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Original article

Spermiogenesis in the African sideneck turtle (*Pelusios castaneus*): Acrosomal vesicle formation and nuclear morphogenesis ☆

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ABSTRACT

Testicular samples were collected from African sideneck turtles (*Pelusios castaneus*) at the peak of spermiogenesis in order to describe spermatid acrosomal vesicle formation and nuclear morphogenesis. Acrosomal vesicle formation commences with a Golgi transport vesicle attaching to a round spermatid, followed by the emergence of an acrosome granule. This is followed by the development of the subacrosomal space, which becomes enlarged as nuclear elongation and condensation continue. The round spermatid elongates and the emerging elongating spermatid successively becomes surrounded by circular, longitudinal and slanting microtubules of the manchette. The acrosomal vesicle becomes visible with an acrosome granule resting on the base of the electron dense material. Acrosomal vesicle morphogenesis in the African sideneck turtle results in a highly compartmentalized acrosome divisible into the acrosomal cortex and medulla. The future position of the flagellum starts to develop, being encircled by mitochondria while the distal centriole becomes obvious and the emerging flagellum grossly divisible into the connecting piece, midpiece, principal piece and endpiece. Although acrosomal vesicle formation and nuclear morphogenesis during spermiogenesis in the turtle are consistent with other reptilian species, a few differences were observed. The major difference observed was the formation of a single acrosome granule, which manifests prior to the attachment of the acrosomal vesicle to spermatid nucleus. The other differences observed were the emergence of two endonuclear canals in the elongating spermatid and the presence of slanting microtubules of the manchette. The observed developmental variations are expected to be valuable in future phylogenetic studies and potentially serve to test certain hypotheses concerning the reproductive status of turtle species. Findings from this study add to the growing database of spermatid morphology in turtles, thereby providing insights into variations in mature sperm morphology in the species.
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1. Introduction

The African sideneck turtle (*Pelusios castaneus*) is a freshwater turtle widely distributed in West Africa (Kirkpatrick, 1995). The turtle is small to medium in size, with a relatively extensive plastron that may have a hinge present between the pectoral and abdominal scutes (Olukole et al., 2010).

Studies on the ultrastructure of spermatozoa of reptiles have provided knowledge on the development and functional significance of many spermatozoal organelles (Teixeira et al., 1999; Al-Dokhi, 2004; Al-Dokhi et al., 2007, 2015) while elucidating the relationship between these components and reproductive activities (Gribbins, 2011). Similar to the mammalian, the reptilian spermatozoon consists of a head region containing the nucleus and the acrosomal structures, a midpiece, and a tail region which is subdivided into principal and end-pieces (Teixeira et al., 1999; Al-Dokhi and Al-Wasel, 2001a).

Extensive ultrastructural studies on spermiogenesis in reptiles have been conducted within the last decade. This include Jamaican gray anole, *Anolis lineatropus* (Rheubert et al., 2010a); black swamp snake, *Seminatrix pygaea* (Rheubert et al., 2010b); lizard *Iguana iguana* (Vieira et al., 2004, 2005, 2007); American alligator, *Alligator mississippiensis* (Gribbins et al., 2010) and Mediterranean gecko, *Hemidactylus turcicus* (Rheubert et al., 2011).

Ultrastructural studies on spermiogenesis in turtles have been carried out on the freshwater turtle, *Maurymes caspica* (Al-Dokhi

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and Al-Wasel, 2001a,b) as well as the soft shell turtles- *Trionyx sinensis* (Chen et al., 2006) and *Pelodiscus sinensis* (Zhang et al., 2007). Nevertheless, there is a scarcity of information on the ultrastructure of spermiogenesis in freshwater turtles of African origin. At present, spermatogenesis in the African sideneck turtle has only been studied at the light microscope level (Olukole et al., 2013, 2014). The current study, being the first of this nature performed in a turtle species of African origin, aims to describe acrosomal vesicle formation and nuclear morphogenesis during spermiogenesis in the African sideneckwith the view of generating data useful in the reproductive biology of the turtle.

2. Materials and methods

2.1. Experimental animals

Ten adult male African sideneck turtles (*Pelusios castaneus*) with an average bodyweight of 0.72 kg and average curved carapace length of 26.4 ± 1.87 cm, were sampled in August and September, a period of peak spermiogenesis (Olukole et al., 2014). The ten turtles were parts of sixty turtles sampled on the basis of five turtles per month for a calendar year during the process of investigating the annual spermiogenic cycle in the turtles (Olukole et al., 2014). The turtles were collected from river drainages in Ibadan (Ogunpa, Odo-Ona, Odo-Oba and Oke-Ayo), Nigeria. Carapacial and plastral characteristics of the turtle, as described by Kirkpatrick (1995), were used in the determination of adulthood in the turtles. The turtles were anaesthetized with an intramuscular injection of ketamine-HCl (25 mg/kg body-weight). The animals were subsequently sacrificed by cervical decapitation. The testes were removed after separating the plastron from the carapace. All procedures were carried out according to the guidelines for the care and use of experimental animals (National Institute of Health (NIH), USA. The study was approved by the University of Ibadan Animal Care and Use Research Ethics Committee (UIA-CUREC: 12/13/05).

