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ORIGINAL ARTICLE

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

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KEYWORDS

Acrosomal vesicle;
Manchette;
Implantation fossa;
Mitochondrial sheath;
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.

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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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.

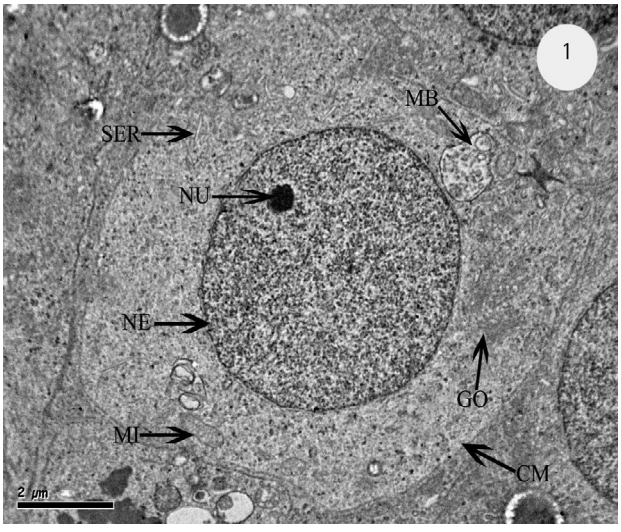
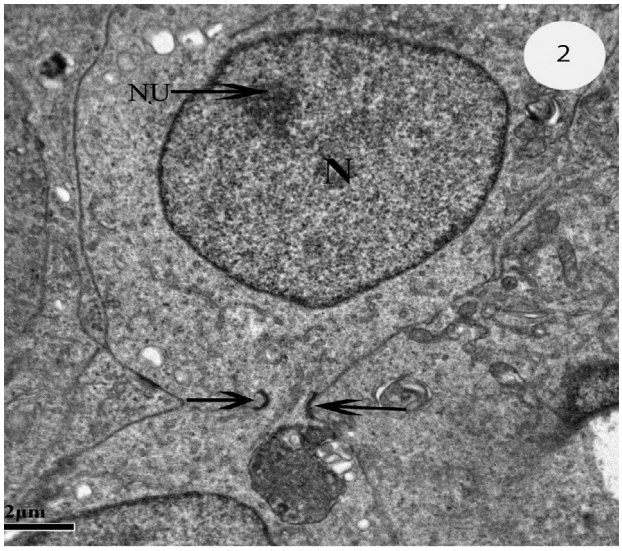


Figure 1 Primary spermatid showing a round nucleus with a distinct nuclear envelope (NE) and a dark nucleolus (NU). The other organelles in spermatids were well developed such as the Golgi complex (GO) and mitochondria (MI). Numerous smooth endoplasmic reticula (SER) were also distributed throughout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



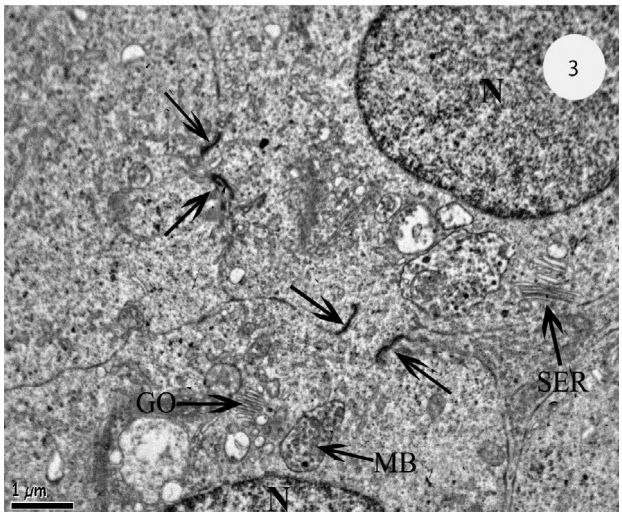


Figure 2 & 3 Early spermatids interconnected by cytoplasmic bridges (arrows). The other organelles were well distributed in the region such as nucleus (N) Golgi complex (GO), mitochondria (MI), smooth endoplasmic reticulum (SER), and multivesicular body (MB).

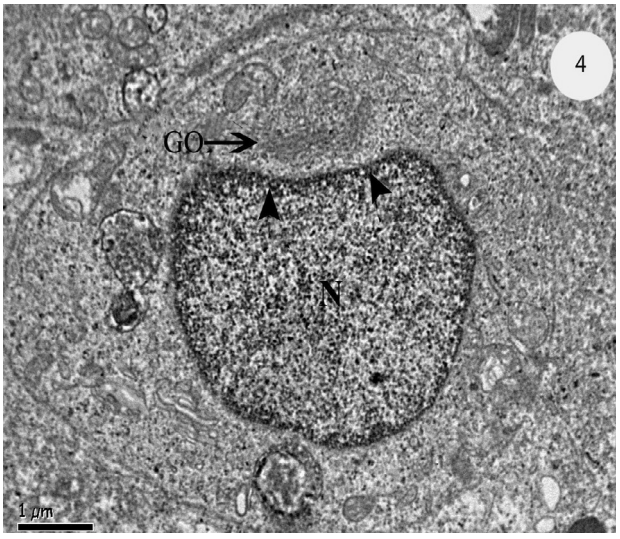


Figure 4 Commencement of spermatid differentiation. Golgi complex (GO) numbers were increased, forming microvesicles. Proacrosomal vesicles (arrows) were the result of microvesicle combination and close proximity to spermatid nuclei (N).

