and statistical techniques could allow classifying the overall sperm population of semen samples into homogeneous separated subpopulations, grouping spermatozoa with similar morphometric characteristics [8].

There are apparently no reports regarding the examination of sperm morphometric subpopulations on primate spermatozoa as a tool to assess and classify individuals and sperm samples with different characteristics. Therefore, the objectives of this work were to: (1) study the main morphologic differences intermarmoset individuals for analyzing the variability regarding sperm morphometric dimension and shape parameters; (2) characterize marmoset spermatozoa by using sperm head morphometry analysis; and finally (3) conduct a general and individual study of sperm morphometric subpopulations in marmoset ejaculates as a validated method to classify specific sperm subpopulation groups with similar characteristics.

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, Ltda., São Paulo, SP, Brazil). The experiment was carried out at the Deutches Primatenzentrum, Göttingen, Germany, and at the University of São Paulo, Pirassununga, SP, Brazil. All procedures were performed in accordance with the German animal protection law (Animal Experiment Permission # AZ 509.42502/08-01.03).

2.2. Donors and semen collection

The study was conducted using 20 ejaculates collected from four healthy reproductively mature common marmosets (genetically heterogeneous). Semen samples were collected on a regular basis by penile vibrostimulation apparatus (FertiCare Personal; Multicept ApS, Rungsted, Denmark), with slight modifications of a published protocol [12] (one collection per week). The modification consisted of stimulation phases of 2 min followed by resting phases of 30 sec. The first intensity in each stimulation phase was the same as the last one before the resting phase. Initial stimulation intensity was 70 Hz and 1-mm amplitude for 1 min, then increased to 80 Hz and same amplitude for 1 min. After the resting phase, stimulation was repeated with 80 Hz and 1-mm amplitude for 2 min. Stimulation was continued with 80 Hz and 1-mm amplitude for 1 min and 70 Hz and 1.5-mm amplitude for 1 min. If ejaculation had not occurred, stimulation intensity was increased to 80 Hz and 1.5 mm, 90 Hz and 1.0 mm, and 90 Hz and 1.5 mm [13], pooling two successive ejaculates from the same animal per day to obtain homogeneous samples. All marmosets were maintained under uniform nutritional and environmental conditions to minimize external factor differences and effects on semen quality.

2.3. Semen processing and sample staining

Immediately after semen collection (at Deutches Primatenzentrum) into a dry handmade glass tube, the semen was diluted into 50 μL of modified TALP-HEPES medium (TALP-HEPES +3 mg/mL BSA V, 0.25 mM Na pyruvate, pH 7.33) at 37 °C in a water bath and sperm quality of each ejaculate was assessed (volume, sperm concentration, and sperm motility) with a phase-contrast Nikon Eclipse E200 microscope (Nikon, Tokyo, Japan). Slides were prepared by placing