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Fine Structure of the Epididymal Sperm of the Snake *Eryx jayakari* (Squamata, Reptilia)

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Abstract: The ultrastucture of the snake *Eryx jayakari* (*E. jayakari*) mature sperm has not been published. Therefore, the present study was conducted to elucidate the sperm ultrastructure of this species in comparison with that of other reptilian species. The present investigation has shown that mature spermatozoa of *Eryx jayakari* are filiform in shape averaging 45 μm (mean of 10) in length with a curved head measuring 9 μm. The acrosomal complex, which involves the acrosomal cap and subacrosomal material, measures 2.5 μm. The nucleus is 6.5 μm in length, the neck approximates 1 μm and the tail (midpiece, principal piece and endpiece) is maximally 35 μm in length. The different tail pieces vary in diameter, being the largest at the midpiece (0.66 μm), diminished at the principal piece (0.36 μm) and the least at the endpiece (0.25 μm). The midpiece, the longest tail piece, is composed of mitochondrial and fibrous sheaths encircling the microtubular axoneme. The mitochondria are rod-shaped having linear cristae but no dense bodies interrupt their arrangement. The principal tail piece consists of the microtubular axoneme surrounded by the fibrous sheath. The endpiece involves only an axoneme enveloped within the plasmalemma of the spermatozoon. The present findings were discussed in relation to other relevant studies. It was concluded that the general ultrastucture of the *E. jayakari* spermatozoon conforms to that reported to be typical of squamates.

Key words: Snake, epididymal sperm, ultrastructure

INTRODUCTION

The processes of spermatogenesis and spermiogenesis have been comprehensively described in mammalian species (Fawcett, 1975, 1981, 1991; Philips and Asa, 1993). However, these processes have received relatively limited attention in reptiles. The ultrastucture of spermatozoa has been described for the major groups of squamate reptiles (Jamieson and Scheltinga, 1993; Jamieson, 1995; Jamieson *et al*., 1996; Oliver *et al*., 1996). The relevant studies have improved the understanding on organelles responsible for sperm motility and on adaptations accounting for fertilization and egg activation (Teixeira *et al*., 1999b). Additionally, the spermatozoal ultrastructure has been utilized in phylogenetic analyses of squamates (Jamieson, 1995; Jamieson *et al*., 1996; Teixeira *et al*., 1999 b,c). Nevertheless, spermatozoa of several squamate families await ultrastructural description (Jamieson, 1995; Teixeira *et al*., 1999 b,c).

Ultrastructure of spermiogenesis and mature sperm of the snake species was the objective of some previous studies (Austin, 1965; Boisson and Mattei, 1966; Hamilton and Fawcett, 1968; Philips and Asa, 1993; Dehlawi and Ismail, 1994; Al-Dokhi, 1996; Oliver *et al*., 1996). However, ultrastructure of the entire snake spermatozoa has been reported in relatively few studies (Furieri, 1965, 1970; Jamieson and Koehler, 1994). The ultrastucture of the snake *Eryx jayakari* (*E. jayakari*) mature sperm

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has not been published. Therefore, the present study was undertaken to illustrate the fine structural morphology of the spermatozoon of this snake species. The ultrastructural features characterize the sperm of this species from other reptiles are also considered.

MATERIALS AND METHODS

Ten adult males of the snake *Eryx jayakari* (*E. jayakari*) were collected during the period of sexual activity (from March to May, 2002) from Thumamh area (25°30′ N, 49°40′ E), north-east of the city of Riyadh, Saudi Arabia. After decapitation, the snakes were dissected and their epididymses (head and tail regions) were removed and diced into small pieces that were immediately fixed by immersion in 3% buffered glutaraldehyde (0.1 M sodium cacodylate buffer at pH 7.2) for 4 h at 4°C. The fixed epididymal tissue specimens were thoroughly washed 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 and the tissues were then transferred to epoxy resin (Epon/Araldite mixture) via propylene oxide. Thin sections were cut with a diamond knife on an ultramicrotime (Leica, UCT), double stained with uranyl acetate and lead citrate and examined under a transmission electron microscope (JEOL, 100 CX) operating at 80 kV.

RESULTS

Spermatozoa of *E. jayakari* are filiform in shape (Fig. 1) averaging 45 μm (n = 10) in length. The head is long, attenuated and curved measuring 9 μm and contains an elongated cylindrical nucleus 6.5 μm in length. The total length of the tail is maximallly 37 μm. Throughout its length, the spermatozoon is circular in cross section and its diameter maximizes at the midpiece (0.66 μm), decreases at the principal piece (0.36 μm) and is the least at the endpiece (0.25 μm). For the purpose of illustrating the ultrastructure of *E. jayakari* spermatozoon, it was divided into three main parts; head, neck and tail.

Head

The head of an *E. Jayakari* mature epididymal spermatozoon is subdivided into acrosomal and nuclear regions (Fig. 2).

