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Electron Microscopic Study of Sperm Tail Differentiation of the Jordanian Lizard *Agama stellio*

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ABSTRACT. The sperm tail of *A. stellio* consists of a middle piece, a principal piece, and an end piece. The tail is connected to the nucleus with an extremely short neck, composed of a capitulum and a connecting piece with striated elements; no neck cylinder is present. The middle piece comprises mitochonrdial sheath, fibrous sheath, nine dense fibers, and an axoneme of nine doublets and two singlets. Dense intermitochondrial plaques are present between the mitochondria, which are arranged end-to-end, in a longitudinal fashion. The fibrous sheath consists of fibrous rings which extend from the posterior two thirds of the middle piece till the end of the principal piece. The latter is made of an axonemal core within the fibrous sheath. The end piece is just a plain axoneme. The sperm plasma membrane surrounds all parts of the tail.

A limited number of investigations has been devoted to ultrastructural studies of reptilian spermiogenesis, with special emphasis on either the acrosome (Boisson and Mattei 1966, Del Conte 1976, Da Cruz-Landim and Da Cruz-hoffling 1977; and Butler and Gabri 1984), or the whole mature sperm (Clark 1967, Furieri 1970, 1974, Del Conte 1976, and Da Cruz-Landim and Da Cruz-Hoffling 1977). Very little attention has been paid to differentiation of reptilian sperm tail. In this connection, the first fine structural study was reported by Austin (1965) who described mature sperm tail in six species of snake. This was followed by the work of Hamilton and Fawcett (1968) who studied the neck and the middle piece in two other species of snake. In these studies, special features of snake sperm tail were emphasized, such as the occurrence of two centrioles rather than one, the presence of a neck cylinder, and the concentric arrangement of a mitochondrial coat and a fibrous sheath in the middle piece. In addition, the latter authors reported, for the first time, a feature which is peculiar to reptilian sperm tail, namely, the presence of conspicuous intermitochondrial plaques. Such plaques and a neck cylinder were

Early in the tail differentiation of *A. stellio* sperm, the mitochondria are scattered within the cytoplasm (Fig. 1). Later, they accumulate around one side of the nucleus (Fig. 2), and still later, these organelles gather around the centriolar apparatus of the neck region of the sperm to form a primordium of the mitochondrial sheath of the middle piece (Fig. 5). Some mitochondria move posteriorly and occupy more of the middle piece (Fig. 6). The cristae of these mitochondria are parallel to each other (Figs. 5, 6).

In addition to the above-mentioned changes, the plasma membrane of the spermatid tail invaginates and retracts to form a tube which houses the flagellum (Figs. 1, 2, 3, 5). An oval-shaped annulus (Jensen's ring) of intermediate electron density is observed at the site where the invaginated plasma membrane is withdrawn posteriorly (Figs. 1, 2, 3). With further differentiation of the tail, this ring migrates posteriorly and marks the end of the middle piece (Fig. 6). In addition, a granular body is shown near the nucleus (Fig. 5). Apparently, fragments of this body move backward and assume intermitochondrial positions (Fig. 6). A mass of lipid droplets, which in very early sptermatids is scattered throughout the cytoplasm, surmounts the nuclear region (Fig. 6). Later, around one side of the nucleus, this mass together with other follicular elements, forms a cytoplasmic droplet (Fig. 7) which then migrates posteriorly to lie one side of the middle piece (Figs. 10, 13, 14).

With advanced differentiation of the sperm tail, the middle piece becomes well established. At this stage, the neck has become extremely short and no neck cylinder is present. The mitochondria form a sheath of about 3.9 μm, whose elements are arranged end-to-end (Figs. 7, 10, 14). In cross sections of the middle piece, 8 mitochondria could be observed with parallel cristae (Figs. 11, 12, 14). Interposed among the mitochondria, at almost equal distances in the middle piece, are intermitochondrial plaques (Figs. 7, 10, 14). These plaques are either oval or triangular (Figs. 10, 11); they are not limited by membranes. They are composed of granular-textured electron dense material, and approximate to the mitochondrial sheath in their thickness (Figs. 7, 10). Longitudinal sections of the middle piece reveal four (Fig. 10) and five (Fig. 7) of these plaques. At this stage, nine dense (coarse) fibers are observed as a ring between either the mitochondrial sheath and the 9 doublets of the axoneme (Fig. 11), or between the fibrous sheath and the axonome (Fig. 12). These fibers are of variable diameters, and are oval in cross section (Figs. 11, 12, 14). Their sides facing the doublets are slightly concave. Each dense fiber is made of a cortex which is separated from a medulla by an electron-translucent rim (Figs. 12, 14). These fibers are present in the anterior two thirds of the middle piece only; they are absent in the rest of the tail. Two of these fibers (arrow heads, Fig. 12) are twice as thick as the rest, and they are the only ones which appear in the posterior region of the middle piece (Figs. 9, 14c). These

observed in different species of squamata and chelonia (Furieri 1970) and in few species of iguanidae (Furieri 1974). In the lizard *Tropidurus torquatus,* a poorly differentiated neck and lack of intermitochondrial plaques were reported (Da Cruz-Landim and Da Cruz-Hoffling 1977). Finally, results similar to those obtained by Hamilton and Fawcett (1968) were described by Camps and Bargallo (1977) in the snake *Elaphe scalaris.*