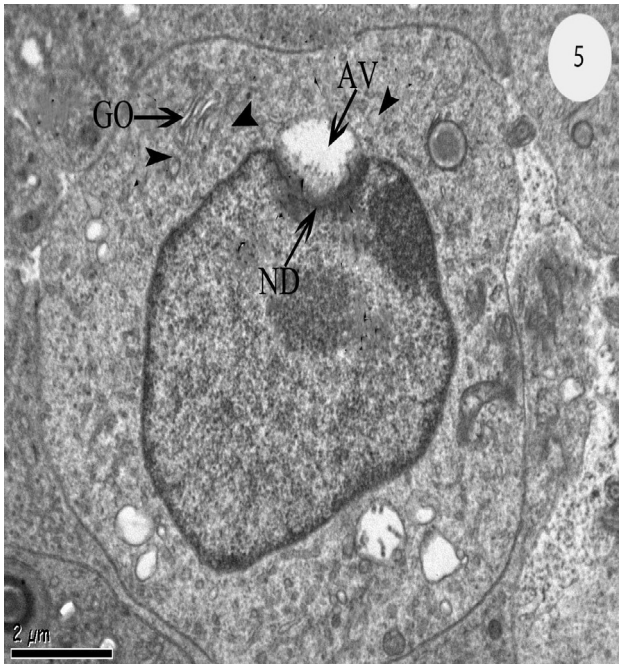


Figure 5 In this stage, the large vesicle was found in direct contact with the nuclear envelope and formed an acrosomal vesicle (AV). A distinct nuclear demarcation is shown (ND) in the figure. The Golgi complex (GO) was also present near the invagination side.

Furthermore, a small nuclear depression was discerned subjacent to the basal membrane of the acrosomal vesicle. Nuclear elongates were seen in Sertoli cells and enclosed in various membranes including (from outside to inside) those of Sertoli cells, spermatids, and acrosomal vesicles. Thereafter, the acrosomal vesicle was flattened and concentrically overlaid the subacrosomal nuclear space (SS), which was devoted to the proximal end of the nucleus. The SS became thicker and more densely stained, was devoid of organelles, and subsequently increased in size and assumed a cap-shape over the nuclear apex Fig. 10.

The chromatin (prolong nuclei) progressively underwent steady condensation. At the beginning of condensation, it was seen as packed coarse filaments, which formed a meshwork. The transverse and longitudinal sections of nuclear elongates, with either filamentous or condensed chromatin, were encircled by various microtubules, which were associated with the nuclear envelope (Figs. 11 and 12). Both longitudinal and circular microtubular manchettes were detected. The circular manchettes typically encircled the nuclei with filamentous chromatin, whereas the longitudinal manchettes accompanied the elongated nuclei with condensed chromatin. In the latter case the manchettes appeared to consists of a cylindrical structure ensheathing the nuclei and having an anterior-posterior

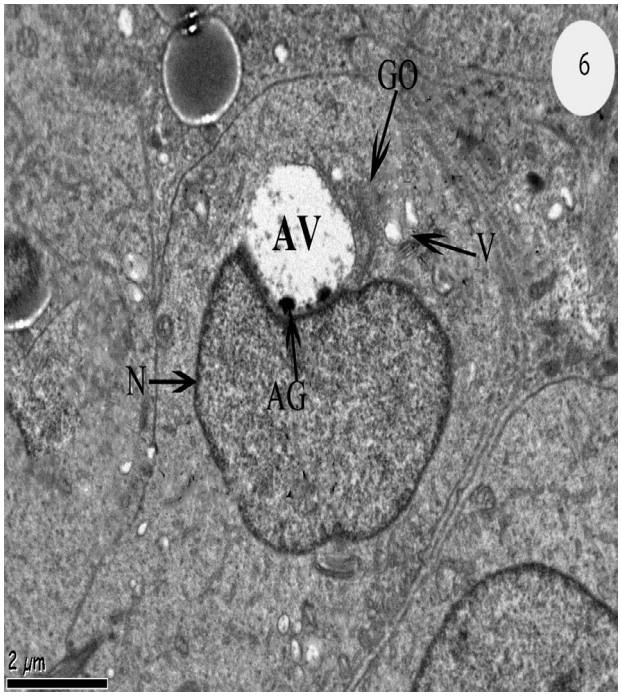


Figure 6 At the site of contact, an acrosomal granule (AG) was found. This marks the future anterior pole of the spermatid nucleus (N). A large acrosomal vesicle was present and formed a cup-shaped proximal nuclear depression (anterior nuclear concavity). The Golgi complex (GO) and small vesicles were also present near the acrosomal side.

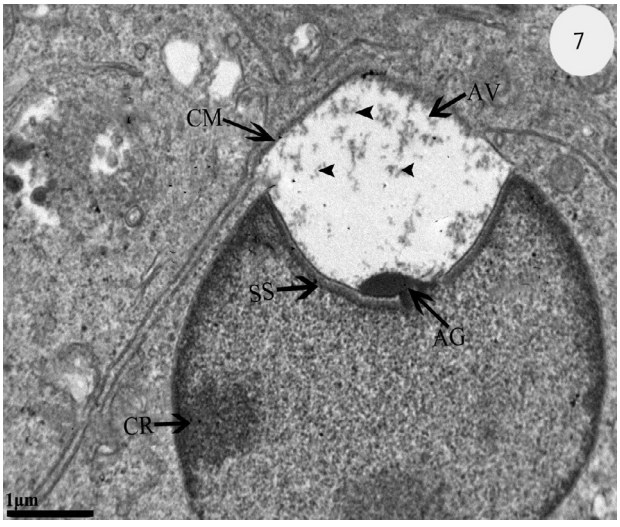


Figure 7 In nuclear envelope, a single large electron-dense granule was developed at the base of the acrosomal vesicle (AV), called the acrosomal granule (AG). In between the nuclear envelope and vesicular membrane there was a space called the subacrosomal nuclear space (SS), which showed the presence of dense material. In a corner of the nucleus, patchy chromatin is distributed. The whole spermatid is covered with the cytoplasm membrane (CM).

orientation. The proximal sides of the manchettes were attached to the posterior end of the acrosomal vesicle as lateral arms Fig. 13.