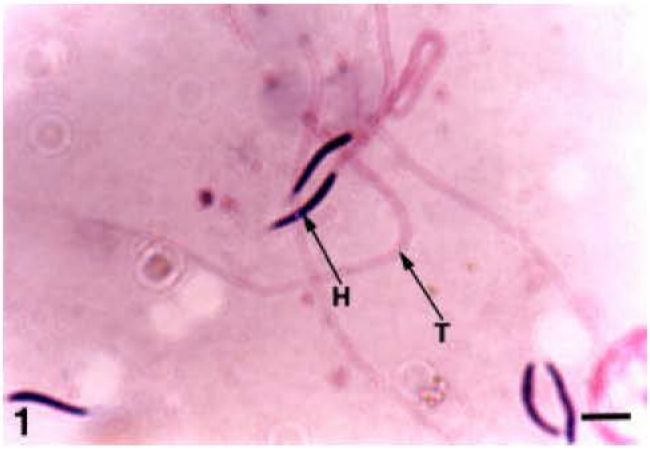

Fig. 1: Filliform-shaped spermatozoa of *E. Jayakari* showing a curved Head (H) and noticeably long tail (T). HE. Scale bar = 10 μm

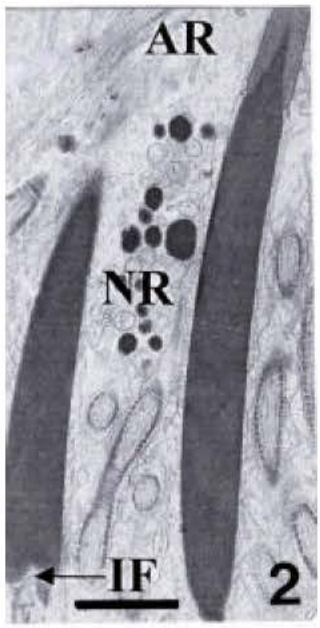

Fig. 2: Head of the *E. jayakari* spermatozoon showing Acrosomal Region (AR) and Nuclear Region (NR). Nucleus on the left has a basal depression, the Implantation Fossa (IF). Scale bar = 1 μm

Acrosomal Region

This region, averaging 2.5 μm in length, is located at the proximal portion of the head and seen in longitudinal sections as a coat covering the anterior portion of the nucleus (Fig. 3a). The acrosome complex consists of an acrosome vesicle which appears as an elongate cone-shaped hollow structure, an underlying subacrosomal cone and a rod-shaped putative perforatorium. The anterior attenuated end of the acrosome vesicle, which constitutes about one third of its total length, forms hollow cone with a thick wall and a narrow lumen housing the perforatorium. The remaining portion of the acrosomal vesicle extends posteriorly as a sleeve-like thin walled continuation investing the subacrosomal material. The subacrosomal cone material reveales a fine lattice due to presence of intersecting striations and thus presents a paracrystalline appearance (Fig. 3b). The subacrosomal cone invests the tapered anterior end of the nucleus (nuclear rostrum) and extends distally as a posterolateral flange behind the base of the acrosomal vesicle. The most distal portion of the acrosomal sleeve rests on the flange-like posterior end of the subacrosomal cone. The nuclear rostrum terminates within the anterior portion of the subacrosomal cone at a tiny electron lucent region (epinuclear electron lucent region). The perforatorium, measuring 1 μm in length, is a slender rod of moderate electron density extending anteriorly from the subacrosomal material through the thick walled portion of the acrosomal vesicle (Fig. 3b). It is housed in a narrow lumen internal to the inner acrosomal vesicle membrane. This lumen is contained within the acrosomal medulla which is represented by an attenuated portion in which the acrosomal granule underwent dissolution. The perforatorium has a contact at its posterior end with the subacrosomal cone, however no perforatorial basal plate is recognizable. The longitudinal axis of the perforatorium appears to be slightly tilted relative to that of the acrosomal vesicle.

Nuclear Region

The nucleus constitutes the main part of the head region and it is elongated and cylindrical in shape averages 6.5 μm in length (Fig. 1). It is curved and gradually pointed anteriorly and its tapering begins at the level of the basal region of the acrosome (acrosomal sleeve). The well developed and

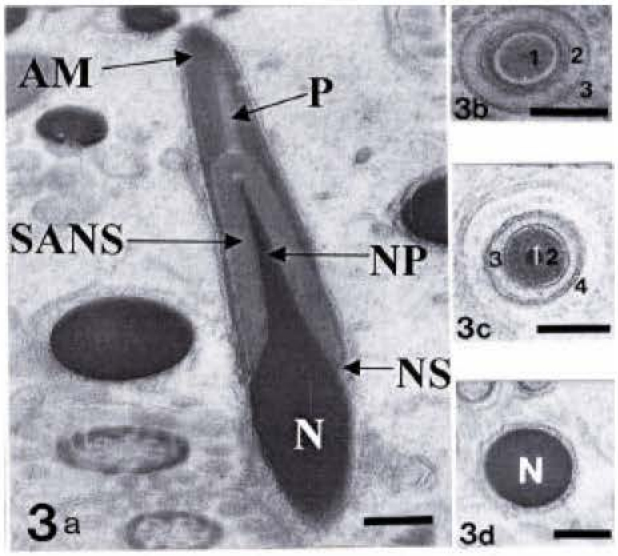

Fig. 3a: Acrosomal complex forming a cap over the Nucleus (N). Nuclear Rostrum (NR), the tapered nuclear proximity, is extended into the Subacrosomal Cone Material (SAC). The Perforatorium (P) is an elongated rod-like structure extending into the Acrosomal Medulla (AM). The lateral arms of the acrosome vesicle are resting on the flanges of subacrosomal cone. Note the Nuclear Shoulders (NS). Scale bar = 0.5 μm. b, Transverse section through the acrosomal complex at the level of the perforatorium (1). Acrosomal vesicle (2) and sperm plasmalemma (3). Scale bar = 0.5 μm. c, Transverse section at the level of the nuclear rostrum (1). Subacrosomal cone (2), acrosomal vesicle (3) and sperm plasma membrane (4). Scale bar = 0.5 μm and d Transverse section at the level of the highly compact dense nucleus (N). Scale bar = 0.5 μm

smoothly sloping nuclear shoulders (nuclear curvature on each side) marking the transition from the longer cylindroid region to the tapered tip of the nucleus (nuclear rostrum) which extends into the subacrosomal space (Fig. 3c). The nuclear rostrum approximates 1.5 μm in length while the nucleus from the base of acrosomal vesicle to its base is approximately 5 μm long. The total length of the nucleus includes the nuclear rostrum capped by the subacrosomal cone and the free nuclear region. The nucleus slightly increases in width towards its posterior end with the maximum diameter approximates 0.4 μm. However, in transverse section, the nucleus is circular throughout its length (Fig. 3d). The nuclear chromatin is strongly electron dense and lacks lacunae. At the nuclear base, there is a moderately deep conical fossa (nuclear fossa) which partially accommodates the proximal centriole (Fig. 4).