This paper reports the development of the sperm tail in the Jordanian lizard *Agama stellio.* The differentiation of the sperm head of this reptile was the subject of a previous investigation (Al-Hajj *et al.* 1987).

Materials and Methods

Adult males of *A. stellio* were collected monthly in the period extending from February 1983 to August 1983. Six very small (1×1×1 mm) pieces of testes and epididymes were fixed in a buffered mixture of 5% glutaraldehyde and 8% paraformaldehyde to which sucrose (3%) was added. Specimens were washed in 0.1 M sodium cacodylate buffer at pH 7.4, post-fixed in 1% osmium tetroxide for 1 hr at 4°C, washed in the same buffer, dehydrated in a graded series of acetone, and finally infiltrated and embedded in Spur's resin. Ultrathin sections (60 mm thick) were cut using a Sorval MT-2B ultramicrotome with a diamond knife, picked up on uncoated copper grids, stained in uranyl acetate and lead citrate, and examined in a Zeiss EM 10B electron microscope, at 60 KV.

Results

Concomitant with the changes which encompass nuclear and acrosomal differentiation of *A. stellio* sperm, simultaneous changes occur in the spermatid cytoplasm of this lizard, in preparation for tail differentiation. One of the early signs of this differentiation is the appearance of an implantation fossa in the posterior nuclear envelope, opposite to the site of acrosome development (Fig. 1). This starts as a small concavity which later deepens and becomes arch-shaped (Fig. 3). Lining the implantation fossa and assuming its contour is a fairly dense layer, called the basal plate. A connecting piece made of dense striated columns, links the head of the sperm with the anterior portion of the flagellum (Figs. 4, 14, inset of Fig. 6). This structure has a dense convex articular region, the capitulum (Figs. 4, 6, 14), which conforms to the concavity of the basal plate. The capitulum is made of homogeneous slightly electron dense material. A fine filamentous material traverses the space between the capitulum and the basal plate (Figs. 4, 14). Embedded in the concavity of the basal plate is a proximal centriole, which makes an angle of about 60° with the distal centriole (Figs. 2, 4).

are believed to be fibers 3 and 8. An extra dense fiber is associated with one singlet of the axoneme (Figs. 11, 14).

At the stage of mitochondrial grouping around the axoneme, a fibrous sheath, of high electron density is interposed between the mitochondrial sheath and the ring of the nine dense fibers (Figs. 6, 10, 12). The elements of this sheath are rib-like which run in a circular to spiral fashion (Figs. 7, 8, 9, 10), extending from the posterior two thirds of the middle piece (Figs. 6, 10, 14) to the end of the principal piece (Figs. 8, 14). Longitudinal sections of the principal piece show that the fibrous sheath comprises fibrous rings (Fig. 8) with hollow core (arrow heads in Figs. 6 and 9).

In addition to the mitochondrial sheath, dense fibers and fibrous sheath the middle piece consists of a core of nine doublets and two singlets of microtubules (Figs. 9, 9a, 14). Dynein arms extend from the α-subunit of each doublet. A sheath of light electron density surrounds the two singlets. Nine spokes radiate from this sheath towards the α-subunits of the doublets (Figs. 8, 9, 9a). An electron dense longitudinal column (cut in cross section) is associated with the α-subunit of each doublet (Figs. 11, 14A). The principal piece comprises the axonemal core and a fibrous sheath, surrounded by a plasma membrane (Figs. 8, 14). The end piece includes the same structures as the principal piece, except that it lacks a fibrous sheath (Figs. 9a, 14E).

