Tissue and Cell xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Tissue and Cell

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

Structural features of the spermatozoon of a passeridan bird, the Carib grackle, *Quiscalus lugubris*

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ARTICLE INFO

Article history:
Received 3 February 2017
Received in revised form 17 February 2017
Accepted 18 February 2017
Available online xxx

Keywords:
Spermatozoon
Morphology
Avian
Passerine bird
Carib grackle
Quiscalus lugubris

ABSTRACT

The spermatozoon of the Carib grackle, *Quiscalus lugubris*, a member of the family Icteridae, is generally similar in organization to the passerine-type of spermatozoon, in being highly elongated and displaying a helical structure of the acrosome, nucleus and principal piece of the tail. There are subtle variations in acrosomal structural features between this organelle in the grackle and that in some of the very few passerine species of birds in which the spermatozoon has been studied. The proximal centriole is present, and, thus, the Carib grackle is the third passeridan bird in which this organelle, hitherto regarded as absent in passerine birds, has been described in the spermatozoon. The spermatozoon of this bird also possesses a granular helix, which feature has been found variably even in the scanty available reports on passerine spermatozoa. It is advocated that the spermatozoon be studied in many more species of this large clade of birds. This report provides a basis for the study of spermiogenesis in the Carib grackle, with the aim of exposing, *inter alia*, a number of developmental features and processes of certain organelles that have received attention, recently, in the spermatozoa of passerine birds.

1. Introduction

Sperm structure has been studied in only a few birds, and of these, non-passerine birds have had the lion share (Jamieson, 2007; Aire, 2014; Aire, 2007) although passerine birds constitute more than half of all birds (Jamieson, 2007). From the few available reports, it is remarkable that there is considerable variability in the presence and expression of certain organelles and structures in the spermatozoa of passerine birds studied, to date (Jamieson, 2007; Birkhead et al., 2007; Jamieson et al., 2006; Aire and Ozegbe, 2012). For example, not all passerine birds have been reported to possess the granular body or the proximal centriole. Also, the structure and composition of the acrosome seems to vary in certain particulars between passerine birds (Tripepi and Perotta, 1991; Jamieson et al., 2006; Birkhead et al., 2006; Aire and Ozegbe, 2012; Aire, 2014). What is the significance of this variability? Molecular analyses have not cleared difficulties in avian

phylogenesis. Indeed, according to Jamieson et al. (2006), they have produced widely conflicting results in these animals that constitute over 50% of all vertebrates (Jamieson, 2007), and hence morphological characters now appear to assume special significance when testing the validity of different phylogenetic hypotheses in birds (Jamieson et al., 2006). Subsequently, sperm morphology is attaining more and more significance in phylogenesis, especially in birds. The disposition of spermatozoal structural features is one major contribution in this effort. As a result, the study of sperm structure in passerine birds has been advocated to determine the prevalence and possible significance of these variably expressed and displayed organelles/features (Koehler, 1995; Jamieson, 2007; Aire, 2014). The present report on the structure of the spermatozoon of the Carib grackle seeks to contribute to, and supplement, knowledge of sperm structure in passerine birds, by exposing morphological features that are variably displayed in some other passerine birds. The colour of the male Carib grackle is black with a bronze hue. The bird belongs to the family Icteridae, it is fearless, and very cantankerous.

2. Materials and methods

Five, adult, sexually-active, male Carib grackle, *Quiscalus lugubris,* birds obtained from the wild with permission of the Government of Grenada, and approval by the Institutional Animal Care and Use Committee (IACUC) of the St. George's University, were sacrificed soon after misty net capture by an overdose of halothane anaesthetic. The thoraco-abdominal cavity of each bird was opened quickly, in two birds, and the testes, epididymides and ducti defer-

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http://dx.doi.org/10.1016/j.tice.2017.02.006
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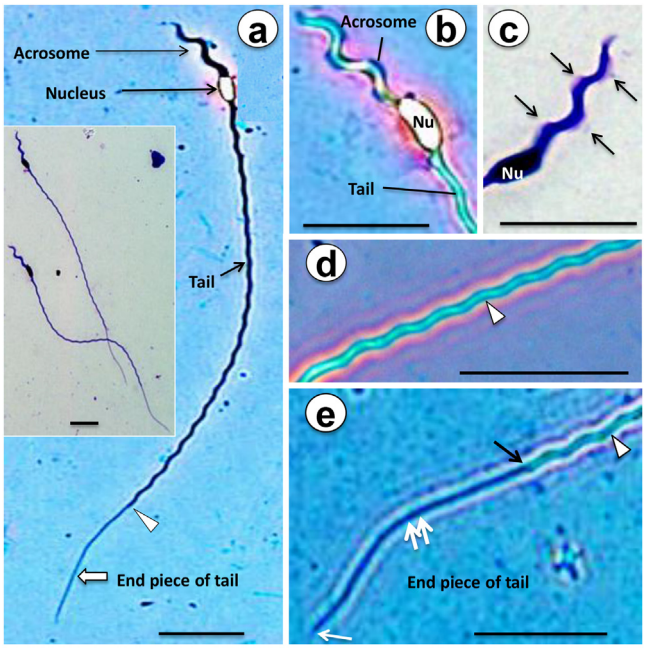

Fig. 1. Phase-contrast and bright field microscopy of spermatozoa of the Carib grackle. **(a)** and its *Inset* – show the various segments of the whole spermatozoon. Arrowhead = junction between the principal piece and end-piece of the tail. **(b)** and **(c)** display the helical acrosome and the slightly curved nucleus, the latter being much shorter than the former; the keel of the acrosomal helix is darker in colour in the phase contrast **(b)** but lighter in the dark field **(c)** pictures, and lies on the convex aspect of the four gyres of the helix (arrows in **(c)**). **(d)** shows part of the regularity of the helical nature of the principal piece of the tail (arrowhead), and **(e)** is a higher order of the distal part of the tail, showing the junction (black thin arrow) between the principal piece (arrowhead) and the end-piece (double white arrows); white arrow = end of the tail. Bars = 10 μm.