Neck

The neck is a short region, averaging 1 μm in length. It is the region where the nucleus joins the midpiece and its internal components include the proximal and distal centrioles (Fig. 4). The neck is surrounded by electron dense material which is in contact with the fibers connecting the proximal and distal centrioles (connecting piece). The dense material encircling the neck together with the connecting piece form the neck cylinder. The proximal centriole is located immediately anterior to the distal one and the two centrioles are nearly perpendicular to each other. The proximal centriole lies immediately behind the basal nuclear fossa and seems to be partially enclosed within a dense material (pericentriolar material) which is also accommodated in the nuclear fossa and separated from it by a narrow space

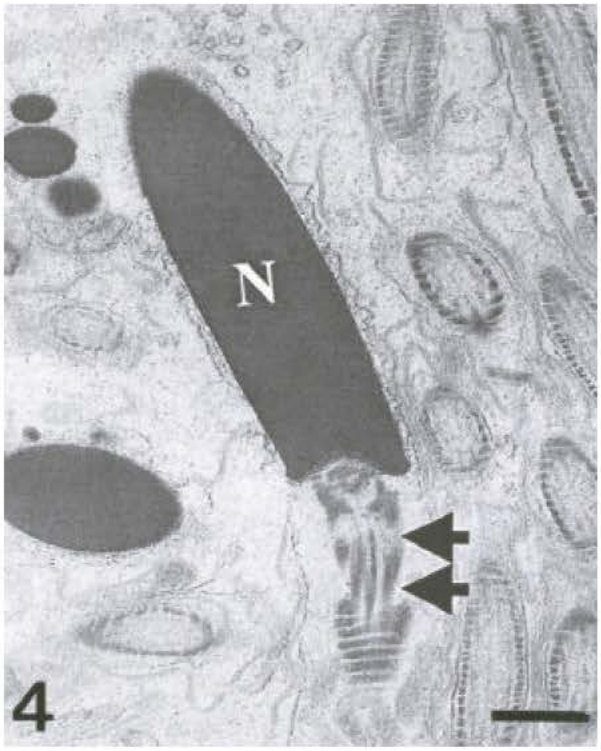

Fig. 4: Neck region (arrows) of a mature spermazoon. Neck is ensheathed by electron dense material which is in contact with the fibers connecting the proximal and distal centrioles (connecting piece). Nucleus (N). Scale bar = 0.5 μm

(Fig. 4). The latter dense structure has distal extensions which are inserted between the proximal and distal centrioles. The distal centriole forms the basal body of the flagellum and its longitudinal axis is in the long axis of the axoneme. Longitudinal sections of the neck region show that the central microtubular singlets of the axoneme extends proximally into the region of transition between the distal centriole and the axoneme.

Tail

The mature spermatozoon of *E. jayakari* has a relatively long tail which averages 35 μm in length. The main constituent of the tail is the axoneme which extends from the distal centriole throughout the whole tail length. The axoneme consists of two central singlets of microtubules encircled by nine peripheral microtubular doublets (the typical 9+2 arrangement). The tail is recognizable into three pieces, the midpiece, principal piece and endpiece:

Midpiece

The midpiece extends from just distal to the neck region and constitutes the longest region of the tail. It is composed of an axoneme surrounded by a fibrous sheath which is, in turn, surrounded by a sheath of rod-shaped mitochondria. In the longitudinal section of the midpiece, the profiles of mitochondria are arranged in a single file on each side of the axoneme (Fig. 5). The profiles of mitochondria in transverse sections are mostly rounded and their number varies, but the majority of the transverse sections show nine mitochondrial profiles (Fig. 6). The mitochondrial cristae are predominantly linear in arrangement. The fibrous sheath extends proximally around the axoneme to the

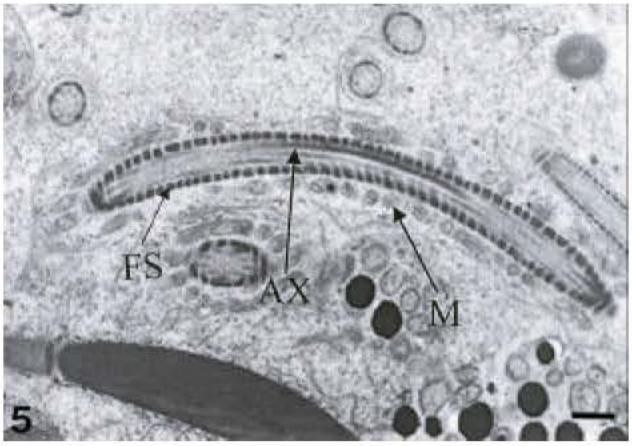

Fig. 5: Longitudinal section through the spermatozoon midpiece. The microtubular Axoneme (AX) is surrounded by the Fibrous Sheath (FS) which in turn ensheathed by the Mitochondria (M). Scale bar = 0.2 μm