Discussion

The tail of *A. stellio* sperm comprises three major regions: middle piece, principal piece, and end piece. A very short and simple neck links the tail with the head, as is found in the sperm of the lizard *T. torquatus* (Da Cruz-Landim and Da Cruz-Hoffling 1977). In this species, the neck lacks a neck cylinder, and is composed of a connecting piece, the elements of which are cross-striated. In addition, a basal plate immediately lines the implantation fossa, and is separated from the capitulum by fine filamentous material. This situation is similar to that in the neck region of mammalian sperm (Fawcett 1975). A basal plate has not been reported in the neck region of other reptilian sperm (Austin 1965, Kaplan *et al.* 1966, Hamilton and Fawcett 1968; Furieri 1970, 1974; Camps and Bargallo 1977; and Da Cruz-Landim and Da Cruz-Hoffling 1977). According to Austin (1965) a reduced neck cylinder of snake sperm could be homologous to the basal plate of mammalian sperm. The connecting piece in sperm of snakes lacks cross striations, as reported by Austin (1965) and Hamilton and Fawcett (1968).

The short and simply-structured neck of *A. stellio* sperm contrasts with the complex neck region found in sperm of other species of reptiles, particularly

snakes, thus far investigated (Austin 1965, Hamilton and Fawcett 1968, and Camps and Bargallo 1977). In these cases, sperm possess a neck cylinder in the form of a chamber with a wall of variable thickness. It is closed anteriorly, except in *L. getulus* and *C. constrictor* (Hamilton and Fawcett 1968) and houses the proximal centriole. Posteriorly, it forms a pocket into which fit the proximal ends of the fibrous sheath. Da Cruz-Landim and Da Cruz-Hoffling (1977) referred to a neck cylinder in sperm of the lizard *T. torquatus*, and from the information they presented (their Figs. 6a and 6b; p. 159), we believe that what they described as a neck cylinder is nothing more than a capitulum of the connecting piece. In addition to the previously-mentioned elements of the neck region of *A. stellio* sperm, a proximal centriole, as well as a distal one, at an angle of about 60°, are also present, as in the neck region of other reptilian sperm.

The middle piece of *A. stellio* sperm includes a mitochondrial sheath, a fibrous sheath, dense (coarse) fibers, and an axoneme. The mitochondrial sheath starts immediately behind the nucleus and terminates at the annulus. Unlike the highly contorted and spirally arranged mitochondria of the snake sperm tail (Austin 1965, and Hamilton and Fawcett 1968), those of *A. stellio* are of uniform thickness, regularly arranged, and run longitudinally. As first reported in the snakes *L. getulus* and *C. constrictor* (Hamilton and Fawcett 1968), the mitochondria of *A. stellio* middle piece are separated by intermitochondrial plaques. Similar results were reported in different species of squamata and chelonia (Furieri 1970), in few species of iguanids (Furieri 1974), and in the snake *E. scalaris* (Camps and Bargallo 1977). Surprisingly, such plaques were not observed among mitochondria of sperm from the lizard *T. torquatus* (Da Cruz-Landim and Da Cruz-Hoffling 1977).

The nine dense fibers observed between the mitochondrial sheath and the doublets of the middle piece in different reptilian sperm so far studied are also present in sperm of *A. stellio*. They form a ring, and each fiber lies external to a doublet. As reported by Austin (1965), Hamilton and Fawcett (1968), Furieri (1970), Camps and Bargallo (1977), fibers 3 and 8 are the thickest, and apparently become fused to the dorsal and ventral portions of the fibrous sheath. This contrasts with the situation in mammals, where these fibers are the smallest and the first to terminate. In the lizard *T. torquatus* (Da Cruz-Landim and Da Cruz Hoffling 1977), the dense fibers are present only in a short segment close to the neck cylinder, being absent in the rest of the flagellum. No reference to fibers 3 and 8 was reported by the latter authors.

A point worth mentioning is the presence of nine dense columns embedded as a ring among the nine doublets of the axoneme of *A. stellio* sperm. In addition, a dense column is associated with one of the two central singlets. This feature has not been reported in any reptilian sperm tail so far investigated. However, Austin (1965) and Hamilton and Fawcett (1968) described a row of nine slender dense

columns central to the triplets of the distal centriole, in addition to a single column close to one singlet. Whether there is any resemblance between the two cases or not remains to be evaluated. The functional significance of the dense fibers could be supportive, in addition to a suggested role in motility of the flagellum (Fawcett 1970).

The fibrous sheath of *A. stellio* sperm tail extends throughout most of the middle piece and the whole length of the principal piece. Although this is in agreement with other reports (Austin 1965, Hamilton and Fawcett 1968, Furieri 1970, 1974, Camps and Bargallo 1977), it contrasts with the situation in the sperm tail of lizard *T. torquatus* (Da Cruz-Landim and Da-Cruz-Hoffling 1977) where the fibrous sheath is found in the middle piece only. A generally accepted function attributed to the fibrous sheath is that it acts as a spring, due to its elastic structure, thus offering resistance to the contraction waves of the axoneme.

