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An Electron Microscope Study of Sperm Tail Differentiation of the Lizard, *Acanthodactylus boskinus* (Squamata: Reptilia)

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ABSTRACT

The major ultrastructural features of the sperm tail differentiation in the lizard, *Acanthodactylus boskinus* (*A. boskinus*) have been described. The initial step is the caudal migration of the centrioles followed by insertion of the proximal centriole in the nuclear fossa and extension of the distal centriole to form the microtubular axoneme. Thereafter, tail differentiation involves the development of neck region and middle, main and end pieces. The later three tail pieces along their extension encompass the axonemal complex which reveals the typical (9+2) arrangement of microtubules. The axonemal complex in the middle piece is enveloped within two successive sheaths, the mitochondrial and fibrous ones while its extension in the main piece is only encircled by the fibrous sheath. The end piece only manifestes the existence of the axonemal complex surrounded by the plasmalemma of the fully differentiated spermatids.

Key words: Ultrastructures, spermatid growth, fibrous sheath, spermiogenesis, sperm differentiation

INTRODUCTION

Relatively limited reports on the ultrastructure of sperm tail differentiation in reptilian species have been published (Austin, 1965; Boisson and Mattei, 1966; Kaplan *et al*., 1966; Furieri, 1970, 1974; Hamilton and Fawcett, 1968; Camps and Bargallo, 1977; Saita *et al*., 1987; Sprando and Russel, 1988; Hess *et al*., 1991; Healy and Jamieson, 1994; Al-Dokhi, 1997, 2009; Jamieson *et al*., 1997; Al-Dokhi and Al-Wasel, 2002). Lizards have received comparatively less attention as evidenced by the relevant fewer studies (Da Cruz-Landim and da Cruz-Hoffling, 1977; Courtens and Depeiges, 1985; Dehlawi *et al*., 1990; Ismail and Dehlawi, 1992, 1994; Ismail *et al*., 1995; Jamieson, 1995a, b; Teixeira *et al*., 1999a, b).

So far, there are no published ultrastructural data on the process of spermiogenesis in the lizard *Acanthodactylus boskinus* (*A. boskinus*). Therefore, the present study aimed at investigation of the ultrastructure of sperm tail differentiation in *A. boskinus*.

MATERIALS AND METHODS

Five adult males of the lizard, *A. boskinus* were collected during April and May (period of sexual activity), from desert (25°11' N, 46°51' E), north-east of the city of Riyadh, Saudi Arabia. After decapitation, dissection of the lizards was carried out and their testes were removed and diced into appropriate small pieces that were immediately fixed by immersion in 3% buffered glutaraldehyde (0.1 M sodium cacodylate buffer at pH 7.2) at 4°C for at least 4 h. The fixed tissue specimens were washed thoroughly in the same buffer and then post-fixed in 1% osmium tetroxide (OsO_4) in 0.1 M sodium cacodylate buffer (pH 7.2) for 2 h. Subsequent dehydration of the fixed

tissues was done in ascending grades of ethanol before final embedding in Epon/Araldite mixture. Thin sections were cut on an ultramicrotome (Leica, UCT), mounted on copper grids, double stained with uranyl acetate and lead citrate and observed by a transmission electron microscope (JEOL, 100 CX) operating at 80 kV.

RESULTS

Before the appearance of the acrosomal vesicle and its attachment to the proximal end of the spermatid nucleus, centrioles migrate to the opposite posterior direction, i.e., the caudal nuclear pole. A little invagination in the envelope of the spermatid nucleus appears and gradually deepened.

At a later stage, the two centrioles which are perpendicular to each other, adapted themselves to the nuclear invagination (implantation fossa) (Fig. 1). The proximal centriole is oriented perpendicular to the longitudinal nuclear axis while the distal one is parallel to this axis. At that early stage of differentiation, mitochondria are obviously aggregated at one side of the spermatid cytoplasm.

An insertion site, lined with an electron dense layer (the basal body or plate), serves as a connection between the proximal centriole and spermatid nucleus. After fitting of the proximal centriole in the insertion site and the development of a neck region, the distal centriole is directed posteriorly to initiate the formation of a microtubular structure (axoneme) (Fig. 2). The microtubular axoneme is running parallel to the cell longitudinal axis. The neck region constitutes the insertion site for the growing axonemal microtubules.

