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Morphometry characterisation of European eel spermatozoa with computer-assisted spermatozoa analysis and scanning electron microscopy

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Abstract

The aim of the present study was to characterise European eel spermatozoa morphometrically, as a basis for future studies on the morphological effects of methods for sperm cryopreservation and sperm quality. This characterisation was carried out measuring several spermatozoa morphology parameters (head length, width, area and perimeter) by scanning electron microscopy (SEM), in comparison with measurements developed in European eel spermatozoa with computer-assisted morphology analysis (ASMA).

Spermatozoa head morphology showed differences in width (1.15 ± 0.01 μm versus 1.12 ± 0.01 μm), perimeter (14.68 ± 0.13 μm versus 13.72 ± 0.19 μm) and area (5.36 ± 0.06 μm^2 versus 1.12 ± 0.01 μm^2) for ASMA and SEM, respectively. When head length was evaluated, significant differences were found, being higher for SEM methodology (5.09 ± 0.04 μm versus 4.29 ± 0.03 μm). The curved and elongated spermatozoa head in eels means a problem for

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the ASMA system (Sperm Class Analyser®, Morfo Version 1.1, Imagesp, Barcelona, Spain), causing an error in the length measurements. However, similar results were obtained by both techniques when spermatozoa head length was considered as the greater length between two points within the object (4.29 ± 0.03 μm versus 4.31 ± 0.04 μm for ASMA and SEM, respectively). In conclusion, this is one of the first applications of ASMA in fish and the first in this species, and confirms this system as a useful tool with wide applications in future fish spermatozoa studies. Width, perimeter and area could be used as parameters for the spermatozoa morphology evaluation, whereas the length requires a new programming of the Imagesp software.

Keywords: Eel; Spermatozoa; Sperm; ASMA; CASA

1. Introduction

Previous reports have described the spermatozoa ultrastructure of European and Japanese eels and examined its morphology by transmission electron microscopy (TEM) [1–4] or scanning electron microscopy (SEM) [3,5]. Other techniques to examine and analyse fish spermatozoa head are laser light-scattering spectroscopy and stroboscopic illumination [6]. Results obtained with these techniques are subjective, time-consuming and highly variable. The search for methods of accurate, objective and repeatable assessment of sperm fertility still remains the aim of many studies. One of these developed computer-assisted applications is an automated system for spermatozoa head morphometry analysis (ASMA), developed and validated for mammals [7] and fish [6].

Abnormal spermatozoa head morphometry has been associated with reduced fertility in the bull, boar and stallion [6,8]. ASMA has increasingly been used with mammalian species, such as man [9], rat [10], rabbit [11], bull [12], dog [13], monkey [14] and alpaca [15]. ASMA measurements have shown toxic effects on human spermatozoa head [16] and the effect of mercuric chloride on goldfish sperm [6]. This technique has also been used in the field of cryopreservation, in which cryoprotectants or frozen-thawed protocols are known to cause morphological damage to the spermatozoa [17,18]. Kruger et al. [19] found that spermatozoa head morphometry, determined by ASMA, was predictive of in vitro fertilisation rates, and it has also been reported to result in detection of fertile and subfertile stallions [20] and rabbit [21]. ASMA systems have never been used in eel species.

ASMA has provided a series of objective parameters, which have facilitated the standardisation of morphological semen evaluation [22]. However, different problems have arisen, such as sample preparation, staining procedure and the settings of the spermatozoa morphology analyser, which must be optimised for each species [9–11,23–25]. The computer-assisted morphometry analysis requires the standardisation of preparation, staining and sampling methods [9].

The main aim of the present study was to characterise the European eel spermatozoa morphometrically, comparing the results obtained by computer-assisted spermatozoa analysis and by scanning electron microscopy.