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Electroejaculation and semen buffer evaluation in the microbat *Carollia perspicillata*

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ABSTRACT

Scientific interests and conservation needs currently stress the necessity to better understanding bat reproductive biology. In this study, we present the first, safe, inexpensive, and reliable method to obtain sperm from a microbat species (*Carollia perspicillata*) by electroejaculation. This method revealed to be highly efficient (100% success rate). We obtained ejaculates composed of two characteristically different fractions. We compared three buffers and recommend using an Earle's balanced salt solution as a semen extender. Earle's balanced salt solution provided significant repeatable measure of swimming ability (intraclass correlation coefficient: 0.74, $P < 0.01$) and proportion of motile sperms (intraclass correlation coefficient: 0.08, $P = 0.01$) and allowed sperm to maintain optimal swimming capacity over time. None of the buffers could dissolve all the coagulated sperm. Although the trypsin buffer freed a larger fraction of spermatozoa in the ejaculate, it impaired swimming ability without improving motility, viability, and stamina. We thus argue that the sperm population analyzed with Earle's balanced salt solution is a representative of the ejaculate. Finally, we found that the mean sperm velocity of *C perspicillata* (78.8 μm/s) is lower than that predicted by regressing sperm velocity on relative testes mass, a proxy of sperm competition. The question as to whether *C perspicillata* is an outsider for sperm velocity, or whether bats evolved yet another unique mechanism to cope with sperm competition deserves more investigations.

1. Introduction

Although long overlooked, bats are currently a promising research model. Indeed, they represent around a fifth of all mammalian species and are distributed worldwide and reveal a complex and diverse biology [1,2]. The broad ecological services offered by bats range from pest control to forest regeneration and pollination, with a potentially enormous economical value [3,4]. Moreover, conservation strategies are currently urgent, as anthropogenic threats have already caused large reductions of certain populations, driving species toward local or global extinctions [5,6]. Despite those dramatic threats, bat semen preservation for assisted reproduction has seldom been addressed as an appropriate method for species and genetic conservation [7–9]. Such an approach would, however, appear most applicable in species breeding poorly in captivity and for which *ex situ* conservation is not a reasonable option. Besides those conservation perspectives, bat semen studies may be valuable for fundamental research. Indeed, the sperm of some bats species is already known for its extraordinary ability to sustain fertility for periods lasting up to 7 months [10,11]. Additionally, with their rich variety of social systems and ecological niches, bats are ideal models for evolutionary studies of mammalian male reproductive physiology [12–14].

One of the reasons why semen collection methods have not yet been properly undertaken in microbats may be the

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difficulty to sample ejaculates from living wild or captive individuals. However, methods have already been developed for other mammals of similar size and for flying-fox species [15,7]. After the collection, adequate ejaculate processing should provide suitable samples for subsequent analyses or utilization. Semen processing efficiency and measurement reliability depend on species-specific sperm biochemical and physiological characteristics [16]. Consequently, numerous buffers are currently available for mammalian models.

Previously obtained ejaculates from *Carollia perspicillata* were small in volume and heterogeneous, as they comprised coagulated sperm that did not spontaneously dissolve (unpublished data). Semenogelin is a protein that mechanically traps spermatozoa and inhibits sperm motility and capacitation [17]. The addition of trypsin, a serine protease, in the seminal extender can precipitate the dissolution of the sperm coagulum [18–20]. However, although the enzymatic reaction allows the dissolution of coagula and the liberation of bound spermatozoa in some species, it can impair sperm survival and motility in others [21].

With this study, we describe the first method of electroejaculation (EE) for a microbat species, which may permit repeated collection of semen without detrimental effects on the animals. Second, we compare three different buffer solutions as sperm extender to get optimal sperm survival and ejaculate fluidity allowing adequate computer-assisted sperm analysis (CASA).