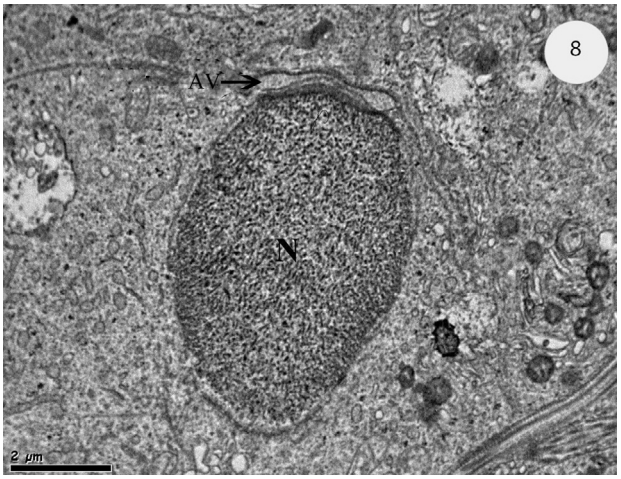


Figure 8 This figure shows a sign of early chromatin condensation, which takes place inside the nuclear membrane. Thereafter, nuclear elongation became a regular phenomenon and the elongated nuclei (N) pushed the acrosomal vesicle (AV) against the spermatid plasma membrane.

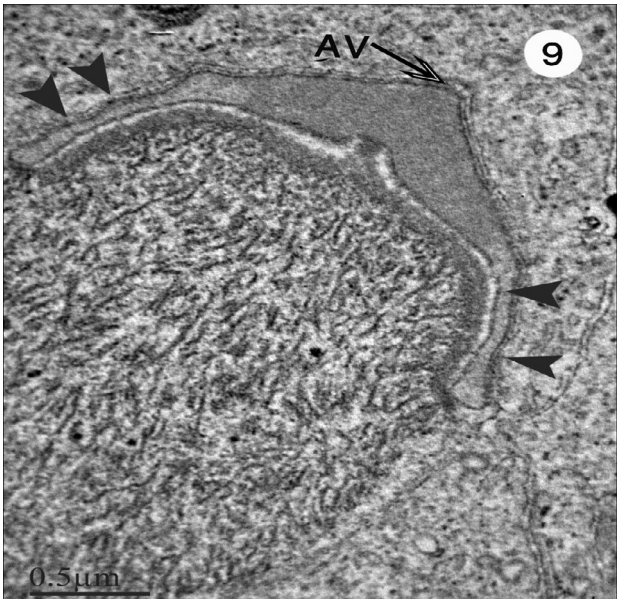


Figure 9 This figure shows the anterior nuclear pole attached to the acrosomal vesicle (AV), which became convex. The dense acrosomal granule disappeared due to a compressed acrosomal vesicle. The acrosomal vesicle membrane (arrows) becomes closely opposed to the spermatid plasma membrane. In the latter case, the manchette appeared to be a cylindrical structure ensheathing the nuclei and having an anterior–posterior orientation parallel to the longitudinal axis of the nucleus. The proximal end of the longitudinal manchette was attached to the posterior end of the acrosomal vesicle as lateral arms.

The manchette microtubules appeared in the spermatid cytoplasm immediately before chromatin granulation. The manchette encircled the nucleus in an anterior-posterior position as a helix (Fig. 14; arrows). With further differentiation, numerous microtubules were increasingly present around the

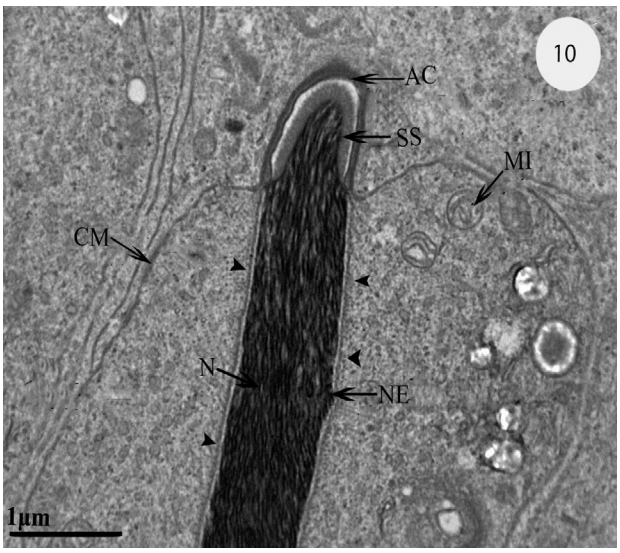
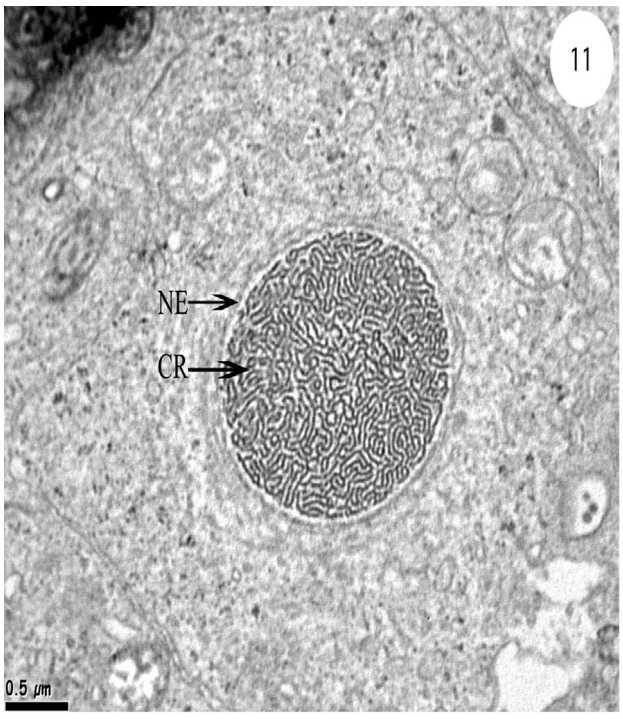


Figure 10 The nuclear elongates embedded in the Sertoli cells, which were enclosed by successive membranes. Thereafter, the acrosomal vesicle was flattened and concentrically overlies a subacrosomal nuclear space (SS), which advances the proximal end of the nucleus (N). The SS becomes thicker and assumes a cap-shape over the nuclear apex. A distinct acrosomal cap (AC) was also seen above the SS.

lateral sides of the nucleus, forming the helical manchette. The chromatin morsels surges in size and finally form a compact homogenous mass of chromatin. Later chromatin material becomes tightly packed to finally form the dense mass found in mature sperm. Furthermore, the manchette maintained the shape of the nucleus during the process of spermiogenesis, compressed the nuclear membrane, and possibly served to orient the subunits of the condensing chromatin. It is also responsible for the initial transformation of the spermatid nucleus shape, by squeezing the nucleus and causing elongation (Fig. 14). In late differentiated spermatid, a sheath of longitudinally aligned microtubules surrounded the nucleus and had highly condensed contents. The longitudinal manchette appeared to help in the posterior translocation of cytoplasmic substance during spermiogenesis.