entia, along with the seminal glomus, were removed and fixed in 3% glutaraldehyde buffered in cacodylate or Millonig's phosphate buffer, for electron microscopic study. In two other birds, semen was gently milked out of the ducti deferentia into the same fixative, and gently swirled around to suspend spermatozoa in the fluid. In one bird, the entire body was intravascularly perfused with the fixative through the heart (Aire and Malmquist, 1979). Tissues from the ducti deferentia and seminal glomera as well as the milked semen were prepared routinely, sectioned and stained for electron microscopy. Spermatozoal sections in both the excurrent ducts, especially of the ductus deferens and seminal glomus, as well as the milked semen material from the latter two segments of the excurrent ducts, were observed at the electron microscope and appropriate images were photographed for this report. For light microscopy, semen smears were stained with Rapidiff® (Clinical Sciences Diagnostics, Johannesburg, South Africa) and examined with an Olympus BX63 light microscope (Olympus Corporation, Tokyo) using a 100x oil immersion lens (bright field as well as phase contrast illumination).

The dimensions, in μm, of the various parts of the spermatozoon were obtained from light microscopy smears, and the ratio of the acrosome: nucleus length was determined. It is pertinent to state that, in this study, the acrosome length has been measured as is, and not the straightened structure using the 'straight helix length' method described by Birkhead et al. (2005).

3. Results

The spermatozoon is typically sauropsid in shape, and its acrosome is evidently spirally- or helically-shaped (Figs. 1–3 and 5). This is followed, respectively, by the nucleus and tail, both of which are also helically shaped. For the purpose of orientation, the proximal centriole is regarded as being on the anterior aspect of the spermatozoon, while the distal centriole is on the posterior aspect of the cell. The acrosome is, thus, on the cranial/rostral or proximal aspect of the spermatozoon, and the tail on the caudal or distal aspect.

The main light morphological features of the Carib grackle spermatozoon are displayed in Fig. 1. Spermatozoa are approximately 102.7 ± 1.8 μm in length, with the acrosome accounting for 10.8 ± 0.5 μm (or 10.5% of the total sperm length), the nucleus, 4.2 ± 0.3 μm (4.1%), the principal piece, 66.2 ± 1.2 μm (64.5%) and endpiece, 21.5 ± 0.8 μm (20.9%).

The highly elongated spermatozoon displayed the usual structural features of a helical acrosome, nucleus and tail, arranged rostro-caudally, in that order (Fig. 1). The acrosome is strongly helical, with four gyres, which bear on their convex surfaces a light-staining 'fluted' keel, at the phase-contrast microscope level (Fig. 1). In light microscope smears, the diameter of the acrosome appears smaller than that of the nucleus but slightly greater than that of the tail. The pitch of the gyres of the helix is similar in both the acrosome and tail segments of the spermatozoon. However, the diameter of the tail decreases quite gradually, proximo-distally. The mitochondrial helix ends at about the distal four-fifth of the length of the spermatozoon, and thereafter, the tail maintains a rather regular round profile that gradually tapers to an end.

3.1. The acrosome

The acrosome is approximately twice as long (2.1) as the nucleus. It is also much less electron-dense than the nucleus. The caudal end or base of the acrosome fits into a v-shaped trough or concavity in the nucleus, when viewed from the side of the spermatozoon, or makes an *en-face*, relatively regular and flat contact with the nucleus, when viewed from the anterior or posterior surface of the spermatozoon (Fig. 3). The acrosome is slightly broader than the nucleus at the contact site or acrosomo-nuclear junction (Fig. 3). In using the proposition by Jamieson et al. (2006), the acrosome consists of two parts, the crest and core. In this description, the crest is regarded as the outer membranous envelope investing the moderately and homogenously electron-dense core. The rostral part of the crest terminates at a sharply pointed projection, and extends into the main body or caudal part, named the acrosome crest sleeve by Jamieson et al. (2006). In the Carib grackle, the acrosome core completely fills the crest which extends and terminates rostrally in a sharp-pointed spur, just below which commences the keel (Fig. 2). The homogenous, moderately electron-dense, acrosome core makes contact with, but does not appear to extend into, the keel that is of greater electron-density (Fig. 2). The acrosome crest therefore fully adheres to the core, leaving no space between them. Occasionally, and usually in cross-sections, but not longitudinal sections, of the acrosome, some space occurs between the core and the crest (Fig. 2b).

3.2. The nucleus

The nucleus is relatively short and appears only slightly curved in light micrographs (Fig. 1). Ultrastructurally, the helically-shaped nucleus is strongly electron-dense (Figs. 2, 3 and 5), and presents two slight surface impressions on the convex helical curvatures where the microtubular helix made contact with the nucleus during spermiogenesis (Fig. 3). The nucleus is elongated, roughly oval in transverse profile (Fig. 3), nearly uniform in diameter throughout its length, and intimately attached to the acrosome rostrally/proximally and the mid-piece caudally/distally. It is homogenous except for a few electron-lucent areas (Fig. 3). The implantation fossa of the nucleus may appear regular and nearly

Please cite this article in press as: Aire, T.A., et al., Structural features of the spermatozoon of a passeridan bird, the Carib grackle, *Quiscalus lugubris*. Tissue Cell (2017), http://dx.doi.org/10.1016/j.tice.2017.02.006