Following these morphological changes, spermatid nucleus is progressively elongated and its chromatin is gradually condensed. At the beginning of the condensation process, chromatin of the nuclear elongates is seen as long coarse filaments oriented in an anterior-posterior direction. Chromatin filaments are gradually thickened and packed and finally become highly condensed. At

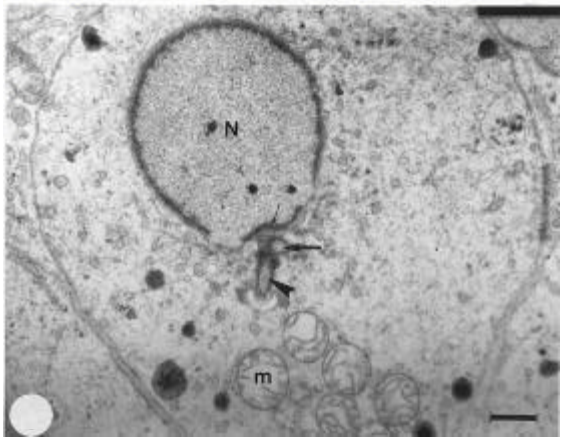

Fig. 1: Transverse section through an early spermatid showing nucleus (N) and cytoplasm. The proximal centriole (arrow) and the distal one (arrowhead) are perpendicular to each other and in a close proximity to the implantation fossa (thin arrow) at the caudal pole of the spermatid nucleus. m: The early aggregated mitochondria at one side of cytoplasm, Scale bar = 0.5 μm

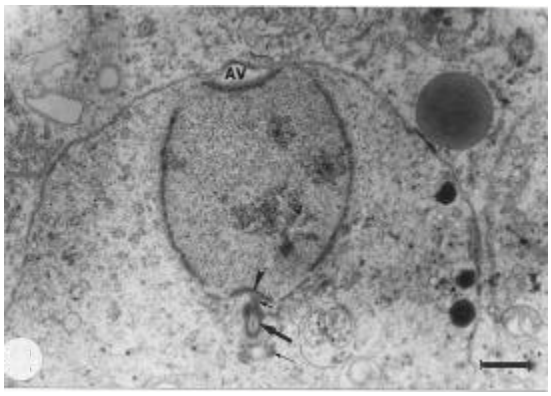

Fig. 2: Transverse section through an early spermatid showing the nuclear invagination (arrowhead) accommodating the proximal centriole (small arrow) which formed the connecting piece, distal centriole (large arrow) is not yet extended, Thin arrow: The early appearance of the annulus, AV: Acrosomal vesicle is directly apposed to the spermatid plasma membrane, Scale bar = 0.5 μm

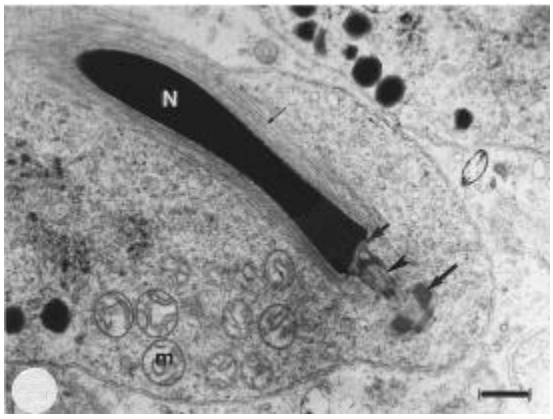

Fig. 3: Longitudinal section through an intermediate spermatid showing a prominent neck lined with a basal plate (small arrow). Arrowhead: The caudally extended distal centriole, annulus (large arrow) is more distinct and seems to accompany the extension of the distal centriole. Nucleus (N) shows highly condensed chromatin and compressed by the longitudinal manchette (thin arrow), Scale bar = 0.5 μm

the stage of filamentous chromatin, the nuclear implantation fossa is gradually deepened and when the chromatin is completely condensed it appears as a cup-shaped depression at the caudal nuclear pole.