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Explanation of the Figures

Fig. 1. An early spermatid of *Agama stellio*. The cell is slightly elongated, the flagellum (F) is developed, mitochondria (M) are scattered in the cytoplasm. An implantation fossa (IF) at the caudal end of the oval nucleus (N) is seen in this section. (A) acrosome; 11,500X.

Fig. 2. A later stage of sperm differentiation. Mitochondria (M) are gathered to one side of the cytoplasm. The proximal centriole (Pc) is embedded in the implantation fossa at the caudal end of the nucleus (N), while the distal centriole (Dc) is seen in continuity with the tail filaments. (F) flagellum, (Mt) microtubules; 10,000X.

Fig. 3. Enlargement of the neck region of the spermatid, showing the basal plate (Bp), part of the proximal, centriole (Pc), and the distal centriole (Dc) in continuity with the axoneme (Ax). Part of the connecting piece (Cp) is seen. (An) annulus, (F) flagellum, (Mt) microtubules; 22,100X.

Fig. 4. Another enlargement of the neck region showing the capitulum (Ca) joining the basal plate (bp) and the connecting piece (Cp). (An) annulus, (Pc) proximal centriole; 38,780X.

Fig. 5. A spermatid showing the formation of the middle piece, with the mitochondria (M) arranged in a sheath around the outer dense fibers (DF) and the inner axoneme (Ax). (FS) fibrous sheath, (Gb) granular body, (Pc) proximal centriole; 11,600X.

Fig. 6. An advanced stage of spermiogenesis. Mitochondria (M) are arranged in a sheath around the axoneme in the middle piece. Arrow head indicates the presence of a hollow core in the ribs of the fibrous sheath (FS). A higher magnification of the proximal centriole area (Pc) is clearly shown in the inset of this figure. The segmented columns (Sc) are connecting the capitulum (Cp) to the dense fibers (DF) below. (Ld) lipid droplet, (N) nucleus. Fig. 6, 15,380X; inset, 42,000X.

Fig. 7. A stage of spermiogenesis at which the middle piece is completely formed, and only a cytoplasmic droplet (CD) is present around the nucleus (N). Intermitochondrial plaques (IMP) are present in between the mitochondria of the mitochondrial sheath (MS). The ribs of the fibrous sheath (FS) are reaching about midway in the middle piece; 15,380X.

Fig. 8. A longitudinal section of the principal piece of the tail. The fibrous sheath (FS) is present at this level surrounding the axoneme (Ax). A cross section of this area is demonstrated in the inset of this figure. Fig. 8, 21,000X; inset, 46,440X.

Fig. 9. A cross section of the middle piece. The dense fibers just ended at this point, where only portions of two dense fibers are seen at arrows. The fibrous sheath (FS) is surrounding the axoneme (Ax). The hollow core inside the ribs of the fibrous sheath is seen at arrow heads; 30,000X.

Fig. 9a. Cross section of the end piece of the tail showing the axoneme (Ax) surrounded by the cell membrane (CM); 41,100X.