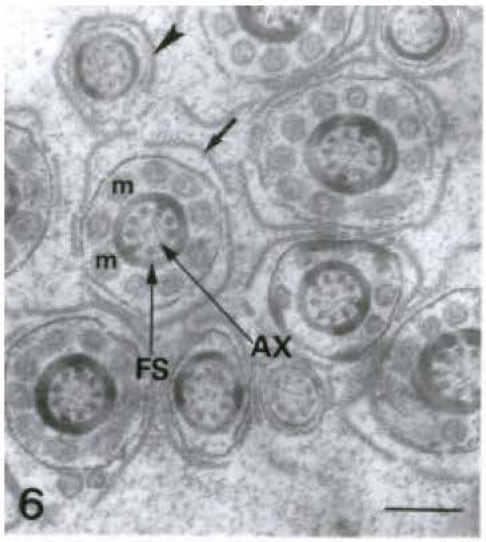

Fig. 6: Transverse sections through the midpiece exhibiting the microtubular Axoneme (AX), surrounded by the Fibrous Sheath (FS) and ensheathed by Mitochondria (M). Mitochondrial profiles are circular and have linear cristae. Note the extracellular microtubules (arrow) which are arranged as circlets of rings attached to the sperm plasmalemma at the regions of midpiece (arrow) and principal piece (arrowhead). Scale bar = 1 μm

point of junction between the distal centriol and the axoneme. In the longitudinal sections, the fibrous sheath exhibits regularly arranged dense blocks which in transverse sections are noticed to form rings around the microtubular axoneme (Fig. 7). This appearance of the fibrous sheath suggests that it consists of annular dense structures. Relative to the axonemal axis, these annular structures are tilted, but anastomoses of the adjacent structures are not detectable. Transverse section of the midpiece reveal the existence of nine peripheral dense fibers, each of them is attached to one of the peripheral microtubular doublets. Longitudinal sections reveal the extension of the peripheral dense fibers along the whole recognizable length of the midpiece. The annulus, which marks the distal end of the

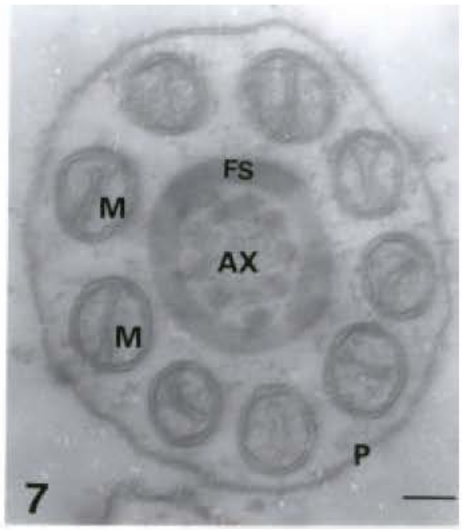

Fig. 7: Higher magnification for a transverse section through the midpiece which comprises the microtubular Axoneme (AX), the extending Fibrous Sheath (FS), the ensheathing Mitochondria (M) and the spermatozoon Plasmalemma (P). Note the linear mitochondrial cristae. Scale bar = 0.2 μm

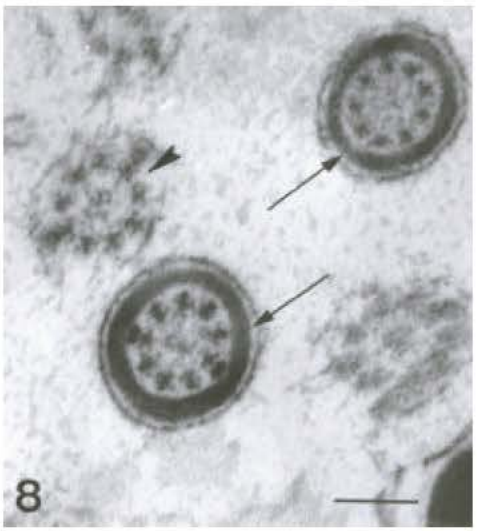

Fig. 8: Transverse sections through the principal piece (arrows) which encompass the microtubular Axoneme (AX) encircled by the fibrous sheath. Note the transverse sections of endpiece (arrowhead) which only involve the microtubular axoneme and the surrounding spermatozoon Plasmalemma (P). Scale bar = 0.5 μm

midpiece, is present as a small dense ring structure which is closely applied to the inner surface of the sperm plasma membrane. Exracellular microtubules, seen as a circlet of rings, are present adjacent to the external surface of the sperm plasmalemma at the region of the midpiece.

Principal Piece

The principal piece is much shorter than the midpiece and it lacks the mitochondrial sheath. It consists of the continuation, behind the midpiece, of the axoneme with its enveloping fibrous sheath

and the plasmalemma (Fig. 8). It begins immediately behind the annulus and from this point and downwards the plasmalemma is closely approximated to the fibrous sheath. The thickness of the fibrous sheath decreases gradually toward the distal end of the principal piece. The extracellular microtubules are also detectable in some transverse sections of the principal piece.

End Piece

The endpiece is the shortest portion of the tail and represents the part of the spermatozoon tail that displays the projection of the axoneme behind the fibrous sheath. It consists only of the microtubular axoneme and the limiting plasmalemma of the spermatozoon (Fig. 8).

Observation of a large number of transmission electron micrographs led to a visualization of the general ultrastructure of *E. jayakari* spermatozoon (Fig. 9 and 10).