During spermiogenesis, and after acrosomal vesicle formation, the two centrioles gradually moved to opposite poles of the nucleus (Fig. 15). As differentiation proceeded, a nuclear invagination appeared in the posterior surface of the nucleus; with further differentiation, a deep invagination formed an arch shape structure that acted as an implantation fossa Fig. 16.

The implantation fossa was lined by an electron dense layer called the basal plate, which connects the proximal centriole to the nucleus. The proximal centriole fit in a shallow invagination in the posterior surface of the nucleus (the implantation fossa), whereas the distal centriole made an 80-degree angle with the proximal centriole in a parallel position to the long axis of the cell. The nuclear envelope surrounded the centriolar complex in a crescent (arch) shape that could provide a strong connection (Figs. 17 and 18). As differentiation proceeds, the middle piece of the tail started to develop behind the neck region. First, the dispersed mitochondria in the cytoplasm



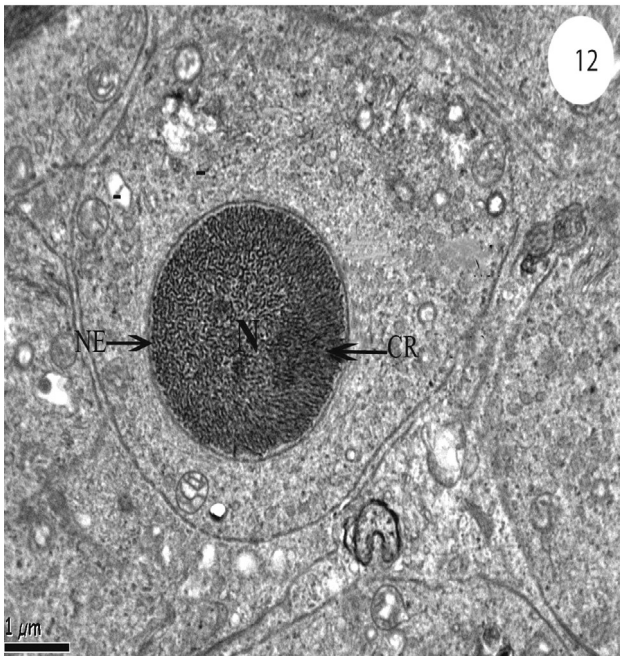


Figure 11 & 12 The chromatin material (CR) progressively undergoing gradual condensation. At the beginning of condensation, it was seen as closely packed coarse filaments, which formed an interconnected meshwork (filamentous chromatin). The transverse sections were encircled by numerous microtubules, which were closely associated with the nuclear envelope (NE).

started to accumulate around the axoneme of the middle piece in gradual stages. The mitochondria were uniform in thickness and regularly arranged around the axoneme of the middle piece. The first crown was bonded to the basal plate, whereas the last was attached to the annulus (Figs. 18 and 19). Later distal centriole gives rise to the axoneme. Elongation of the

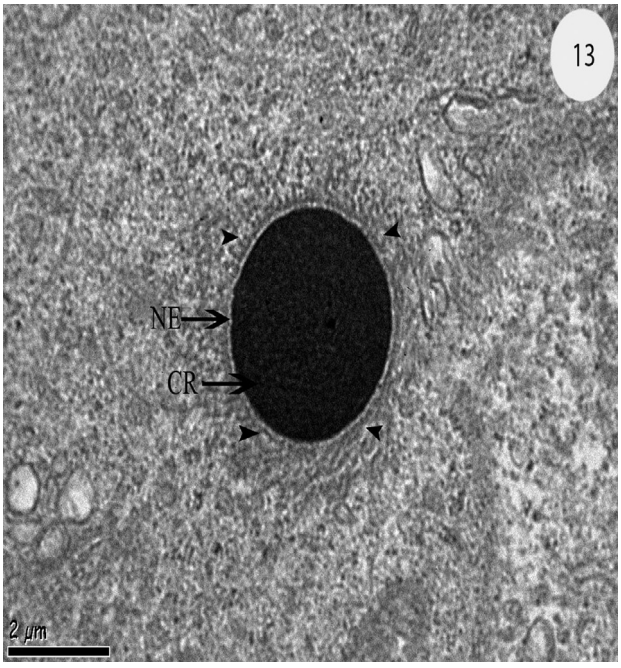


Figure 13 The chromatin granules increased in size and eventually merged to form the compact homogenous mass of chromatin. The chromatin material became tightly packed to finally form a dense mass typical of the mature sperm. The circular manchette was usually encircled by nuclei with filamentous chromatins.

