Animal Reproduction Science 195 (2018) 309–320

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Bottlenose dolphin (*Tursiops truncatus*) sperm revisited: Motility, morphology and ultrastructure of fresh sperm of consecutive ejaculates

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ARTICLE INFO

Keywords:
Atlantic bottlenose dolphin
Tursiops truncatus
CASA
Sperm kinematics
Sperm speed
Sperm morphometrics

ABSTRACT

Computer aided sperm analysis systems allow detailed examination of sperm motility and morphology variables, which are important for the understanding of the spermatology of a species and the development of assisted reproductive techniques. Cetacean biology is too complex to study in the wild and data from captive individuals provide an important alternative for the conservation of these charismatic animals. The present study evaluates ejaculate and sperm characteristics, including sperm motility, kinematic variables and quantitative sperm morphology and ultrastructure, of consecutive ejaculates from Atlantic bottlenose dolphins (*Tursiops truncatus*). Sperm concentrations and total and progressive motilities were greater in the second than the first ejaculate, with all ejaculates being of very high quality (6.9–1127 × 10^6/ml sperm concentration, 75% to 91% total motility and 89% to 96% normal sperm). Most sperm in an ejaculate (≥84%) were highly (VCL > 150 μm/s) and progressively motile with very few abnormal sperm. The sperm have small heads, a short but very bulky midpiece and a long tail. Detailed sperm morphometrics using CASA indicated there were similarities from one ejaculate to the next. The large mitochondria with extensive cristae mitochondriales are tightly packed in the midpiece resulting in a large midpiece volume. All the semen and sperm characteristics indicate high quality sperm and support the assumption that a multimale mating system is present in *T. truncatus*.

1. Introduction

Most cetaceans are threatened and all species are listed in Appendix II of CITES as populations face a number of threats among which are commercial hunting, incidental captures in fisheries and habitat degradation (Reeves et al., 2003). The nature of the cetacean habitat makes it extremely difficult to study these animals and makes captive populations especially valuable for research of

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https://doi.org/10.1016/j.anireprosci.2018.06.009
Received 27 March 2018; Received in revised form 31 May 2018; Accepted 21 June 2018
Available online 22 June 2018
0378-4320/

their biology (O'Brien and Robeck, 2010b). Due to ethical considerations cetaceans, however, are becoming rarer in captivity, which makes it more difficult to obtain material and data and makes any information exceedingly valuable for cetacean research.

Exchange of cetaceans between captive facilities is problematic because of the risks and regulations involved (O'Brien and Robeck, 2010b)., Accordingly, sperm cryopreservation and artificial insemination (AI) methods, have been developed for a number of cetacean species (Robeck and O'Brien, 2004; Robeck et al., 2004; O'Brien et al., 2008; Robeck et al., 2009; O'Brien and Robeck, 2010a).

A thorough understanding of the basic semen and sperm variables of a species is essential. Sperm quality varies not only between individuals of the same species, but also between consecutive ejaculates of the same individual. In some mammals a reduction in semen volume and sperm concentration was observed from the first to subsequent ejaculates (Ritar et al., 1992; Ambriz et al., 2002). In contrast, some aspects of sperm quality were greater in the second ejaculate of both Indo-Pacific (*Tursiops aduncus*) and Atlantic bottlenose dolphins (Yuen et al., 2009; Sánchez-Calabuig et al., 2015). Fresh Atlantic bottlenose dolphin ejaculates are in general of very high quality with excellent sperm motilities, little sperm DNA fragmentation and few abnormal sperm (Fleming et al., 1981; O'Brien and Robeck, 2006; Montano et al., 2012; Sánchez-Calabuig et al., 2015).

The present study evaluated macro and micro sperm characteristics (sperm motilities and kinematics) of fresh ejaculates from three sexually mature Atlantic bottlenose dolphins using a computer aided sperm analysis (CASA) system. It was hypothesized that subsequent ejaculates would be of greater quality than first ejaculates and as such would be a more reliable indicator of a dolphins' reproductive capacity as well as have greater value for ART. Results from three recent studies in humans indicate that the second ejaculate collected a few hours after the first yielded sperm with greater functional characteristics (Alipour et al., 2017; Ayad et al., 2018a, 2018b). The main reason appears to be that sperm age very quickly in the cauda epididymis and sperm from subsequent ejaculates would have been stored *in vivo* for less time and as such be of greater quality. Furthermore, in species with greater amounts of sperm competition it will be advantageous that sperm are of high quality in consecutive ejaculates. This is the first study where this aspect has been quantified by CASA. The present study also complimented previous studies that focused on sperm motility through assessment of sperm sub-populations (rapid, medium and slow). This aspect has not been sufficiently addressed in the past assuming that averages of kinematic values represent the fertilizing sperm population. Eleven sperm morphometric features of the sperm head as well as tail and midpiece length, using CASA was measured for the first time, and evaluation of ultrastructural features by means of transmission electron microscopy.

Data collected in the present study provided further and more comprehensive quantitative information and knowledge on the association of semen and sperm characteristics. Many of the aspects addressed in the current study have been published, but because of the small numbers of dolphins used in all these previous studies, including some from the laboratory where the present research was conducted, it is important to further assess and establish similarities, differences and potential shortcomings.

2. Materials and methods

2.1. Research animals

Two Atlantic bottlenose dolphins, *Tursiops truncatus*, (henceforth referred to as TT1 and TT2) and a *truncatus*-male/*aduncus*-female hybrid (TA1) of uShaka Marine World, Durban, South Africa were trained for semen collection. Conditioning of the dolphins to the voluntary semen collection took approximately 3 weeks. The age of the dolphins varied from 18 (TA) to more than 32 years (TT1) and both TT1 and TT2 successfully sired offspring. Ethical clearance for this project was granted by uShaka Marine World, Durban, South Africa as well as by the Research Ethics and Scientific Committee of the National Zoological Gardens of South Africa (RESC/P06/34).

2.2. Semen collection

Part of the conditioning involved that the dolphins presented themselves with the ventral side up and the trainer tapping lightly by hand on the abdominal wall in front of the sheath that surrounds the penis. This resulted in an erect penis being exposed and it was possible to rapidly rinse the penis with 0.9% saline to exclude potential contamination with sea water. After rinsing the penis, a sterile plastic bag was placed over the erect penis which was in all cases followed by ejaculation. The semen was immediately removed from the plastic bag and the volume measured in Falcon tubes to the closest mL. Semen color and viscosity were also estimated using a subjective manual scale and a rough estimate of pH was performed using Panpeha pH paper strips (Whatman, GE Healthcare Life Sciences, UK). These aspects constituted the macro semen/sperm characteristics. Consecutive ejaculates (three) were sampled at 30 min intervals and repeated after 24 h.

2.3. Sperm concentration and sperm motility (micro sperm characteristics)

The sperm concentration and motility module of the Sperm Class Analyzer (SCA) version 4.1 (Microptic SL, Barcelona, Spain) was used to determine sperm concentration, the percentage sperm motility, progressive sperm motility and sperm kinematics (see Table 2 for more details). For sperm concentration assessments, semen was diluted from 1:20 and up to 1:100 using Ham's F10 culture medium (Gibco laboratories, Buffalo, USA). The diluted sperm suspension was introduced in a Leja 20 µm deep chamber (Leja Products, The Netherlands). An Olympus CH2 microscope with negative phase optics (x10 objective) and a temperature controlled stage (37 °C) was used in conjunction with a Basler 312FC firewire camera. The sperm concentration was determined using the SCA system calibrated for this Leja chamber. At least three different microscopic fields were used counting at least 500 motile sperm.