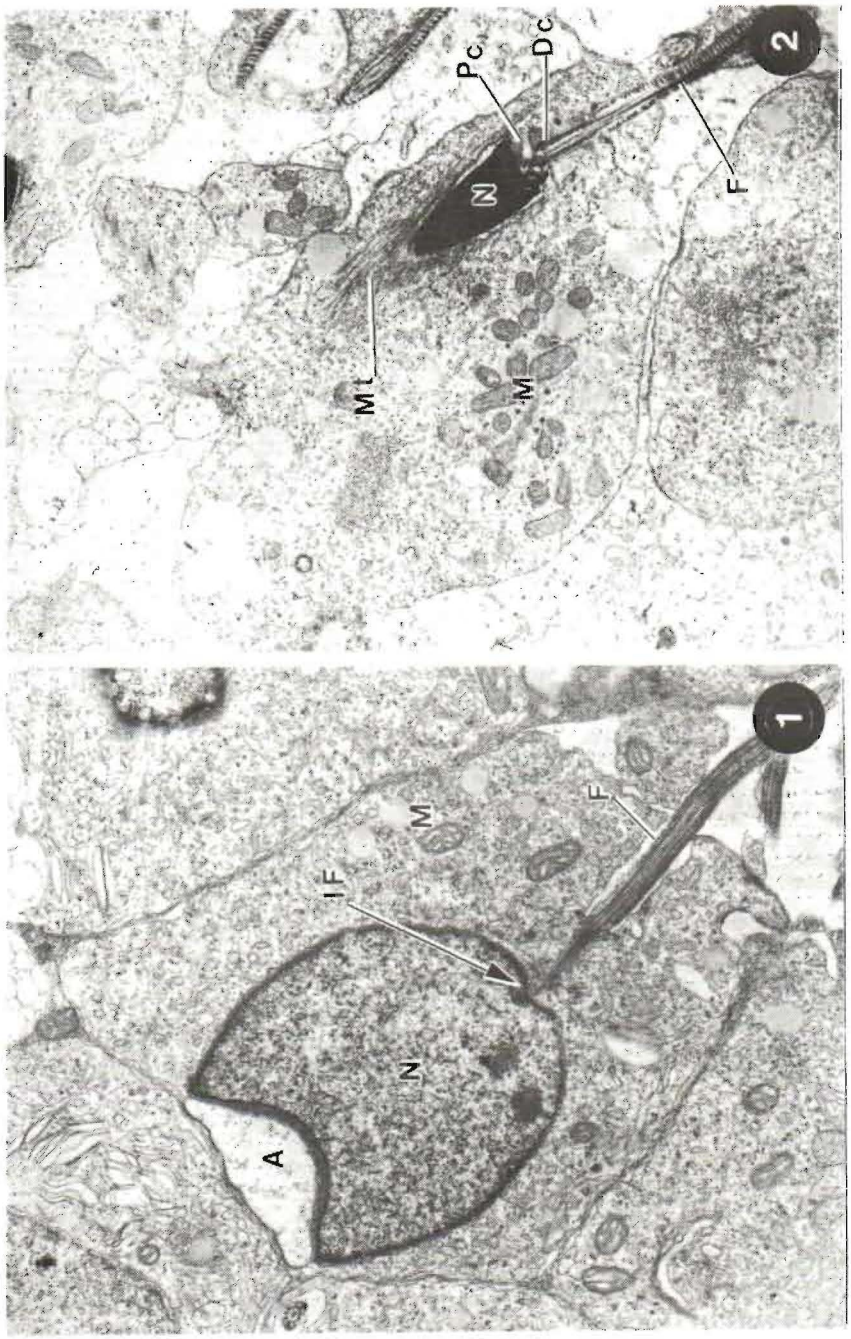
Fig. 10. Late spermatid showing a cytoplasmic droplet (CD) which has migrated backward to the end of the middle piece. The dense fibers (DF) are apposed to the fibrous sheath (FS), seen in this figure in the area enclosed between the two arrows. (Ax) axoneme, (MS) mitochondrial sheath, (N) nucleus, (Pc) proximal centriole; 14,000X.

Fig. 11. Cross section of the middle piece showing the nine doublets of the axoneme (Ax) with a dense amorphous mass in close attachment to each doublet (arrows). Nine dense fibers (DF) are surrounding the axoneme doublets. An extra dense fiber lies in the center of the axoneme beside the two singlets. (IMP) intermitochondrial plaques, (MS) mitochondrial sheath; 57,570X.

Fig. 12. Cross section of the middle piece showing the dense fibers (DF) and the fibrous sheath (FS) around the axoneme (Ax), and inside the mitochondrial sheath (MS); 55,280X.

Fig. 13. Cross section of the middle piece with a cytoplasmic droplet (CD) on one side of the mitochondrial sheath (MS). (Ax) axoneme, (FS) fibrous sheath; 28,560X.