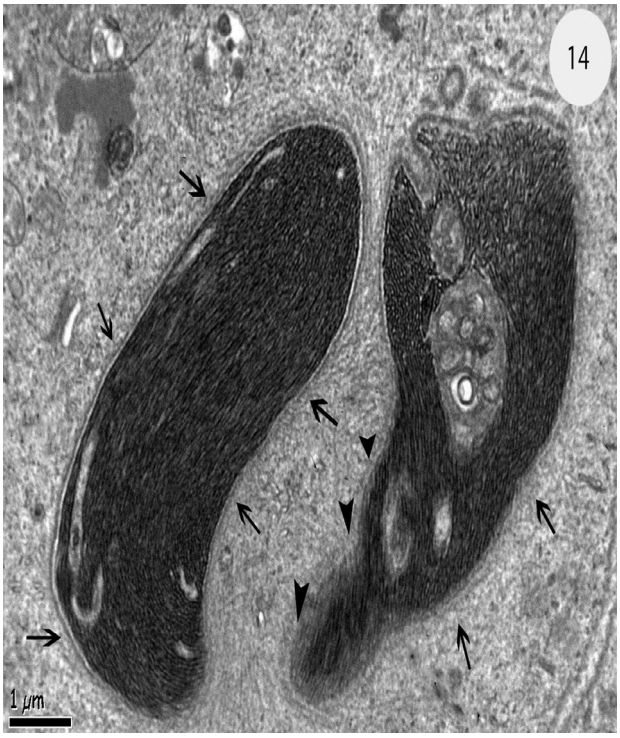


Figure 14 The manchette (arrows) encircled the nucleus in an anterior-posterior position as a helix, parallel to the longitudinal axis of the nucleus. With further differentiation, numerous microtubules were increasingly present around the lateral sides of the nucleus, forming the helical manchette (arrow heads). Furthermore, the manchette maintained the shape of the nucleus, as the process of spermiogenesis was happening, to compress the nuclear membrane, and possibly, to orient the subunits of the condensing chromatin. It is also responsible for the initial transformation in shape of the spermatid nucleus, by squeezing the nucleus and causing elongation.

flagellum continued as tubulin dimers were added to the distal ends of microtubules. In a subsequent stage, the annulus (the distal centriole) developed as electron dense material closely associated with the plasmalemma, where the flagellum emerged from the cell. The appearance of the annulus confirmed the end of the middle piece at the plasma membrane as a dense circular material around the axoneme Fig. 19.

At the late stages of spermatid differentiation, the main piece of tail developed, which was composed of a typical 9 + 2 axoneme (consisting of nine doublets and two central singles) surrounded by a thick fibrous sheath and the plasma membrane. The fibrous sheath was thick at the beginning of the middle piece and gradually became thinner before it disappeared completely at the start of the end piece. The end piece was composed of axoneme only surrounded by the plasma membrane Figs. 20–22.

Mature spermatids had no extra cytoplasm surrounding the tailpieces of the distal annulus. Plasmalemma was specific to the tail segment and was associated with a marginal cytoplasmic layer.

4. Discussion

In most lizards, the development of spermatids has considerable variations in the arrangements, size, and degree of compartmentalization (Talbot, 1991). All *Squamata* have a common acrosomal layer enveloping the nuclear tip for instance in *Tropidurus torquatus* (Da Cruz-Landim and Da Cruz-Hoffling, 1977). In this lizard, a homogeneous electron-dense acrosome is present; similar observations were also noted in *S. scincus*. Furthermore, two visible acrosomal layers might indicate differences according to their maturation capacity. The early development of the acrosome complex in *S. scincus* testis was similar to that observed in other reptiles (Al-Dokhi et al., 2013, 2015). The single acrosome vesicle originates from the Golgi apparatus and is similar to that of many other limbed lizards and squamates (Al-Dokhi, 1996, 2012; Al-Dokhi et al., 2013; Dehlawi and Ismail, 1991; Dehlawi, 1992; Dehlawi et al., 1993; Ismail and Dehlawi, 1994).

The nucleus of the *S. scincus* had uniformly distributed chromatin with a smaller amount of heterochromatin, which is considerably different from Sphenodon heterochromatin (Healy and Jamieson, 1994) and that of chelonians (Zhang et al., 2004). The subacrosome space formed in round spermatids of *S. scincus* continued to expand during the elongation process, and this space accumulated granules which are dark in color and were reported in other sauropsids (Al-Dokhi et al., 2013; Gribbins et al., 2007, 2009). After completion of acrosomal growth, the nuclei of the spermatids linked with the cell membrane. This condition flattened the acrosome vesicle on

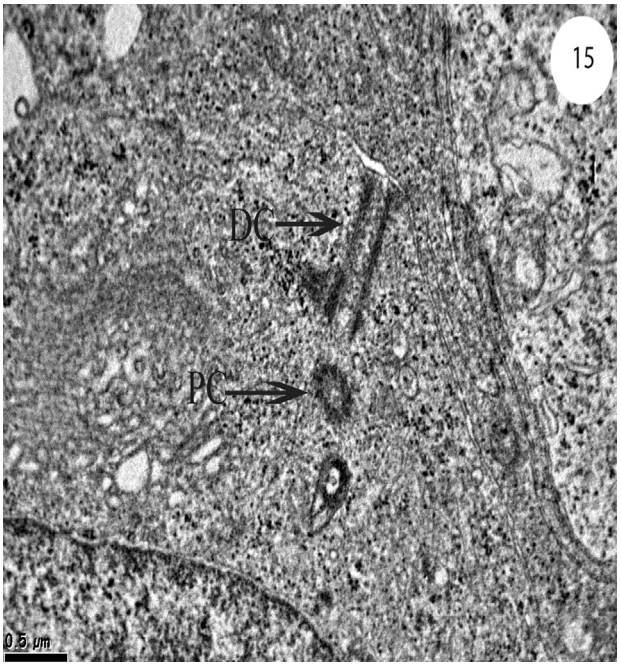


Figure 15 After acrosomal vesicle formation, the two centrioles, the distal and primary centrioles (DC and PC) gradually moved to opposite poles of the nucleus.

