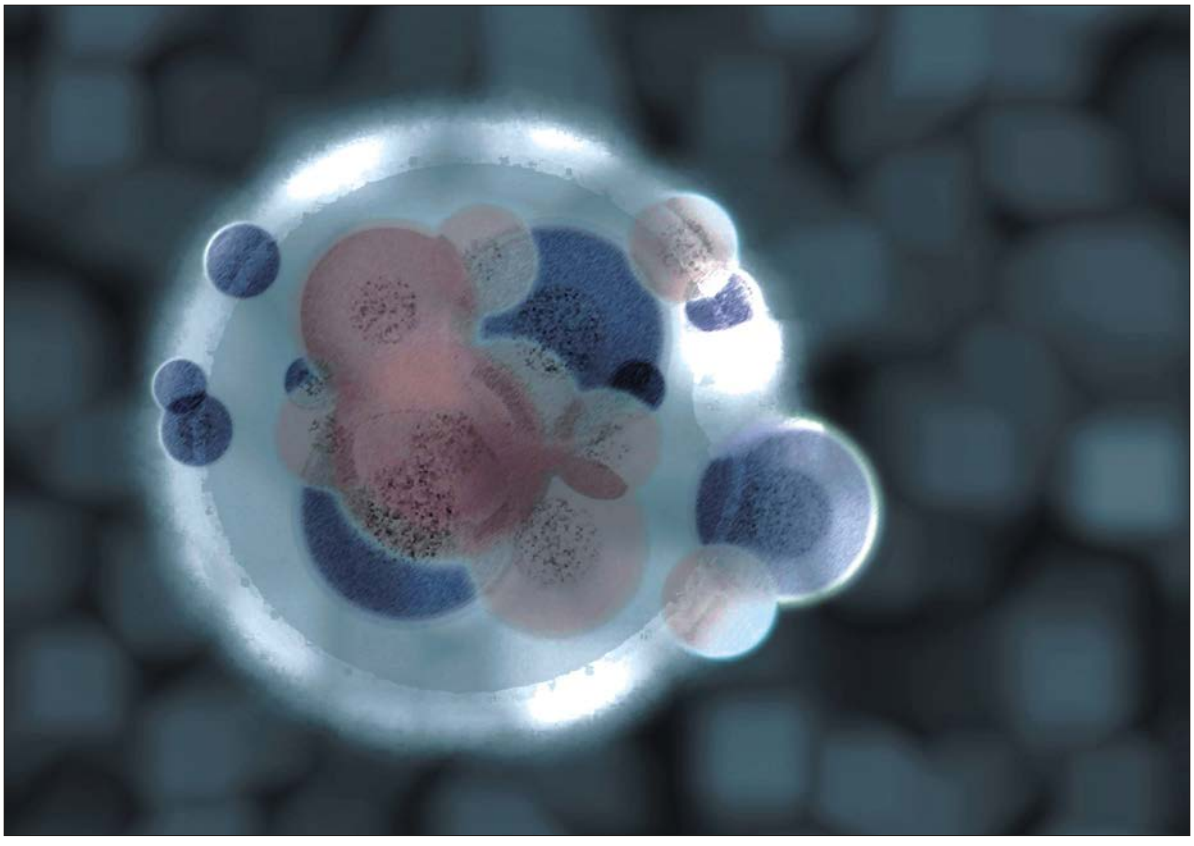

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Ultrastructure of Sperm Tail Differentiation of the Lizard *Stenodactylus dorie* (Squamata, Reptilia)

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Abstract: The ultrastructural features of the sperm tail differentiation in the lizard, *Stenodactylus dorie* have been described. The initial event was the caudal migration of the centrioles followed by implantation of the proximal centriole in the nuclear fossa and extension of the distal centriole to form the microtubular axoneme. Thereafter, the neck region and middle, main and end tail pieces were developed. The later three tail pieces along their length encompassed the axonemal core which revealed the typical 9+2 arrangement of microtubules. The axonemal core in the middle piece was enveloped within two successive sheaths, the mitochondrial and fibrous ones while in the main piece it was only encircled by the fibrous sheath. End piece only revealed the existence of an axonemal core surrounded by plasmalemma of the fully differentiated spermatid.

Key words: *Stenodactylus dorie,* sperm tail differentiation

INTRODUCTION

Relatively limited studies on the electron microscopy of sperm tail differentiation in reptilian species have been published[1-14]. Sperm tail differentiation of lizards was the focus of some previous studies[15-26].

So far there is no published report on the process of spermiogenesis in the lizard *Stenodactylus dorie (S. dorie)*. Therefore, the present study aimed at investigation of the ultrastructure of sperm tail differentiation in *S. dorie*.