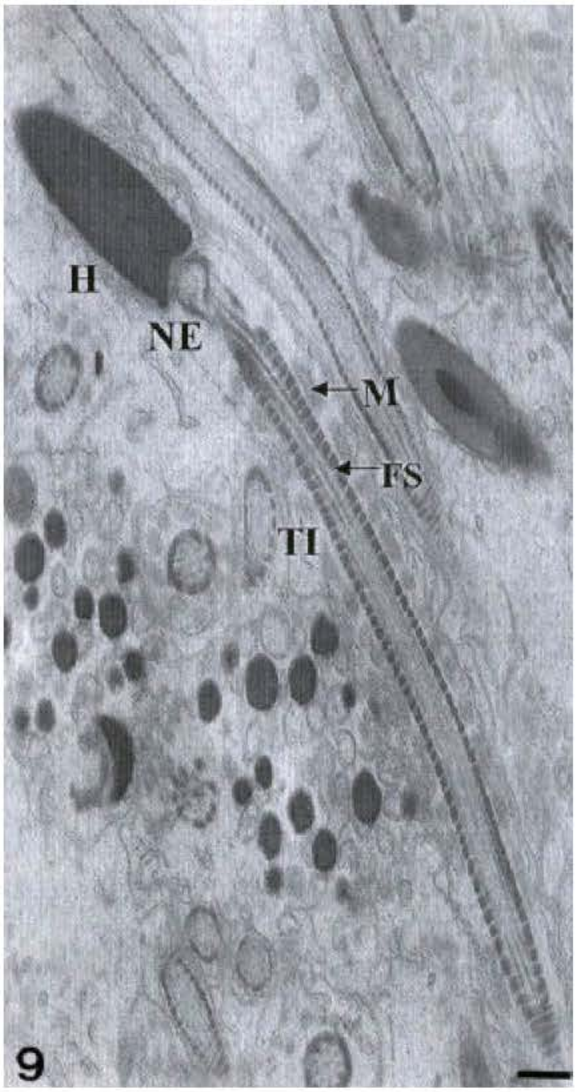

Fig. 9: Longitudinal section through the maximally obtainable lenghth of the Head (H), Neck (NE) and Tail (TI) regions. Note the Mitochondria (M) which ensheath the long midpiece. Fibrous Sheath (FS). Scale bar = 0.5 μm

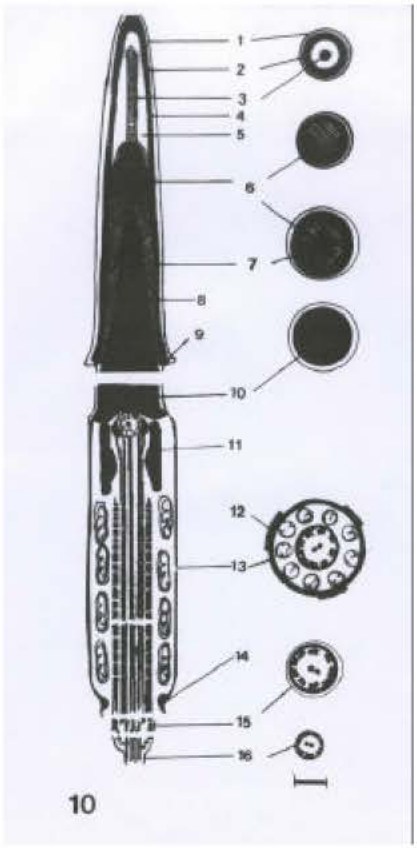

Fig. 10: Diagrammatic representation of the *E. jayakari* mature spermatozoon. The longitudinal section through the maximal length of the spermatozoon is accompanied by the corresponding transverse sections at different levels. Plasmalemma (1), acrosome vesicle (2), perforatorium (3), acrosome cortex (4), acrosome medulla (5), subacrosomal cone (6), nuclear rostrum (7), sleeve of acrosome vesicle (8), flange of subacrosomal cone (9), nucleus (10), neck cylinder (11), extracellular tubules (12), midpiece (13), annulus (14), principal piece (15), endpiece (16)

DISCUSSION

The general ultrastucture of the *E. jayakari* spermatozoon conforms to that reported to be typical of squamates (Jamieson *et al*., 1996). The head region of the *E. jayakari* mature spermatozoon is curved and cylindrical in shape with a pointed anterior end. Its morphology resembles that described in many reptiles (Oliver *et al*., 1996; Courtens and Depeiges, 1985; Healy and Jamieson, 1994; Al-Dokhi, 2004; Vieira *et al*., 2004). The differentiation of the sperm head of *E. jayakari* into acrosomal and nuclear regions separated by the subacrosomal cone mirrors the situation in other reptilian species (Furieri, 1974; Butler and Gabri, 1984; Courtens and Depeiges, 1985; Al-Hajj *et al*., 1987; Dehlawi and Ismail, 1990; Jamieson and Koehler, 1994; Ismail *et al*., 1995; Al-Dokhi, 1996; Al-Dokhi and Al-Wassel, 2001a, b; Al-Dokhi, 2004). The tapered end of the acrosome, presence of the subacrosomal cone and nuclear tip constriction are reported in Chelonia, Crocodilia, Sphenodon and Squamata (Jamieson and Scheltinga, 1993). As the case in all amniote classes, the acrosomal vesicle in *E. jayakari* spermatozoon forms an elongate narrow cone laying on the tip of the nucleus. The

acrosome in this form consricts the nucleus and encloses a similarly shaped subacrosomal cone. The paracrystalline nature of the subacrosomal cone is also a feature of other squamates (Furieri, 1974; Butler and Gabri, 1984).