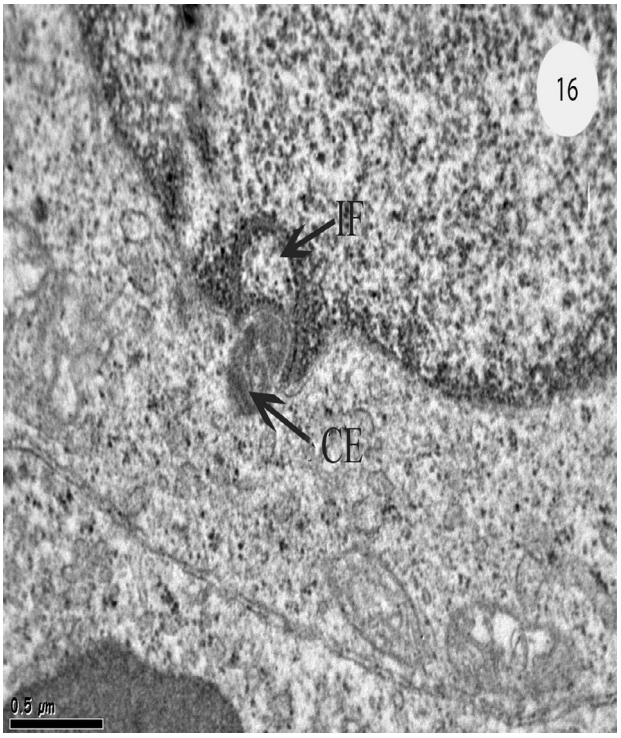
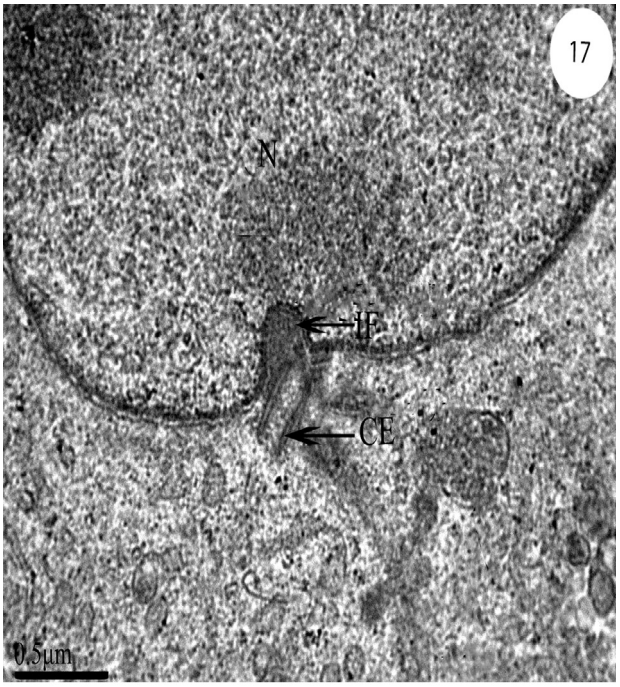


Figure 16 As differentiation proceeds, a nuclear invagination appeared in the posterior surface of the nucleus; with further differentiation it penetrated the deep invagination and formed an arch shape structure that acted as an implantation fossa (IF), which was attached to the centrioles (CE).

the anterior surface nucleus, which in turn aids in relocation of the acrosomal shoulders laterally over the apical nuclear head.



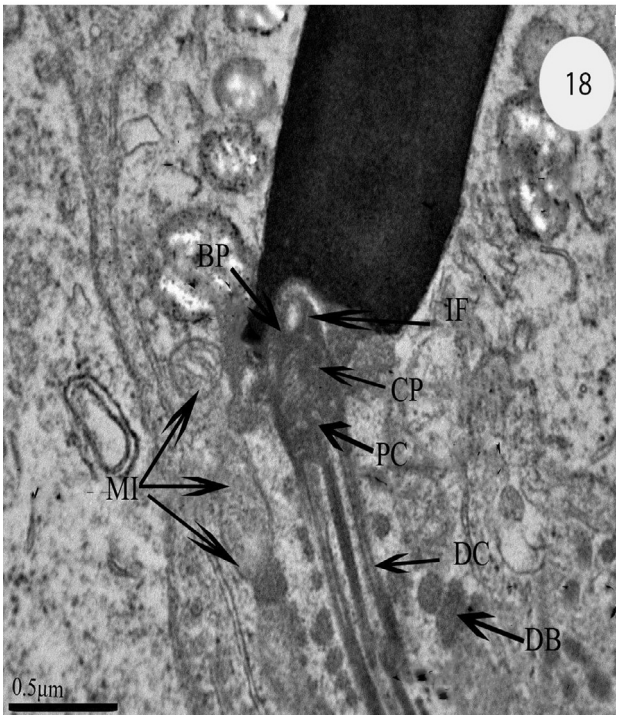


Figure 17 & 18 The implantation fossa (IF) was lined by an electron dense layer called the basal plate (BP), which connected through the connecting piece (CP) to the proximal centriole (PC) and nucleus. The proximal centriole fit in a shallow invagination in the posterior surface of the nucleus of the implantation fossa (IF), whereas the distal centriole (DC) existed at an angle of 80° to the proximal centriole in a parallel position to the long axis of the cell. A large number of mitochondria (MI) and dark bodies were also found in the vicinity.

Additionally, some reptiles such as chelonians, *Diplometopon zarudnyi* (Al-Dokhi et al., 2013, 2015; Zhang et al., 2004) and other crocodilians (Wang et al., 2008), an endonu-

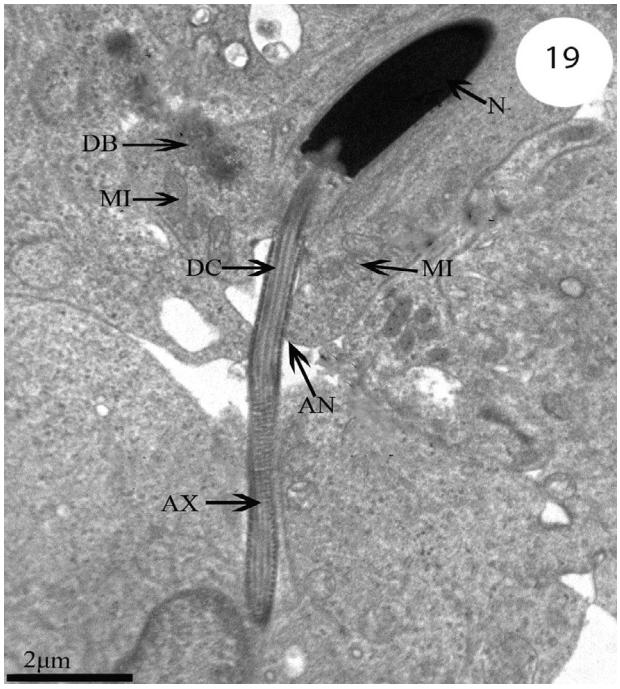


Figure 19 As differentiation proceeds, the middle piece of the tail started to develop behind the neck region. First, the dispersed mitochondria (MI) in the cytoplasm started to accumulate around the axoneme (AX) of the middle piece in gradual stages. The mitochondria were uniform in thickness and regularly arranged around the axoneme in the middle piece. The first crown was bonded to the basal plate, whereas the last was attached to the annulus (AN). The distal centriole gave rise to the axoneme (AX). In a successive stage, the annulus (AN) developed as an electron dense material, closely applied to the plasmalemma. The appearance of the annulus marked the end of the middle piece at the plasma membrane as a dense circular material around the axoneme.

