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ORIGINAL ARTICLE

Ultrastructural differentiation of spermiogenesis in *Scincus scincus* (*Scincidae*, *Reptilia*)

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KEYWORDS

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Implantation fossa;
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Axoneme

Abstract *Background:* Knowledge of spermiogenesis in reptiles, especially in lizards, is very limited. Lizards found in Arabian deserts have not been considered for detailed studies due to many reasons and the paucity of these animals. Therefore, we designed a study on the differentiation and morphogenesis of spermiogenesis, at an ultrastructural level, in a rare lizard species, *Scincus scincus*.

Results: The spermiogenesis process includes the development of an acrosomal vesicle, aggregation of acrosomal granules, formation of subacrosomal nuclear space, and nuclear elongation. A role for manchette microtubules was described in nuclear shaping and organelle movement. During head differentiation, the fine granular chromatin of the early spermatid is gradually replaced by highly condensed contents in a process called chromatin condensation. Furthermore, ultrastructural features of sperm tail differentiation in *S. scincus* were described in detail. The commencement was with caudal migration toward centrioles, insertion of the proximal centriole in the nuclear fossa, and extension of the distal centrioles to form the microtubular axoneme. Subsequently, tail differentiation consists of the enlargement of neck portion, middle piece, the main and end pieces.

Conclusions: This study aids in the understanding of different aspects of spermiogenesis in the lizard family and unfurls evolutionary links within and outside reptiles.
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1. Introduction

Scincus scincus, generally known as sand skink lizard is found in Saharan Africa and throughout the Middle East region (Al-Shammari and Ahmed, 2012). In many reptilians, due to the paucity of lizards, sometimes it is not possible to study all physiological aspects; this is evident in the study of spermiogenesis. Spermiogenesis is a complex process that involves differentiation and polarization of the round spermatid. Ultrastructural studies of reptile spermiogenesis were relatively few, and include the work in squamates (Al-Dokhi, 1996, 2012; Al-Dokhi et al., 2013, 2015; Al-Hajj et al., 1987; Camps and Bargallo, 1977; Courtens and Depeiges, 1985; Da Cruz-Landim and Da Cruz-Hoffling, 1977; Dehlawi and Ismail, 1991; Dehlawi, 1992; Dehlawi et al., 1993; Furieri, 1974; Ismail and Dehlawi, 1994; Teixeira et al., 1999a; Teixeira

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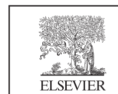

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et al., 1999b), in turtles (Al-Dokhi and Al-Wasel 2001a,b, 2002), in Sphenodontia (Healy and Jamieson, 1994), and in Crocodilia (Jamieson et al., 1997; Saita et al., 1987).

To date, there is no published work on spermiogenesis in the lizard *S. scincus*. Therefore, the goal of this study was to provide ultrastructural analysis of spermiogenesis in *S. scincus* and to compare the evolutionary aspects with other species.

2. Materials and methods

2.1. Animal collections and housekeeping

Ten male adult lizards of *S. scincus* were captured during the active sexual period (April or May), from the northeast region (60 km) (25°30′N, 49°40′E) away from Riyadh, Saudi Arabia. Animals were housed in separate cages and maintained for short periods in plexiglass boxes filled with 10 cm of clean sand. To maintain an optimal temperature (23 ± 1.5 °C) the sand was sprinkled with water. Mealworms and water were accessible *ad libitum* (Al-Quraishy, 2011). Animals were sacrificed according to ethical guidelines outlined by King Saud University.

2.2. Tissue preparation

Animals were euthanized by ether anesthesia and dissected to remove the testes from the lizards; tissues were cut into cubes (1 mm^3) and fixed in 3% buffered glutaraldehyde for 4 h. at 4 °C (0.1 M sodium cacodylate buffer; pH: 7.2). Samples were then fixed in 1% osmium tetroxide (OsO_4) for 1.3 h. Dehydration of the tissues was carried out using ascending grades of ethanol, then cleared in propylene oxide before embedding in pure resin (SPI, Toronto, Canada) (Reyenolds, 1963).