2.1.1. Light microscopy

Samples of the testes were fixed in Bouin's fluid and embedded in paraffin blocks. Sections 2–4 μm thick were stained with Haematoxylin and Eosin, as well as the Periodic Acid Schiff (PAS) (Rao and Shaad, 1985). The slides were then studied under a light microscope (Olympus BX63 with a DP72 camera).

2.2. Transmission electron microscopy

Additional testicular tissues were fixed in glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) for 4 h at 4 °C. The samples were then thoroughly washed in the same buffer, post-fixed in 1% osmium tetroxide, and subsequently dehydrated in a graded series of ethanol solutions. Tissues were then cleared with propylene oxide, infiltrated with a 1:1 solution of propylene oxide: epoxy resin, 1:2 solution of propylene oxide:epoxy resin, and then placed in 100% epoxy resin for 36 h under vacuum. The samples were embedded in fresh epoxy resin and cured at 60 °C for 48 h. Semi-thin sections were stained with toluidine blue and observed under the light microscope (Olympus BX63 with a DP72 camera). Utra-thin sections (70–80 nm) were cut with a diamond knife on an ultramicrotome (Ultracut- Reichert, Austria). The sections were then double stained with uranyl acetate and lead acetate. The copper grids were examined under a transmission electron microscope (Philips CM 10 TEM) operating at 80 kv. Representative micrographs of different stages of spermiogenesis were taken using a Gatan 785 Erlangshen digital camera (GatanInc., Warrendale, PA). Analysis and assembling of composite micrographs were carried out using Adobe Photoshop CS5 (Adobe Systems, San Jose, CA).

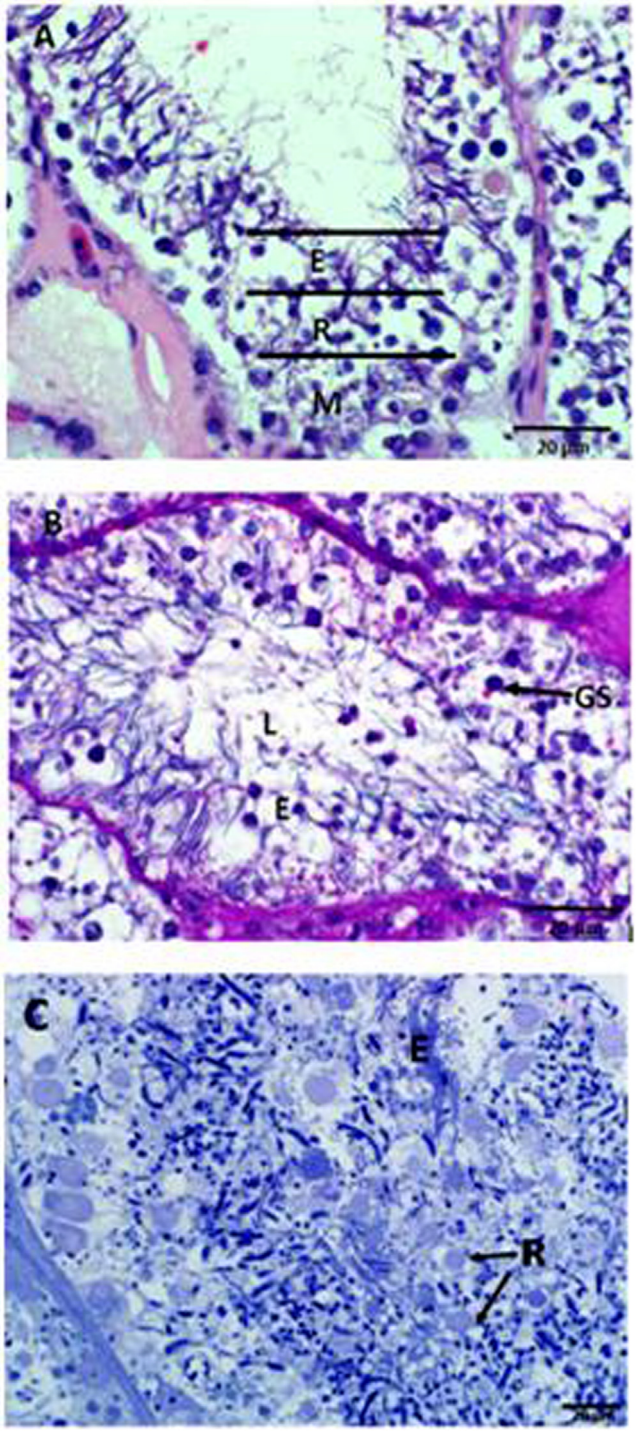

Fig. 1. Light microscope photomicrograph of the seminiferous epithelium in the African sideneck turtle. A. Regional distribution of cells within the seminiferous tubule, M: region of spermatogonia and spermatocytes; R: region of round spermatids; E: region of elongating spermatids; L: lumen, where spermatozoa will be released after the completion of spermiogenesis (H&E). B. PAS-positive Golgi vesicles (GS), elongating spermatids (E) and the lumen (L) of the seminiferous tubule (PAS). C. Toluidine-blue-positive round spermatids (R) and elongating spermatids (E) towards the adluminal compartment of the seminiferous tubule (Toluidine-Blue).

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