MATERIALS AND METHODS

Five adult males of the lizard *S. dorie* 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 dissection of the lizards, their testes were removed and chopped 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 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) using a diamond knife, mounted on copper grids, double stained with uranyl acetate and lead citrateand viewed in a transmission electron microscope (JEOL, 100 CX) operating at 80 kV.

RESULTS

Initially, centrioles migrated to the opposite site of the attached acrosomal vesicle i.e, the caudal nuclear pole. A little invagination in the nuclear envelope of spermatid appeared and gradually deepened.

Later, the two centrioles, which were perpendicular to each other, adapted themselves to the nuclear invagination (implantation fossa) (Fig. 1). Proximal centriole was oriented perpendicular to the longitudinal nuclear axis, while the distal one was parallel to this axis. At that early stage of tail differentiation, mitochondria were aggregated at one side of the spermatid cytoplasm.

Spermatid nucleus was then progressively elongated and its chromatin was gradually condensed. At the beginning of the condensation process, chromatin of the nuclear elongates was seen as long coarse filaments oriented in an anterior-posterior direction. Chromatin filaments were gradually thickened and packed and finally

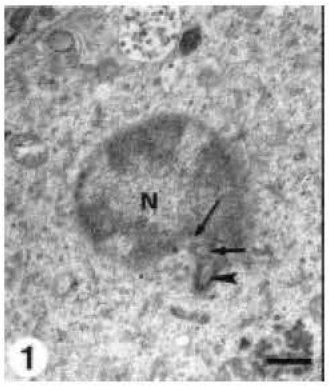

Fig. 1: Transverse section through an early spermatid showing nucleus (N) and cytoplasm. The proximal centriole (arrow) and the distal one (arrow head) are nearly perpendicular to each other and in a close proximity to the implantation fossa (thin arrow) at the caudal pole of the spermatid nucleus. Scale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

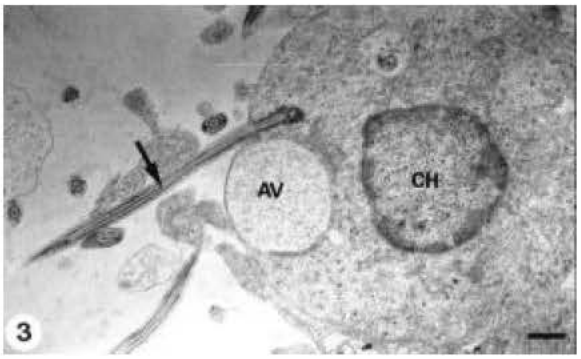

Fig. 3: Transverse section through a differentiating spermatid showing obvious caudal extension of the microtubular axoneme (arrow) which emerged from the spermatid cytoplasm. Note that the nuclear chromatin (CH) is no yet condensed and Acrosomal Vesicle (AV) is still apposed to the spermatid plasma membrane. Scale bar = 1 μm

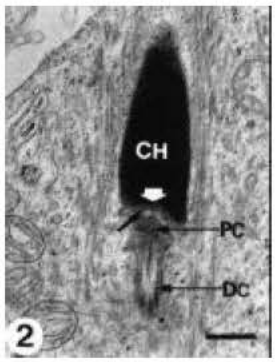

Fig. 2: Longitudinal section through a differentiating spermatid, with condensed nuclear chromatin (CH), showing the caudal nuclear implantation fossa (arrowhead) which accommodates the Proximal Centriole (PC) which formed the connecting piece. Implantation fossa is lined with the basal plate (arrow). Distal Centriole (DC) is not yet extended. Scale bar = 0.5 μm

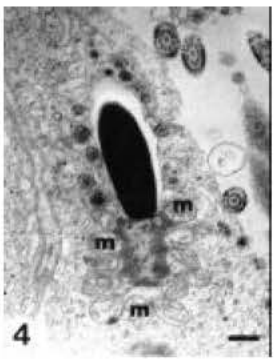

Fig. 4: Longitudinal section through a differentiating spermatid. Mitochondria (m) are arranged regularly around the proximal portion of the axoneme (middle piece) caudal to the neck region. Scale bar = 1 μm.

became highly condensed. Implantation fossa at the stage of filamentous chromatin, was gradually deepened and when the chromatin was completely condensed it appeared as a cup-shaped depression at the caudal nuclear pole.

The implantation site, lined with an electron dense layer (the basal body or plate), connected the proximal centriole to spermatid nucleus. After insertion of the proximal centriole in that site and the development of a neck region, the distal centriole initiated the formation of a microtubular structure (axoneme) (Fig. 2). The extended microtubular axoneme was running parallel to the cell longitudinal axis.