2.3. Ultrastructural analysis

Semithin sections were cut using a glass knife to locate the study area. Ultra-thin sections (50–65 nm) were then cut using an ultra-microtome (Leica, UCT; Germany) with a diamond knife (Diatome, Switzerland); sections were then placed on 300 mesh copper grids and stained with uranyl acetate (20 min) and lead citrate (5 min). The photographs were produced using a transmission electron microscope (JEOL JEM-1011) operating at 80 kV using Tengra™ (Olympus; TEM CCD camera and iTEM software) at the Central laboratory, King Saud University. Electron micrographs were finalized using Adobe Photoshop CS 5.1.

3. Results

Constellations of early spermatids were found in the adluminal compartment of the seminiferous tubules. The primary spermatids were round with an oval or round shaped nucleus and had uniformly distributed euchromatin and sometimes heterochromatin. The prominent organelles in spermatids were well developed and included the Golgi complex, which was composed of compacted cisternae and small vesicles. Mitochondria had linear cristae and some were found near the Golgi complex, whereas others were arranged at the boundary of the cytoplasm. Numerous free ribosomes and polyribosomes were randomly distributed throughout cytoplasm. Occasionally, we observed lipid droplets, multivesicular bodies, and lysosomes scattered in the spermatid cytoplasm (Fig. 1). Early spermatids were interconnected by cytoplasmic bridges that apparently held them together. Thin dense material was noticed in the inner cytoplasm adjacent to the intercellular bridges as shown by arrows in Figs. 2 and 3.

It was obvious that with spermatid differentiation, Golgi complexes were increased in number and microvesicles were developed from them. A larger proacrosomal vesicle was the result of microvesicle coalescence and this large vesicle was in close proximity to the spermatid nucleus (Fig. 4). In the subsequent stage, the large vesicle was found attached to the nuclear envelope and formed an acrosomal vesicle (Fig. 5). This site of contact was evidently a mark for the future anterior pole of the spermatid. With the growth of the acrosomal vesicle, a cup shaped proximal nuclear depression was formed partially to house the vesicle (Fig. 6). At first, no noticeable dense structures were observed within the acrosomal vesicle but after attachment with the nuclear envelope, a single large electron-dense granule was developed at the site of acrosomal vesicle. Markedly flattened cisternae of the Golgi complex were still in close contact with the proximal portion of the lodged acrosomal vesicle. The gap between the nuclear envelope and the vesicular membrane showed the occurrence of dense materials Fig. 7.

Marginalization of condensed chromatin in the peripheral nucleus was a sign of early chromatin condensation. Thereafter, nuclear elongation occurred and the elongated nuclei obviously pushed the acrosomal vesicle against the spermatid plasma membrane (Fig. 8). At this time, the anterior nuclear pole became convex in structure, and was attached to the acrosomal side. The acrosomal granule disappeared simultaneously due to the dissolution of compressed acrosomal vesicles Fig. 9.

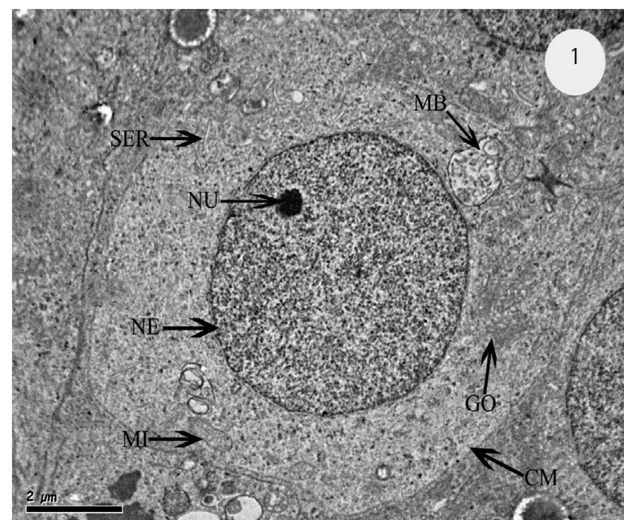

Figure 1 Primary spermatid showing a round nucleus with a distinct nuclear envelope (NE) and a dark nucleolus (NU). The other organelles in spermatids were well developed such as the Golgi complex (GO) and mitochondria (MI). Numerous smooth endoplasmic reticula (SER) were also distributed throughout the cytoplasm. A developed multivesicular body (MB) was also present near the nucleus.

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