Simultaneously, as a consequence of caudal extension of the distal centriole, the microtubular axoneme is obviously elongated (Fig. 3). However, an evidently extended axonemal structure is recognized in some spermatids before the commencement of nuclear elongation while the acrosomal vesicle is still attached to the spermatid nucleus (Fig. 4). Annulus which initially appears as a condensed material, seems to move caudally to accompany and border the extended axoneme.

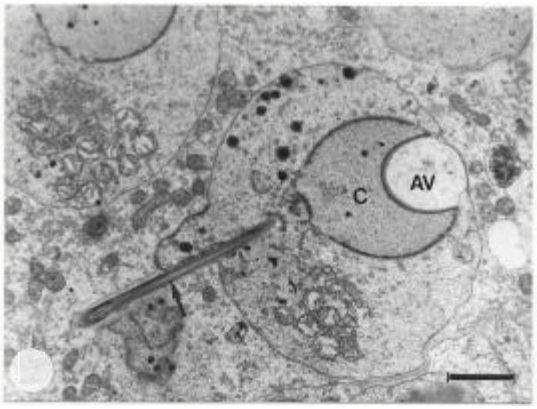

Fig. 4: Transverse section through a differentiating spermatid showing obvious caudal extension of the axoneme (arrow) which emerged from the spermatid cytoplasm, The nuclear chromatin (C) is no yet condensed and acrosomal vesicle (AV) is still apposed to the spermatid plasma membrane, Scale bar = 1 μm

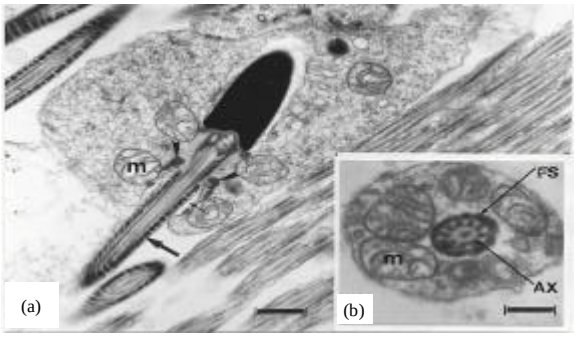

Fig. 5(a-b): (a) Longitudinal section through a late spermatid, Mitochondria (m) start to be arranged regularly around the proximal portion of the axoneme (middle piece) caudal to the neck region, There is a groove (thin arrow) between the annulus (arrowheads) and the axonemal complex, Scale bar = 0.5 μm and (b) Transverse section through the middle piece of a late spermatid, Mitochondria (m) are regularly arranged forming a collar or sheath around the axonemal core, FS: Fibrous sheath, AX: Axoneme, Scale bar = 0.2 μm

As the caudal extension of microtubular axoneme proceeds, mitochondria are arranged around the most proximal segment of the tail, caudal to the neck region (Fig. 5a). Later, mitochondrial collar or sheath develops around the axonemal core in this tail segment (middle piece). This mitochondrial arrangement is apparent in transverse sections of the middle tail piece which manifestes the characteristic microtubular arrangement of the axonemal complex (9 peripheral doublets and 2 central singlets) (Fig. 5b).

Interior to the mitochondrial sheath, a dense fibrous homogeneous material (fibrous sheath) encloses the axonemal microtubules. Inter-mitochondrial electron-dense bodies are recognized consistently in the transverse and longitudinal sections of the middle tail piece (Fig. 6).

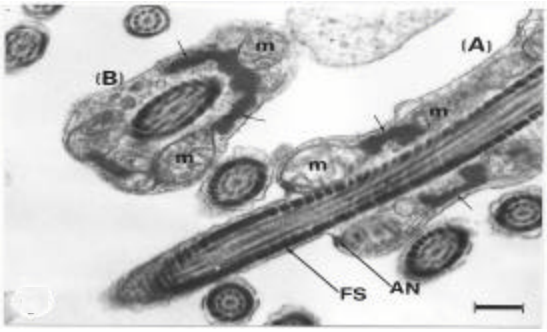

Fig. 6: Longitudinal (A) and transverse (B) sections through the middle piece of a late spermatid tail, there are iner mitochondrial dense bodies or plaques (small arrows) that join the mitochondria (m) together, The fibrous sheath (FS) extends through the middle and main pieces, Annulus (AN) marks the distal end of the middle piece, Scale bar = 0.2 μm

