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Light microscopic features and morphometry of sperm in the emu (*Dromaius novaehollandiae*)

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ABSTRACT

A comprehensive morphologic description of emu sperm at the light microscopy level, an essential prerequisite for the routine evaluation of semen quality in this species, is not currently available. In this study, sperm morphology and morphometry were evaluated using conventionally prepared Romanowsky-stained semen smears of samples collected from the distal ductus deferens from 15 adult birds and fixed in 2.5% glutaraldehyde. Examination of the smears using phase contrast under 100× magnification readily resolved the various components of the cell, namely, the acrosome, nucleus, midpiece, principal piece, and endpiece. This technique was simple to use and produced consistently reproducible results. Normal emu sperm were typically filiform in appearance and closely resembled sperm of the ostrich and other non-passerine species, particularly poultry. A previously undescribed cytoplasmic appendage, associated with the base of the head, was a novel morphologic feature. The acrosome was short (1.84 ± 0.31 μm; mean ± standard deviation), whereas the nucleus measured 11.77 ± 0.93 μm in length. The length of the segments of the flagellum were 2.91 ± 0.4 μm for the midpiece, 47.45 ± 2.8 μm for the principal piece, and 3.69 ± 0.82 μm for the endpiece. The total sperm length was 67.64 ± 3.13 μm (range, 60.14–79.49) and the head:tail ratio was 1:4. Sperm dimensions in the emu were similar to those of other ratites.

1. Introduction

Sperm morphology, motility, and concentration are considered to be the three most important parameters when assessing semen quality [1–5]. Morphologic features serve as a reliable indicator in predicting the fertilizing capacity of sperm and also reflect certain disorders of spermatogenesis [2]. In humans, the accurate assessment of morphologic abnormalities is of prognostic value in predicting the results of assisted reproduction and fertilization when male infertility is compromised. Likewise, artificial insemination, an important and widely used technique in ensuring and maintaining genetic traits in domestic animals such as cattle and horses, relies on a clear understanding of normal sperm structure [2]. The value of sperm morphology in determining reproductive success is reflected in the numerous studies carried out, for example, on man [5–7] and domesticated animals such as the bull [2,8,9], stallion [10–12], boar [13], and dog [14–16].

Avian sperm structure, in particular that of non-passerine birds of economic importance, has likewise received considerable attention. Numerous studies have detailed the morphologic features of both normal and defective sperm in the chicken [4,17–20], turkey [1,21–23], duck [24], goose [25,26], Japanese quail [27,28], pintailed duck [29], and guinea fowl [30,31]. The commercial exploitation of ratite species such as ostrich, rhea, and emu has prompted a renewed interest in ratite sperm structure. Because artificial insemination has been suggested as a means of improving the economic viability of these niche industries [32], a thorough knowledge of normal sperm structure in these birds is of paramount importance. This

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view was further emphasized by Bertschinger et al. [33], who considered sperm morphology to be among the most important factors in predicting fertility in ostriches. The morphology of normal sperm has been well-documented for the ostrich by both light and electron microscopy [33–39], and the ultrastructure of rhea sperm and aspects of their development were reported by Phillips and Asa [40]. Apart from a brief ultrastructural description of the development and morphology of emu sperm [34], little information has been published on the structure of the normal male gamete in this species.

Recent detailed publications on the classification and description of defective sperm in the emu [41–44] have emphasized the need to reevaluate the dearth of published information on normal emu sperm, particularly at the light microscopy (LM) level. This information is required to provide comparative data for the accurate identification of abnormal forms. The objective of the present study was to provide a detailed description of the normal morphology of emu (*Dromaius novaehollandia*) sperm as observed by LM.