The nucleus in *E. jayakari* spermatozoon is elongated and has highly condensed chromatin. The nuclear morphology including the nuclear rostrum resembles that found in many reptiles and also other vertebrates (Nagano, 1962; Porter, 1965; Anderson *et al*., 1966; Boisson and Mattei, 1966; Fawcett, 1975; Da Cruz-Landim and Da Cruz-Hoffling, 1977; Butler and Gabri, 1984; Al-Hajj *et al*., 1987; Dehlawi and Ismail, 1990, 1991).

The endonuclear canals recorded in certain reptilian species by some authors (Al-Hajj *et al*., 1987; Sprando and Russel, 1988; Dehlawi and Ismail, 1990, 1991, 1994; Ismail *et al*., 1995; Jamieson *et al*., 1997; Al-Dokhi and Al-Wassel, 2001a). are absent in the mature sperm of *E. Jayakari*. Absence of the endonuclear canals is a synapomorphy of the squamates (Jamieson and Healy, 1992). Also, absence of such endonuclear canals may support the conclusion that these canals are lacking in lizards and snakes and that the perforatorium may play a similar role (Healy and Jamieson, 1994). The development of rounded nuclear shoulder, as noticed in *E. jayakari* spermatozoon, is considered a basic feature to Squamate sperms (Oliver *et al*., 1996).

The perforatorium in *E. jayakari* sperm head extends as a cylindrical rod-like structure from the subacrosomal cone material into the subacrosomal space. However, it lacks the basal plate existing in some other reptilian species such as the lizards *Anolis carolinensis* (Clark, 1967), *Cnemidophorus lemniscatus* (Del Conte, 1976), *Tropidurus torguatus* (Da Cruz-Landim and Da Cruz-Hoffling, 1977), *Agama stellio* (Al-Hajj *et al*., 1987), *Lacerta taurica* (Butler and Gabri, 1984) and *Stenodactylus selvini* (Dehlawi and Ismail, 1991) and the snakes *Psammophis schokari* (Dehlawi and Ismail, 1994), *Nerodia sipedon* (poorly developed stopperlike base plate) (Jamieson and Koehler, 1994) and *Boiga irregularis* (Oliver *et al*., 1996).

The neck cylinder is a feature in *E. jayakari* mature sperm and is probably formed by aggregation of dense granules around the two centrioles. Similar structure is present in many other snakes and it is speculated that the neck cylinder material first arises as a precipitated material adjacent to mitochondria and later aggregated around the distal centriole at the tail base (Courtens and Depeiges, 1985; Al-Hajj *et al*., 1987).

The tail of *E. jayakari* mature sperm is long (35 μm) and its general structure resembles that of other reptiles. Worth of mentioning is the great elongation of the midpiece which occupies most of the axoneme length of *E. jaykari* sperm. This agrees with the ultrastructural data reported on the sperm tail midpiece of other snakes (Austin, 1965; Boisson and Mattei, 1966; Hamilton and Fawcett, 1968; Furieri, 1970; Dehlawi and Ismail, 1994; Jamieson and Koehler, 1994; Oliver *et al*., 1996). Snake sperm is unique among squamates regarding the immense elongation of the midpiece (Jamieson, 1995) and this feature is considered a synapomorphy of the Serpents (Jamieson and Koehler, 1994). The number of mitochondria seen in the transverse sections of midpiece of *E. jayakari* sperm shows variation. In snakes, the mitochondrial number is reported to show apomorphic increase to as many as 14 (Jamieson and Scheltinga, 1993). Because of the great length of the midpiece it is difficult to count the repeats of the mitochondrial units in *E. jayakari* sperm. The linear arrangement of the mitochondrial cristae observed in *E. jayakari* spermatozoon is a usual feature of amniotes, including most reptiles (Jamieson, 1995). The dense intermitochondrial bodies (mitochondrial transformations) in the sperm tail midpiece described by some workers (Hamilton and Fawcett, 1968; Jamieson and Scheltinga, 1993; Jamieson and Koehler, 1994; Oliver *et al*., 1996). In some reptile species are absent from the midpiece of *E. jayakari* sperm.

Extension of the fibrous sheath into the midpiece of the *E. jayakari* spermatozoon is an autapomorphy of the Squamata (Healy and Jamieson, 1992; Jamieson and Healy, 1992; Jamieson, 1995; Oliver *et al*., 1996). The fibrous sheath in *E. jayakari* spermatozoon has an annulated appearance

similar to that of all amniote classes (Jamieson and Scheltinga, 1993). However, no anastomoses of the adjacent fibrous sheath rings are discernible in *E. jayakari* spermatozoon. Some snakes, such as *Lampropeltis getulus* (Austin, 1965) reveal branching and anastomoses of the adjacent fibrous sheath rings.

The presence of a multilaminar membrane investing the sperm midpiece has been considered a synapomorphy of the Serpents (Furieri, 1970; Jamieson and Koehler, 1994). Snake sperm is characterized apomorphically by multilaminar membranes in place of the normal plasma membrane of the midpiece (Oliver *et al*., 1996). This membrane pattern is not detectable in the *E. jayakari* spermatozoon in which extracellular microtubules are recognizable external to the plasma membrane. Similarly, this membrane form is absent from spermatozoa of some snakes such as *Boiga irregularis* (Oliver *et al*., 1996). Snake sperm shows greater development of extracellular microtubules than is known in any other squamate (Jamieson, 1995). Moreover, it was reported that snakes are the only squamate reptiles which retain microtubules external to the plasma membrane of the mature spermatozoon (Jamieson and Koehler, 1994). There is evidence that extracellular microtubules are transformed into membranes and these microtubules possibly represent a stage in production of the laminated membranes (Oliver *et al*., 1996). It has been suggested that the extracellular microtubules provide a source of endogenous energy for sperm motility (Hamilton and Fawcett, 1968). On the other hand, the microtubules around the sperm tail is regarded as remnants of the spermiogenic manchette (Furieri, 1965).