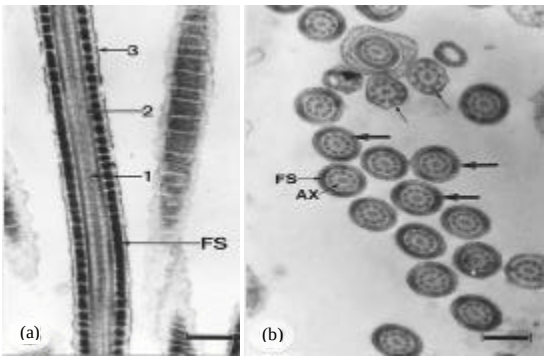

Fig. 7(a-b): (a) Longitudinal section through the main tail piece of a late spermatid, the fibrous sheath (FS) is extended throughout this tail piece and seen as interrupted column of dense material, 1: The central microtubules and 2: Peripheral ones of the axonemal complex and 3: Plasmalemma, Scale bar = 0.2 μm, (b) Transverse sections through the main piece (thick arrows) and the end piece (small arrows) of a fully differentiated spermatids, The main piece is built-up of a fibrous sheath (FS) and an axoneme (AX), The end tail piece consists only of the axonemal complex ensheathed by the plasmalemma, Scale bar = 0.2 μm

Mitochondria in middle tail piece of the differentiating spermatids possess cristae assuming a concentric form unlike the linear cristae of the early spermatid mitochondria. The most distal encircling mitochondria are located in a close contact to a dense structure (annulus) which is attached to the inner aspect of sperm plasmalemma. Between the annulus and the axonemal core, there is a delicate groove.

The developing tail is seen emerging from the late spermatid cytoplasm beyond the site of the annulus which marks distal end of the middle piece. The next tail piece (principal or main piece) has no mitochondrial sheath and only extension of the fibrous sheath envelopes the axonemal complex throughout the whole length of this tail piece (Fig. 7a). Fibrous sheath in longitudinal sections of the main tail piece is discerned as interrupted column of a dense material. The main tail

piece is tapered distally and the fibrous sheath disappears at the beginning of the end piece. Axonemal complex ensheathed by plasmalemma of the late spermatid is the only constituent of the end tail piece (Fig. 7b). In the fully differentiated spermatids, no excess cytoplasm is noticed surrounding the tail segments distal to the annulus site. Plasmalemma is tailored to exactly follow the contour of tail segments and associated with a minimal subjacent cytoplasmic layer.

DISCUSSION

Process of spermiogenesis involves the formation of a flagellum, with the accompanying shedding of excess spermatid cytoplasm and rearranging of spermatid organelles, as an essential feature (Hiatt and Gartner, 1997). The present lizard *A. boskinus* manifests the major morphological features of sperm tail differentiation which are in consistence with those reported in other lizard species (Furieri, 1970; Camps and Bargallo, 1977; Da Cruz-Landim and da Cruz-Hoffling, 1977; Courtens and Depeiges, 1985; Al-Hajj *et al*., 1987; Dehlawi *et al*., 1990; Ismail and Dehlawi, 1992).

Presently, posterior migration of centrioles in the early spermatids is the initial morphological event in the development of sperm tail. This is shortly followed by the appearance of a nuclear implantation fossa at the caudal nuclear pole. Thereafter, proximal centriole is fitted well to the nuclear fossa at a perpendicular orientation to the cell axis. The distal centriole is devoted to the formation of the flagellar microtubular substance. However, such centriolar modifications are not so distinct as those described in the differentiating mammalian spermatids (Fawcett, 1975, 1991).

Currently, the progressively extending axonemal microtubules is strongly linked to the spermatid nucleus via the developed neck region.

The proximal centriole in the present differentiating lizard spermatid is first adapted in a little implantation nuclear fossa which later deepened to form a cup-shaped depression at the caudal nuclear pole. This morphological nuclear change is obviously a modification to establish a firm centriolar-nuclear binding. Other reptiles show different forms of such bindings, for instance the differentiating spermatid of the turtle *Mauremys caspica* (Al-Dokhi and Al-Wasel, 2002) developed an arch-shaped implantation fossa to establish a firm binding.