clear canal is present and it host the perforatorium, and extends deep into the nuclear region of a spermatid. It's confusion whether some sauropsids reptiles have a perforatorium within the endonuclear canals. (Saita et al., 1987); however, in *D. zarudnyi,* (Al-Dokhi et al., 2013) a visible perforatorium has developed an endonuclear canal, as observed in other worm lizards (Saita et al., 1987). In compare with other squamates including that of *S. scincus* have an extra- nuclear perforatorium present in the subacrosome space within their spermatozoa; whereas, no endonuclear canals or no visible canal was found in the present study. (Gribbins et al., 2007, 2009; Rheubert et al., 2010).

In cross sections, the acrosome and subacrosomal cone looks round in the family *Tropiduridae* (Furieri, 1974; Teixeira et al., 1999), elliptical in *Iguania* (Scheltinga et al., 2000, 2001) and *Agamidae* (Oliver et al., 1996); but in *Scincidae* (Jamieson and Scheltinga, 1994) and *Gekkonidae* (Jamieson et al., 1996) the epinuclear electron-lucent zone is absent. *Squamata* species always have varying sizes of epinuclear electron-lucent zones and are described in all *Iguania* species as poorly developed (Oliver et al., 1996; Scheltinga et al., 2000, 2001), whereas they are very well developed in *Pygopodidae*

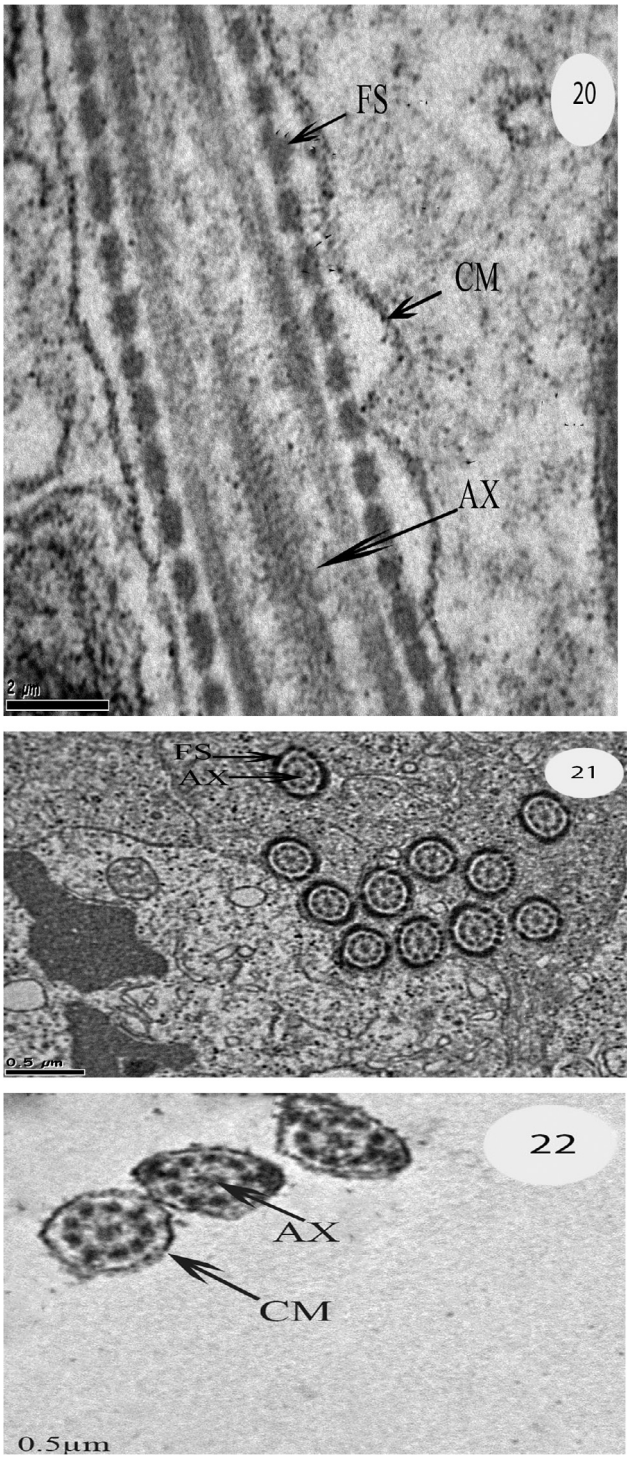


Figure 20, 21 & 22 At late stages of spermatid differentiation, the main piece of the tail was developed, which comprised a typical 9 + 2 axoneme (consists of nine doublets and two central singles) surrounded by a thick fibrous sheath (FS) and the plasma membrane (CM). The fibrous sheath was thick at the beginning of the middle piece and gradually became thinner before it disappeared completely at the start of the end piece. The end piece was composed of the axoneme surrounded only by the plasma membrane.

(Jamieson et al., 1996). In our study, a very poorly developed epinuclear electron-lucent zone was observed.

The origin of perforatorium is not known to date, but it is assumed that it is composed of fibrous material, and mostly cytoskeletal fibrils (Baccetti and Afzelius, 1976). In land faunas, the perforatorium contains actin filaments that undergo conformational changes during acrosomal reaction (Baccetti, 1986; Shiroya et al., 1986). In notochord it is important for sperm penetration (Baccetti et al., 1980), whereas some birds, it not exists and cannot be credited to the perforatorium. As a result, Baccetti et al. (1980) proposed that it only involved in maintenance of the acrosome. In some lizards (Del Conte, 1976), the granule present in pro-acrosomal vesicle in contact with nucleus has been originated from perforatorium. According to Del Conte (1976), the granule may be created by the interaction between pro-acrosomal vesicle and the nucleus. In Sphenodontia (Healy and Jamieson, 1992), crocodilians (Jamieson et al., 1997; Saita et al., 1987), Chelonia (Sprando and Russel, 1988), amphibians (Bao et al., 1991) and some birds (Sprando and Russel, 1988) the perforatorium is located in the interior of intranuclear canals.