2. Materials and methods

2.1. Animals and samples

Semen samples were collected during mid-breeding season (May to July) from 15 healthy (animals approved for slaughter) and sexually active emus after slaughter at commercial abattoirs (Protocol V070/11, Faculty of Veterinary Science, University of Pretoria). The birds ranged in age from 22 months to 5 years. Two groups of birds were sampled. One group ($n = 5$) was sourced from the Rustenburg district in the North West Province, South Africa and slaughtered at the Emu Ranch Abattoir. The second group ($n = 10$) was from the Grahamstown district, Eastern Cape Province, South Africa, and was slaughtered at the Grahamstown Ostrich Abattoir. Samples were collected approximately 60 minutes after the birds had been slaughtered. Drops of semen were gently squeezed from the distal ductus deferens into plastic test tubes containing 2.5% glutaraldehyde in 0.13 mol/L Millonig's phosphate buffer.

2.2. Sperm morphology evaluation

Smears for LM were routinely prepared from the fixed cell suspensions, air-dried for a minimum period of 24 h and stained with Wright's stain (Rapidiff, Clinical Sciences Diagnostics, Johannesburg, South Africa) in Coplin jars. The dried smears were fixed in methanol for 20 seconds, stained with eosin for 30 seconds, blotted, and stained with methylene blue solution for 60 seconds. The smears were then gently rinsed with distilled water and allowed to air dry before mounting with Entellan and a coverslip. One smear from each bird was examined with an Olympus BX63 light microscope (Olympus Corporation, Tokyo, Japan) using a 100× oil immersion objective (bright field as well as phase contrast microscopy) to evaluate normal sperm morphology. The incidence of normal sperm was determined for each bird by evaluating 300 cells. Sperm images were digitally recorded using the Olympus cellSens Imaging Software (Olympus Corporation, Tokyo, Japan). The linear dimensions of the various segments of the sperm (acrosome, nucleus, midpiece, principal piece, and endpiece), as well as the total length of the cells, were determined by measuring a minimum of 20 cells from each bird. The measurements were processed using the Soft Imaging System iTEM software (Olympus, Münster, Germany).

2.3. Statistical analysis

Statistical analysis of the different parameters assessed was performed using Sigma Plot 12.0 and descriptive statistics for each of the sperm segments as well as the total sperm length were generated. Respective data subsets were tested for normality (Shapiro-Wilk) and equal variance, and subsequently, averaged linear sperm dimensions for individuals of the two groups of birds (Grahamstown and Rustenburg) were compared using the nonparametric Mann–Whitney rank-sum test. All tests were two tailed, with the α-level of significance set at 0.05.

3. Results

When viewed by LM, spermatozoa were typically filiform-shaped, consisting of a head and tail connected by an indistinct neck (Fig. 1). The relatively straight or gently convoluted head tapered anteriorly and consisted of a clearly defined nucleus capped by a small conical acrosome, which occasionally appeared pointed, particularly when using bright field illumination. The nuclear material was homogeneous in appearance with no obvious evidence of vacuolation. When using phase contrast microscopy the nucleus was starkly illuminated in white, unequivocally demonstrating the full extent of the nuclear contents (Fig. 1B, C). In most sperm a thin, thread-like appendage could be discerned at the base of the head (Fig. 1C). This appendage varied in length and generally extended vertically from the cell surface. The tail was composed of three distinct segments, namely, the midpiece, principal piece, and endpiece. The base of the head was continuous with the midpiece, the first segment of the tail. The nuclear base and the proximal end of the midpiece were of similar diameter although the midpiece as a unit was thinner than the head. The midpiece revealed no specific features on LM and was often difficult to distinguish from the rest of the tail on the Rapidiff smears using bright field microscopy (Fig. 1A). However, the various components of the sperm could clearly be distinguished from each other using phase contrast microscopy (Fig. 1B, C). After termination of the midpiece, the tail continued as the long undulating principal piece. The principal piece was thinner than the midpiece and terminated in a short, visibly thinner and nonconspicuous endpiece of variable length. In the 15 birds studied, 86.4% of the sperm displayed the normal morphologic features as outlined. Sperm dimensions are presented in Table 1. Based on extrapolation from those measurements, the total head length was determined to be 13.61 μm and the total tail length 54.05 μm, giving a head:tail ratio of 1:4. No significant differences in sperm morphometric parameters between Rustenburg and Grahamstown birds were observed.