The developing sperm tail of *A. boskinus* possesses no frank structure comparable to the neck cylinder described in some lizard species such as *Lacerta vivipara* (Courtens and Depeiges, 1985). Likewise, neck cylinder is lacking in the lizards *Chalcides ocellatus* (Ismail and Dehlawi, 1992), *Agama stellio* (Al-Hajj *et al*., 1987), *Uromastyx philbyi* (Dehlawi *et al*., 1990) and also in *Scincus mitranus* (Al-Dokhi, 1997). The flagellar structure is extended in a regular pattern as a result of the sequential addition and polymerization of tubulin templates to the distal ends of the growing microtubules (Fawcett, 1991). In common with other reptiles, the middle piece of *A. boskinus* sperm tail encompasses a mitochondrial collar or sheath, an axonemal core (motor apparatus) and terminated by an annulus (terminal ring).

Reptiles commonly exhibit fusion of mitochondria in the middle piece of sperm tail (Fawcett, 1981). In the present study, mitochondria in the middle tail piece are attached or fused via inter-mitochondrial dense bodies. Since these dense structures are in an intimate association to mitochondria, it is suggested that these bodies are transformed and/or fused mitochondria. Some reptilians such as *S. mitranus* (Al-Dokhi, 1997) have similar dense bodies or plaques. The inter-mitochondrial dense bodies are one of the character-states that considered as synapomorphies of Squamata (Jamieson, 1995b). The morphology and location of the present solid dense inter-mitochondrial bodies differ from that reported in some other lizard species such as

M. maximiliani (Teixeira *et al*., 1999a) and *P. acutirostris* (Teixeira *et al*., 1999b). The latter lizards reveal granular dense bodies in the middle tail piece of their sperms on contrast to other iguanians which have solid condensed structures (Furieri, 1974; Jamieson, 1995b; Oliver *et al*., 1996).

The present results clearly indicate that mitochondria of *A. boskinus* sperm tail is a persistent structure. This finding contradicts the situation in some other lizards such as *L. vivipara* (Courtens and Depeiges, 1985), *C. ocellatus* (Ismail and Dehlawi, 1992) and also *S. mitranus* (Al-Dokhi, 1997) which manifest disappearance of mitochondria during the late stages of sperm tail differentiation. Al-Dokhi (1997) explained that other energy sources such as glycogen may compensate for the absence of mitochondria. However, mitochondria are known to be the principal site for production of energy essential for sperm tail motility (Junqueira and Carneiro, 1980).

On contrast to *A. boskinus*, some lizards such as *T. torquatus* (Da Cruz-Landim and da Cruz-Hoffling, 1977) and also *S. mitranus* (Al-Dokhi, 1997) lack a fibrous sheath in the middle piece of their sperm tails and reveal its existence only in the main tail piece. Annulus in *A. boskinus* sperm tail exists as a dense structure attached to the sperm plasmalemma with the presence of a groove between it and the axonemal core. Annulus configuration varies among species (Fawcett, 1981), but its function in preventing caudal slipping of mitochondria in the actively motile sperm tails remains crucial (Burkitt *et al*., 1993; Hiatt and Gartner, 1997).

It is worth mentioning that the present sperm tail differentiation of *A. boskinus* displays some uncommon features. The first is the early aggregation of mitochondria at one cytoplasmic side in the early spermatids. The second is the noticeably early appearance of the annulus and its accompanying to the caudally extending axoneme. One additional feature is the occurrence of evident axoneme extension before the stage of nuclear elongation and chromatin condensation. In other words, the steps of tail differentiation may much precede those of the head differentiation.

Conclusively, the initial step in sperm tail differentiation of *A. boskinus* is the posterior migration of centrioles and formation of an implantation fossa at the caudal nuclear pole. This is followed by insertion of the proximal centriole and subsequent progressive caudal extension of the microtubular axoneme. Simultaneous mitochondrial arrangement around the proximal axonemal segment takes place. Annulus marks the distal end of the middle piece. As the case in other amniotes (Jamieson, 1995a), the longest main tail piece is consisted of an axoneme wrapped by a fibrous sheath but lacks mitochondrial sheath. Sperm tail of *A. boskinus* is terminated at the end piece which only encompasses the axoneme delimited by the plasmlemma.

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