The acrosomal vesicle has been synthesized from the cytoplasmic vesicles secreted by endoplasmic reticulum and Golgi complex (Carcupino et al., 1989). Evidently this appears an instance for *S. scincus* spermatid, considering that more vacuoles accumulated with the endoplasmic reticulum, and later could be delivered to the Golgi complex. The nucleus, which forms from the vigorous condensation of chromatin, helps in motion and keeps the genome against any damage during storage and transportation (Krause, 1996). This slender shaped nucleus is recognized throughout spermiogenesis process by manchette (Soley, 1994); by DNA and protein accumulation (Fawcett et al., 1971). A majority reptilian has slender spermatozoa such as in *S. scincus*, except for those of *Eugongylus,* (Jamieson and Scheltinga, 1993) which possess a larger diameter. All *Squamata* including *S. scincus* have a similar pattern for axoneme formation and they form the centrioles (Al-Dokhi et al., 2015; Al-Hajj et al., 1987; Phillips and Asa, 1993). Peripheral dense fibers (nine in numbers) following matching doublets, as well as a single dense fiber attached to one of the central pairs of microtubules, are characteristic of all *Squamata*. Additional features for *Squamata* are the presence of a short distal centriole. It does not extend along the entire midpiece, and ends well above the annulus, within the layer of encircling mitochondria as shown in *D. zarudnyi* (Al-Dokhi et al., 2015). In some reptiles, the distal centriole is long as described by (Healy and Jamieson, 1992; Jamieson and Healy, 1992; Oliver et al., 1996).

The anterior nuclear cup-shaped depression, which partially houses the acrosomal vesicle, is considered as a specific feature in *Squamata* (Butler and Gabri, 1984; Courtens and Depeiges, 1985; Dehlawi and Ismail, 1991; Dehlawi, 1992). This nuclear depression was not reported in the developing spermatids of mammals and birds (Courtens and Depeiges 1985; Dehlawi and Ismail 1991; Healy and Jamieson, 1994; Ismail and Dehlawi, 1994; Ismail et al., 1995), whereas the early spermatids of *Bunopus tuberculatus* have a single big acrosomal granule at the base of acrosomal vesicle. In contrast, some reptilian species such as the lizards *Scincus mitranus* (Al-Dokhi, 1996) and *Agama adramitana* (Dehlawi et al., 1992) revealed the presence of several acrosomal granules either attached or separated.

All *Squamata* spermatozoa show almost linear mitochondrial cristae, inter-mitochondrial dense bodies, a short and distal centriole, and a fibrous sheath, which already begins at the midpiece. Another characteristic of reptiles is the presence of peripheral fibers, distal centrioles, and the axoneme (Al-Dokhi et al., 2015; Jamieson et al., 1997). The fibrous sheath is flexible and helps in mobility (Fawcett, 1970). The annulus that exists in *S. scincus* is similar to that of other limbed lizards (Al-Dokhi et al., 2015; Baccetti and Afzelius, 1976). It consists of filamentous subunits, which firmly adhere to the plasma membrane. Its main function is to inhibit dislocation of mitochondria from the midpiece during flagellar movement (Al-Dokhi et al., 2015; Fawcett, 1970). *Squamata* represent dispersed dense bodies (Jamieson, 1995; Oliver et al., 1996). It is assumed that the dense bodies initiate from mitochondria and are similar to the intra-mitochondrial dense bodies (Carcupino et al., 1989; Healy and Jamieson, 1992). The axoneme pattern of microtubules in the end piece of lizards varies greatly, but constantly maintains the typical 9 + 2 arrangement (Scheltinga et al., 2000, 2001). Similar dense bodies are reported in the present study, and are distributed near the middle region of the spermatozoa.

The early change in the development of the sperm tail was in the migration of centrioles at the posterior side. This was followed by the appearance of a nuclear implantation fossa at the caudal nuclear pole. Subsequently, the proximal centriole was well fitted 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 differentiating lizard spermatid was first adapted in during implantation in the nuclear fossa, which later deepened to form a cup-shaped depression at the caudal nuclear pole. This topological variation in nuclear membrane is clearly a adjustment to form a firm centriolar-nuclear binding. In some reptilians it was different binding; for example, the differentiating spermatid of *Mauremys caspica* (Al-Dokhi and Al-Wasel, 2001a,b) develops an arch-shaped implantation fossa to establish firm binding. Herein, the flagellar structure was extended in a regular pattern and this could be explained by the successive addition and polymerization of tubulin templates to the distal ends of the growing microtubules. In conformity with other reptilians, the middle piece of the *S. scincus* sperm tail involves a mitochondrial sheath, an axonemal core, and is terminated by an annulus. Fusion of the mitochondria in the middle piece of sperm tail is a common feature of reptiles. In this study, mitochondria in the middle piece were bonded via inter-mitochondrial dense bodies, as these dense structures were in an intimate association with the mitochondria. Similar dense inter-mitochondrial bodies were observed in *B. tuberculatus* (Al-Dokhi, 2004) and *Acanthodactylus boskinus* (Al-Dokhi, 2012). The inter-mitochondrial dense bodies are considered a synapomorphic characteristic of *Squamata* (Jamieson, 1995).

5. Conclusion

The spermatogenesis process was studied in detail by electron microscopy in *S. scincus* lizards; even though the initial cell stages were comparable with those of other reptiles. The findings of different phases during early spermatid development have elucidated the progress of the acrosomal complex. The

matured acrosome, the existence of an electron dense medulla, and a cortex was evidently established, which is different from other limbed lizards. In tail region, changes observed are similar to those of all other *Squamata*. The above study could be used to correlate phylogenetic and evolutionary relationships within the reptile family.

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