The presently described ultrastructural morohology of *E. jayakari* spermatozoon conforms closely to the features common to Squamate sperm reported by other researchers (Oliver *et al*., 1996). Possession of a single perforatorium, which is wholly prenuclear or presubacrosomal (synapomorphy), loss of endonuclear canals, presence of a well-developed epinuclear electron lucent region (autapomorphy), linear form of the mitochondrial cristae, extension of the fibrous sheath into the midpiece (autapomorphy) and paracrystalline subacrosomal cone (synapomorphy) represent the most significant and convincing synapomorphies and autapomorphies of Squamates (Benton, 1985; Healy and Jamieson, 1992; Jamieson and Healy, 1992; Jamieson and Koehler, 1994). Also, some of the ultrasructural features of *E. jayakari* sperm such as reduction of the epinuclear lucent region, loss of the perforatorial base plate and the greater development of extracellular tubules are known snake apomorphies (Jamieson, 1995; Oliver *et al*., 1996).

REFERENCES

Al-Dokhi, O.A., 1996. Ultrastructural studies on the differentiation of the sperm head in the sand skink *Scincus mitranus* (Anderson, 1871) (Squamata, Reptilia). Arab Gulf J. Sci. Res., 14: 471-482.

Al-Dokhi, O.A. and S. Al-Wasel, 2001a. Ultrastructure of spermiogenesis in the freshwater turtle *Maurymes caspica* (Chelonia, Reptilia), I: The acrosomal vesicle and the endonuclear canals formation. J. Egypt. German Soc. Zool., 36: 93-106.

Al-Dokhi, O.A. and S. Al-Wasel, 2001b. Ultrastructure of spermiogenesis in the freshwater turtle *Maurymes caspica* (Chelonia, Reptilia), II: The nucleus elongation and chromatin condensation. J. Union Arab Biol. Zool., 15: 355-366.

Al-Dokhi, O.A., 2004. Electron microscopic study of sperm head differentiation in the Arabian horned viper *Cerastes cerastes* (Squamata, Reptilia). J. Biol. Sci., 4: 111-116.

Al-Hajj, H., S. Jankat and F. Mohamoud, 1987. Electron microscopic study of sperm head differentiation in the lizard *Agama stellio*. Can. J. Zool., 65: 2959-2968.

Anderson, W.A., A. Weissman and R.A. Ellis, 1966. A comparative study of microtubules in some vertebra and invertebrate cells. Zeitschrift fur Zellforschung undmikroskopische Anatomie, 71: 1-13.

Austin, C.R., 1965. Fine structure of the snake sperm tail. J. Ultrastructure Res., 12: 452-462.

Benton, M.C., 1985. Classification and phylogeny of the diapsid reptiles. Zool. J. Linn Soc., 84: 97-164.

Boisson, C. and X. Mattei, 1966. La spermiogenese de *Python sebea* (Gmelin), obsevee au microscope electronique. Annal. Sci. Natur. Zool. Biol. Ani. Series, 12: 363-390.

Butler, R. and M. Gabri, 1984. Structure and development of the sperm head in the lizard *Podarcis* (*Lacerta*) *taurica*. J. Ultrastructure Res., 88: 261-274.

Clark, A., 1967. Some aspects of Spermiogenesis in lizard. Am. J. Anato., 121: 369-400.

Courtens, J.L. and A. Depeiges, 1985. Spermiogensis of *Lacerta vivipara*. J. Ultrastruct. Rres., 90: 203-220.

Da Cruz-Landim, C. and M. Da Cruz-Hoffling, 1977. Electron microscope study of the lizard spermiogenesis in *Tropidurus torquatus* (Lacertilia). Caryologia, 30: 151-162.

Dehlawi, G.Y. and M.F. Ismail, 1990. Studies on the ultrastructure of the spermiogenesis of Saudian reptiles, 1-The sperm head differentiation in Uromastyx philbyi. Proc. Zoo. Soc. Egypt, 21: 79-89.

Dehlawi, G.Y. and M.F. Ismail, 1991. Studies on the ultrastructure of the spermiogenesis of Saudian reptiles. 3-The sperm head differentiation in *Stenodactyius selevini*. Egypt. J. Histol., 14: 71-81.

Dehlawi, G.Y. and M.F. Ismail, 1994. Studies on the ultrastructure of the spermiogenesis of Saudian reptiles. 13-The sperm differentiation in the snake. Proc. Zoo. Society, Egypt, 251: 95-106.

Del Conte, E., 1976. The subacrosomal granule and its evolution during spermiogenesis in a lizard. Cell and Tiss. Res., 171: 483-498.

Fawcett, D., 1975. The mammalian spermatozoon. Developmental Biol., 4: 394-436.

Fawcett, D.W., 1981. The Cell. 2nd Edn., W.B. Company Saunders Philadelphia.

Fawcett, D.W., 1991. Spermatogenesis. In: Developmental Biology. Browder, L.W., C.A. Erickson and W.R. Jeffrey (Eds.). 3rd Edn., Saunders College Publishing, New York.