As a consequence of the caudal extension of distal centriole, the microtubular axoneme was obviously elongated (Fig. 3). It was interesting to observe evident extension of the axonemal structure in some spermatids before the commence of nuclear elongation while the acrosomal vesicle was still attached to the spermatid nucleus. Annulus (the terminal ring) which initially appeared as a condensed material, seemed to be gradually dislocated distally to accompany and border the extended axoneme.

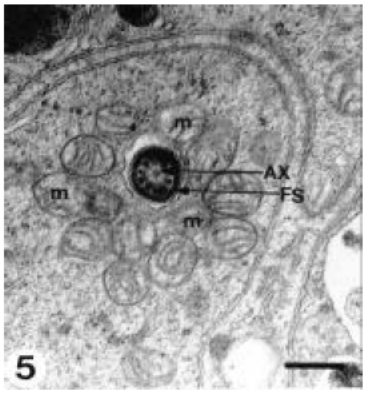

Fig. 5: Transverse section through the middle piece of a middle stage spermatid. Mitochondria (m) are regularly arranged forming a collar or sheath around the Axonemal core (AX). Note the concentrically lamellated mitochondrial cristae and the Fibrous Sheath (FS) enveloping the axonemal core. Scale bar = 0.5 μm

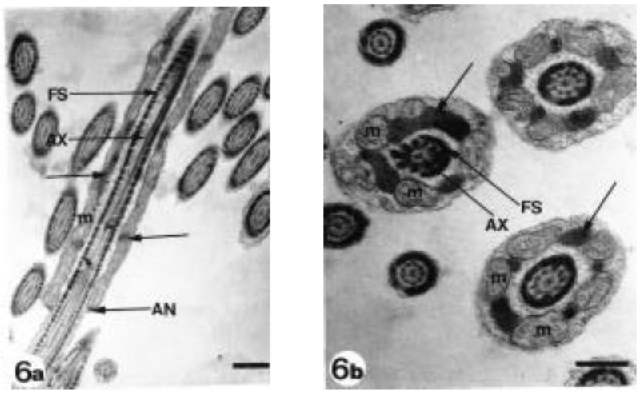

Fig. 6: a and b: Longitudinal (a) and transverse (b) sections through the middle piece of a late spermatid tail. There are inermitochondrial dense bodies or plaques (arrows) that join the mitochondria (m) together. Note the Fibrous Sheath (FS) which extends through the middle and main pieces to envelope the Axonemal core (AX). Annulus (AN) marks the distal end of the middle piece. Scale bar a = 1 μm, b = 0.5 μm

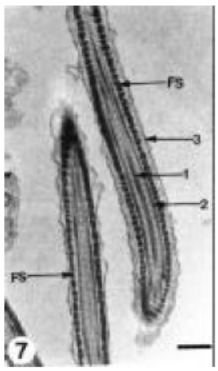

Fig. 7: Longitudinal sections through the main tail piece of a late spermatid. The Fibrous Sheath (FS) is extended throughout this tail piece and seen as interrupted column of dense material. Note the central microtubules (1) and the peripheral ones (2) of the axonemal core and the plasmalemma (3). Scale bar - 0.5 μm

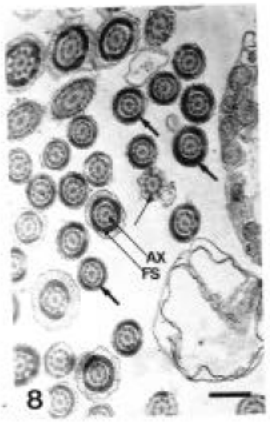

Fig. 8: Transverse sections through the main piece (thick arrows) and end piece (thin arrows) of fully differentiated spermatids. Note that the main piece is built-up of a Fibrous Sheath(FS) and an Axoneme. The end tail piece consists only of the Axonemal Core (AC) ensheathed by the plasmalemma. Scale bar = 0.5 μm

Mitochondria were then arranged themselves around the most proximal segment of the tail (middle piece) caudal to the neck region (Fig. 4). Mitochondrial sheath was thus developed around the axonemal core in this tail segment. The mitochondrial arrangement was apparent in transverse sections of the middle tail piece (Fig. 5) which also manifested the characteristic 9+2 microtubular pattern of the axonemal core (9 peripheral doublets and 2 central singlets). Cristae of mitochondria arranged in the middle tail piece tended to be in a concentric form unlike the linear cristae of the early spermatid mitochondria. The most distal encircling mitochondria were in a close contact with the annulus which was attached to inner aspect of the sperm plasmalemma. A delicate groove between the annulus and the axonemal core was seen.