2. Materials and methods

2.1. Animal welfare and ethics

We monitored animal recovery and welfare as well as possible effects on health during the subsequent days. For the first 20 collections, animals were kept after the manipulation in cages (2.1 m × 0.9 m × 1.4 m for l × l × h) for 5 days with food and water *ad libitum*. We also monitored their post-anesthesia recovery by keeping them for one to three hours in individual cotton bags provided with food *ad libitum* (apple pieces). During this period, we recorded any injury or abnormal behavior that would indicate excessive pain or stress and would require euthanizing the animal in accordance with our guidelines. As no midterm disturbance was detected, the eight bats used for the buffer comparison were released directly after the post-anesthesia monitoring. Experimental setup and detention conditions were authorized by the veterinary office of the Canton of Fribourg after examination by the cantonal ethical committee (FR_2012_15E).

2.2. Study area and model species

Carollia perspicillata (family: phyllostomidae; suborder: yangochiroptera) is a moderate-sized (18.5 g) frugivorous species. This species is common in its natural range (Central and South America) and can easily breed in captivity. Thus, *C perspicillata* is suitable for zoos and research facilities [22]. The present study was performed in the Papiliorama, a tropical zoo (Kerzers, Switzerland) where light cycles are reversed on a 12/12 hour basis. A population of 400 individuals lives in semicaptivity with constant environmental conditions. Bats roost in an artificial cave and can fly freely under a dome, which mimics a tropical environment. A fruit-based mixture is provided twice a day. Males with large scrotal testes are constantly present in captivity and nature, where testes size can vary slightly with reproductive seasons [23]. However, in constant environmental conditions, captive bats do not show any reproductive patterns [22]. Bats were caught with a harp trap (Faunatech Austbat, Australia) or with a hand net, and males with large scrotal testes (approximately 7 mm in length) were detained for semen collection. With a single collection, we tested the efficiency of the method on 20 bats. Then, another eight individuals were used to conduct the sperm buffer comparison on the basis of mobility traits. An insufficient amount of ejaculate (<3 μL) was obtained from one male, reducing the sample size to seven individuals for this analysis.

2.3. Anesthesia

Health status was evaluated before the manipulation by general visual inspection with focus on fur quality, presence of superficial injuries and apathy, and only bats in good condition were kept for the experiment. To avoid hypothermia, animals were laid dorsally on a warming pad. Anesthesia induction was achieved by inhalation of 5% isoflurane (Nicholas Piramal I Ltd, UK) mixed with 0.8 L/min oxygen (Carbagas, Switzerland) through a Rodent Nosecone Non-rebreathing system (Rothacher medical, Switzerland). After the induction phase, anesthesia was maintained during the EE with a lower dose of isoflurane (1.5%–2.5%) mixed with 0.8 L/min oxygen. After manipulation, the bat was provided with pure oxygen until emergence. Throughout the procedure, the respiratory rate was visually monitored.

2.4. Electroejaculation

Any ejaculate containing motile sperm was considered a successful EE. The rectal probe (diameter, 2.5 mm; see Supplementary Fig. 1) was fitted with two 4-mm electrodes situated at 2.5 mm from the probe's distal end (International Canine Semen Bank, USA). After anesthesia induction, the anus and the genitalia were first washed with water-soaked cotton. Then, the probe, coated with an aqueous lubricant (K-Y; Johnson and Johnson), was gently inserted 1-cm deep into the rectum. The electrodes were placed upward to face the prostate and stimulate the nearby nerves, contracting the pelvic muscle. As the nerves passing through this area are responsible for the erection but also activate the leg muscles, we ensured that the probe was correctly positioned by observing the legs' contractions. Three series of stimulations were performed progressively to avoid urinary contamination. Each series consisted of 10 stimulations with increasing intensity (0.3–3 mA, 50 Hz) of 1 second each with a 1-second break in-between. One series ended with a 10-second stimulation at the highest current intensity accompanied with gentle in and out movements of the probe. Two resting periods of 60 seconds were planned between each series. Stimulation series were designed using an audio software (Audacity 2.0.4) and transferred to the probe with an audio amplifier (JVC A-X2). The electrical current was continuously