Furieri, P., 1965. Prime osservasioni al microscopio electronico sulla ultrastructura dello spermatozoo di Vipera aspis aspis. Boll Society Italiano Biologica Spermatozoo, 41: 478-480.

Furieri, P., 1970. Sperm Morphology in Some Reptiles: Squamata and Chelonia. In: Bacceti, B. (Ed), Comparative Spermatology, Academic Naz. Lincei, pp: 115-131.

Furieri, P., 1974. Sperm and spermiogenesis in certain *Agentinean iguanids*. Revista di Biolgia, 67: 233-251.

Hamilton, D. and D.W. Fawcett, 1968. Unusual feature of the neck and middle piece of snake spermatozoa. J. Ultrastruct. Res., 23: 81-97.

Healy, J.M. and B.G.M. Jamieson, 1992. Ultrastructure of the spermatozoon of the tuatara (*Sphenodon punctatus*) and its relevance to the relationships of the sphenodontia. Philosophical Trans. Royal Soci. London Series Biol. Sci., 205: 193-235.

Healy, J.M. and B.G.M. Jamieson, 1994. The ultrastructure of the spermatogenesis and epididymal spermatozoa of the tuatara *Sphenodon punctatus* (Sphenodontida, Amniota). Philosophical Trans. Royal Soc. London Series Biol. Sci., 344: 187-199.

Ismail, M.F., G.Y. Dehlawi and O.M. Sarhan, 1995. Ultrastructure of the spermiogenesis of Saudian reptiles. The sperm differentiation in *Echis carinatus* (Serpents, Viperidae). Proc. Zool. Soc. Egypt, 26 : 59-65.

Jamieson, B.G.M. and J.M. Healy, 1992. The phylogenetic position of the tuatara *Sphenodon* (Sphenodontida, Amniota), as indicated by cladistic analysis of the ultrastructure of spermatozoa. Philosophical Trans. Royal Soc. London Ser., B335: 207-219.

Jamieson, B.G.M. and D.M. Scheltinga, 1993. The ultrastructure of spermatozoa of *Nangura spinosa* (Scincidae, Reptilia). Memoris Queensland Museum, 34: 169-179.

Jamieson, B.G.M. and L. Koehler, 1994. The ultrastructure of the spermatozoon of the northern water snake, *Nerodia stepdon* (Colubridae, Serpents), with phylogenetic considerations. Can. J. Zool., 72: 1648-1652.

Jamieson, B.G.M., 1995. The Ultrastructure of Spermatozoa of the Squamata (Reptilia) with Phylogenetic Considerations. In: Advances in Spermatozoal Phylogeny and Taxonomy. Jamieson, B.G.M., J. Ausio and J.L. Justine (Eds.), Memooires du Museum National dHitoire Naturelle, Paris, France, pp: 359-383.

Jamieson, B.G.M., S.C. Oliver and D.M. Scheltinga, 1996. The ultrastructure of spermatozoa of Squamata. I. Scincidae, Gekkonidae and Pygonidae (Reptilia). Acta Zool., 77: 85-100.

Jamieson, B.G.M., D.M. Scheltinga and A.D. Tucker, 1997. The ultrastructure of spermatozoa of Australian freshwater crocodile, Crocodylus johnstoni kreft (Crocodylidae, Reptilia). J. Submicroscopic Cytol. Pathol., 29: 265-274.

Nagano, T., 1962. Observations on the fine structure of the developing spermatids in the domestic chicken. Biology, 14: 193-205.

Oliver, S.C., B.G.M., Jamieson and D.M. Sceltinga, 1996. The Ultrastructure of spermatozoa of Squamata. II. Agamidae, Colubridae, Elapidae and Biodae (Reptilia). Herpetologica, 52: 216-241.

Philips, D.M. and C.S. Asa, 1993. Strategies for Formation of the Midpiece. In: Comparative Spermatology 20 Years After. Bacetti, B. (Ed.). Raven Press, New York, pp: 997-1000.

Porter, K.R., 1965. Illustration of Cell Fine Structure. In: Moore, J.A. (Ed.), Ideas in Modern Biology, The National History Press, New York, pp: 95-124.

Sprando, R.L. and L.D. Russel, 1988. Spermiogenesis in the red-ear turtle (Pseudomys, Scripta) and the domestic fowl (*Gallus domesticus*): A study of cytoplasmic events including cell volume changes and cytoplasmic elimination. J. Morphol., 198: 95-118.

Teixeira, R.D., G.R. Colli and S.N. Bjo, 1999a. The ultrastructure of the spermatozoa of the lizard *Micrablepharus maximiliani* (Squamata, Gymnophthalmidae), with considerations on the use of sperm ultrastructure characters in phylogenetic reconstruction. Acta Zool., 80: 47-59.

Teixeira, R.D., G.H.C. Vieira, G.R. Colli and S.N. Bjo, 1999b. The ultrastructure of spermatozoa of the lizard *Polychrus acutirostris* (Squamata, Polychrotidae). J. Submicroscopic Cytol. Pathol., 31: 387-395.

Teixeira, R.D., G.H.C. Vieira, G.R. Colli and S.N. Bjo, 1999c. Ultrastructural study of spermatozoa of the neotropical lizards, *Tropidurus semitaeniatus* and *Tropidurus torquatus* (Squamata, Tropiduridae). Tissue and Cell, 31: 308-317.

Vieira, G.H.C., G.R. Coli and N. Bao, 2004. The ultrastructure of the spermatozoon of the lizard *Iguana iguana* (Reptilia, Squamata, Iguanidae) and the variability of sperm morphology among iguanian lizards. J. Anatom., 204: 451-464.