A dense fibrous homogeneous material (fibrous sheath), located interior to the mitochondrial sheath, enclosed the axonemal microtubules. Intermitochondrial electron-dense bodies were recognized consistently in the transverse and longitudinal sections of the middle tail piece (Fig. 6a and b).

The differentiating tail emerged from the late spermatid cytoplasm beyond the site of the annulus which marked the distal end of the middle piece. The next tail piece (principal or main piece) had no mitochondrial sheath and only the fibrous sheath enveloped the axonemal core throughout the whole length of this tail piece (Fig. 7). Fibrous sheath in longitudinal sections of the main piece was observed as interrupted column of dense material. Main piece was tapered distally and at the beginning of the end piece fibrous sheath disappeared. Axoneme ensheathed by the plasmalemma of the late spermatid were the only constituents of the end piece (Fig. 8). In the fully differentiated spermatids, no excess cytoplasm was noticed around the tail segments distal to the annulus site. Plasmalemma exactly followed the contour of all tail segments and it was associated with a minimal subjacent cytoplasmic layer.

DISCUSSION

Formation of a flagellum, with the accompanying shedding of excess spermatid cytoplasm and rearranging of spermatid organelles, is an essential feature of spermiogenesis[27] . The present lizard *S. dorie* revealed the major morphological features of sperm tail differentiation which are in accordance with those reported in other lizard species[4,7,15-18,25,26,28].

The initial morphological event in the development of sperm tail was the posterior migration of centrioles. This was shortly followed by the appearance of a nuclear implantation fossa at the caudal nuclear pole. Thereafter, proximal centriole was fitted well in the nuclear implantation fossa at a perpendicular orientation to the cell axis. The distal centriole was devoted to the formation of the flagellar microtubular component.

The proximal centriole in the present differentiating lizard spermatid was 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 was obviously a modification to establish a firm centriolar-nuclear binding. Other reptiles show different forms of such binding, for instance the differentiating spermatid of the turtle *Mauremys caspic*[14] develops an evident arch-shaped implantation fossa to establish that firm binding.

Sperm tail of *S. dorie* has no distinct structure comparable to the neck cylinder described in some lizard species such as *Lacerta vivipara*[16]. Likewise, neck cylinder is lacking in the lizards *Chalcides ocellatus*[18], *Agama stellio*[28], *Uromastyx philbyi*[17] and *B. tuberculatus*[25]. Currently, the flagellar structure was extended in a regular pattern and this may be explained by the sequential addition and polymerization of tubulin templates to the distal ends of the growing microtubules[29, 30].

In accordance with other reptiles, the middle piece of *S. dorie* sperm tail involves a mitochondrial sheath, an axonemal core (motor apparatus) and terminated by an annulus (terminal ring). Fusion of mitochondria in the middle piece of sperm tail is a common feature of reptiles[31]. In the present study, mitochondria in the middle piece were attached or fused via intermitochondrial dense bodies. Since these dense structures were in an

intimate association to mitochondria, it is supposed that these bodies are transformed and/or fused mitochondria. Similar dense intermitochondrial bodies were observed in the lizards *B. tuberculatus*[25] and *A. boskinus*[26]. The inermitochondrial dense bodies are one of the character-states that considered synapomorphies of Squamata[22]. The morphology and location of the present solid dense intermitochondrial bodies differ from that reported in some other lizard species such as *M. maximiliani*[23] and *P. acutirostris*[24]. The latter lizards reveal granular dense bodies in the middle piece of their sperm tails on contrast to other iguanians which have solid condensed structures[5,22,32].

Mitochondria of *S. dorie* sperm tail seem to be a persistent structure. This contradicts with the situation in some other lizards such as *L. vivipara*[16], *C. ocellatus*[18] and *also S. mitranus*[12] which manifest disappearance of mitochondria during the late stage of sperm tail differentiation. It was interpreted that other energy sources such as glycogen may compensate for the absence of mitochondria[12]. However, mitochondria are known to be the principal site for production of energy essential for sperm tail motility[33].

Some lizards such as *T. torquatus*[15] and also *S. mitranus*[12], unlike *S. dorie*, lack a fibrous sheath in the middle piece of their sperm tails and reveal its existence only in the main tail piece.

It is worth mentioning that the present sperm tail differentiation of *S. dorie* manifested an interesting feature represented by the occurrence of evident axoneme extension before the stage of nuclear elongation and chromatin condensation. This may indicate that steps of tail differentiation may much precede those of the head differentiation.

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