Fig. 14. Schematic drawing of sperm tail of *A. stellio* showing the posterior part of the nucleus (13). The neck region of the tail comprises a basal plate (12), a capitulum (11), striated columns of the connecting piece (9) and a proximal centriole (10). The middle piece comprises a central pair of microtubules (1), nine outer doublets of microtubules (4), nine dense (coarse) fibers (2), a mitochondrial sheath (5), intermitochondrial plaques (7) and a fibrous sheath (8). A cytoplasmic mass containing lipid droplets (6) is still attached to the sperm middle piece. Three cross sections (A, B, C) of this region are illustrated. The principal piece (D) comprises the axoneme (1, 4) and the fibrous sheath (8). The end piece (E) comprises only the axoneme (1, 4) surrounded by the cell membrane. The nine dense columns (3), present in between the nine doublets of the axoneme are only present in the uppermost region of the middle piece, and could only be illustrated in cross sections of this area (A).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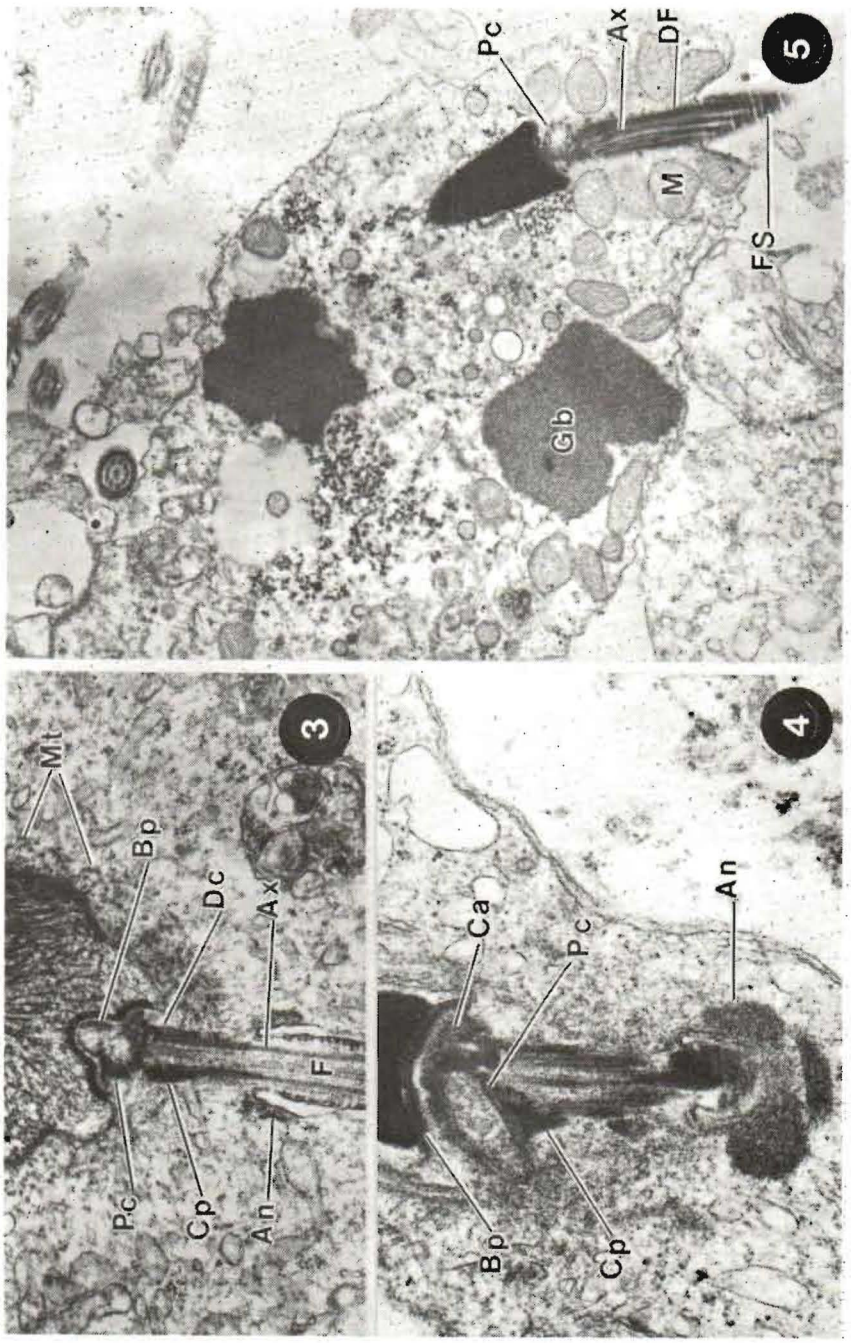
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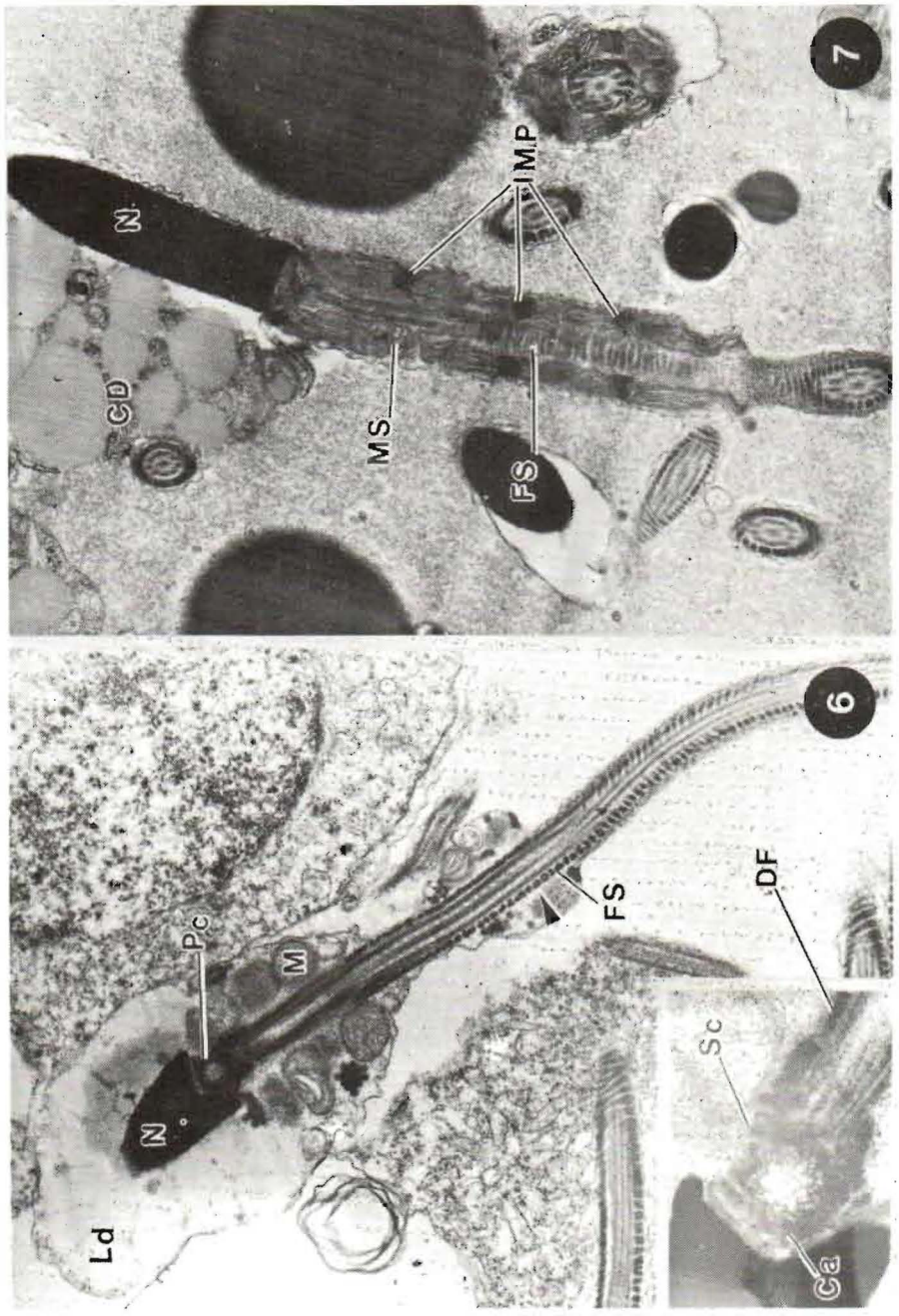
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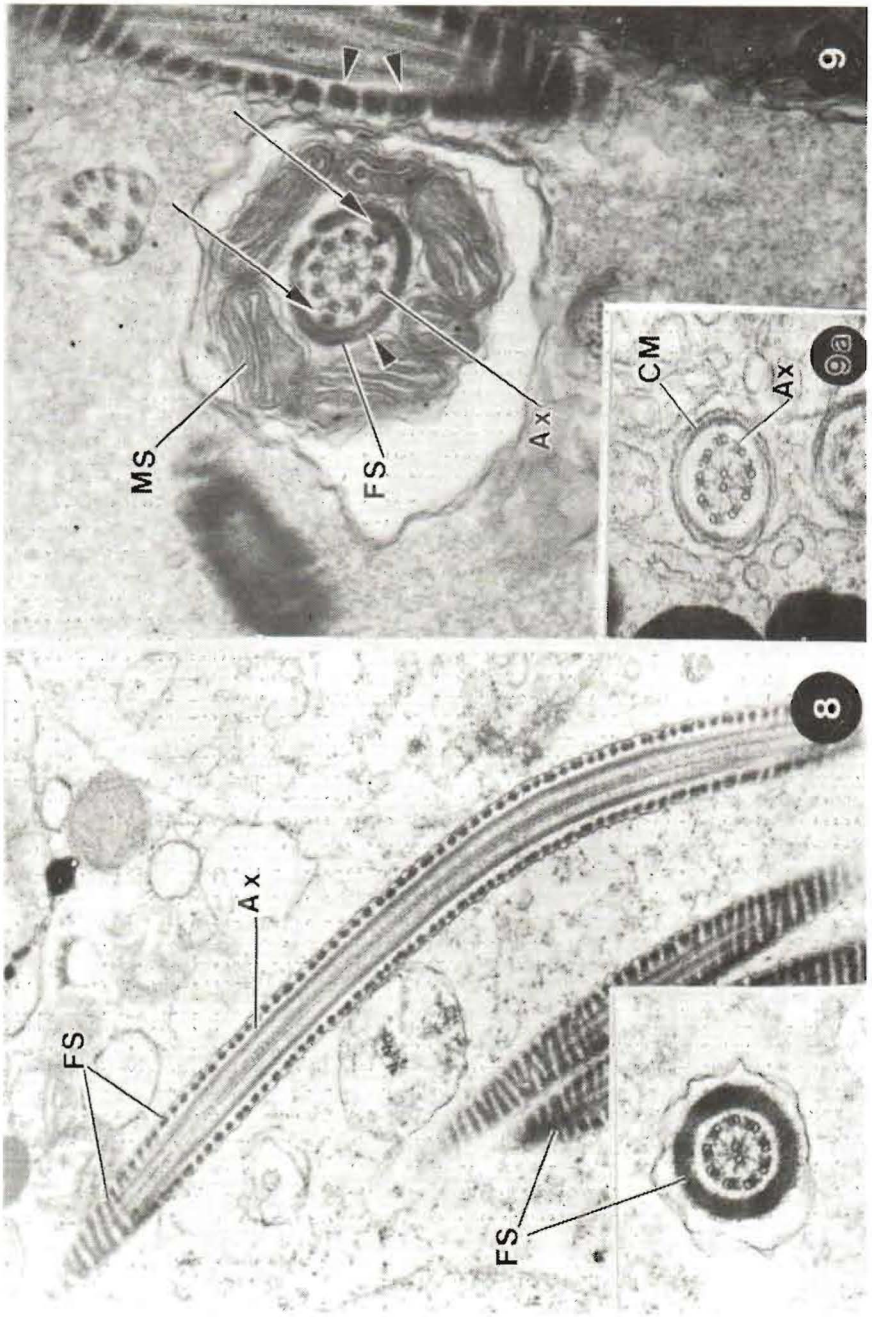
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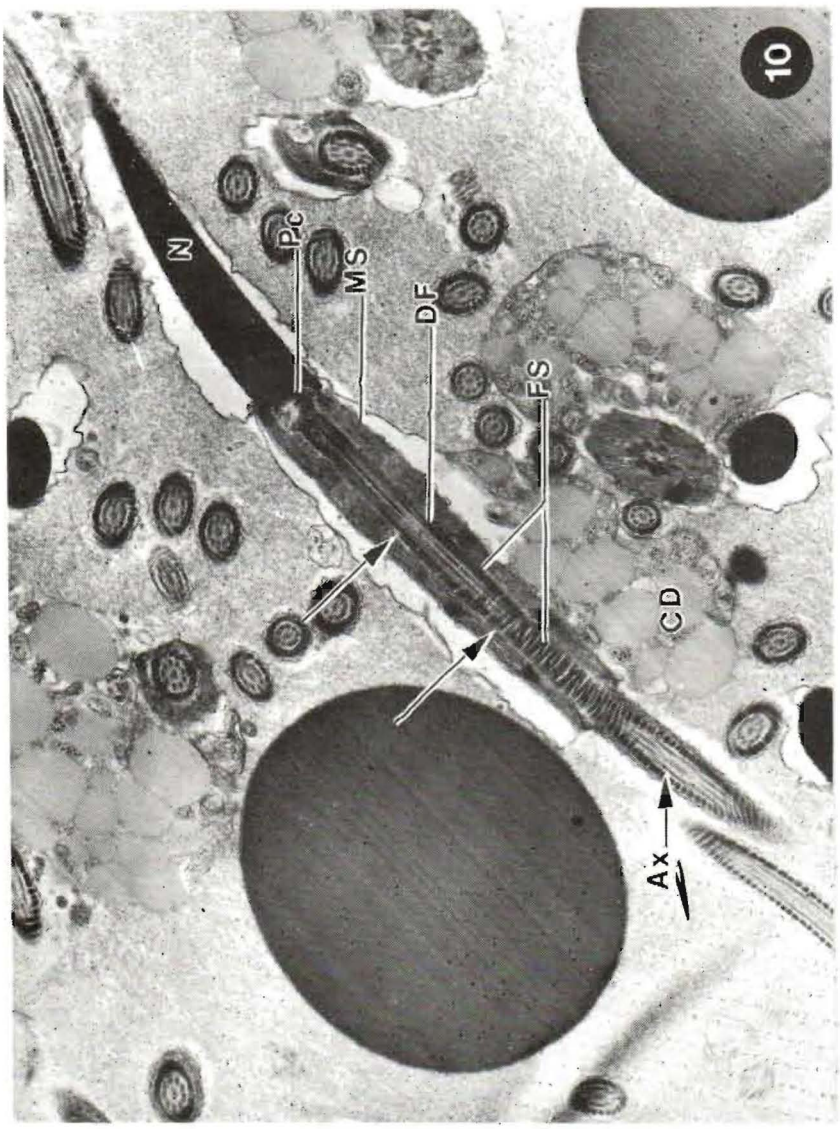
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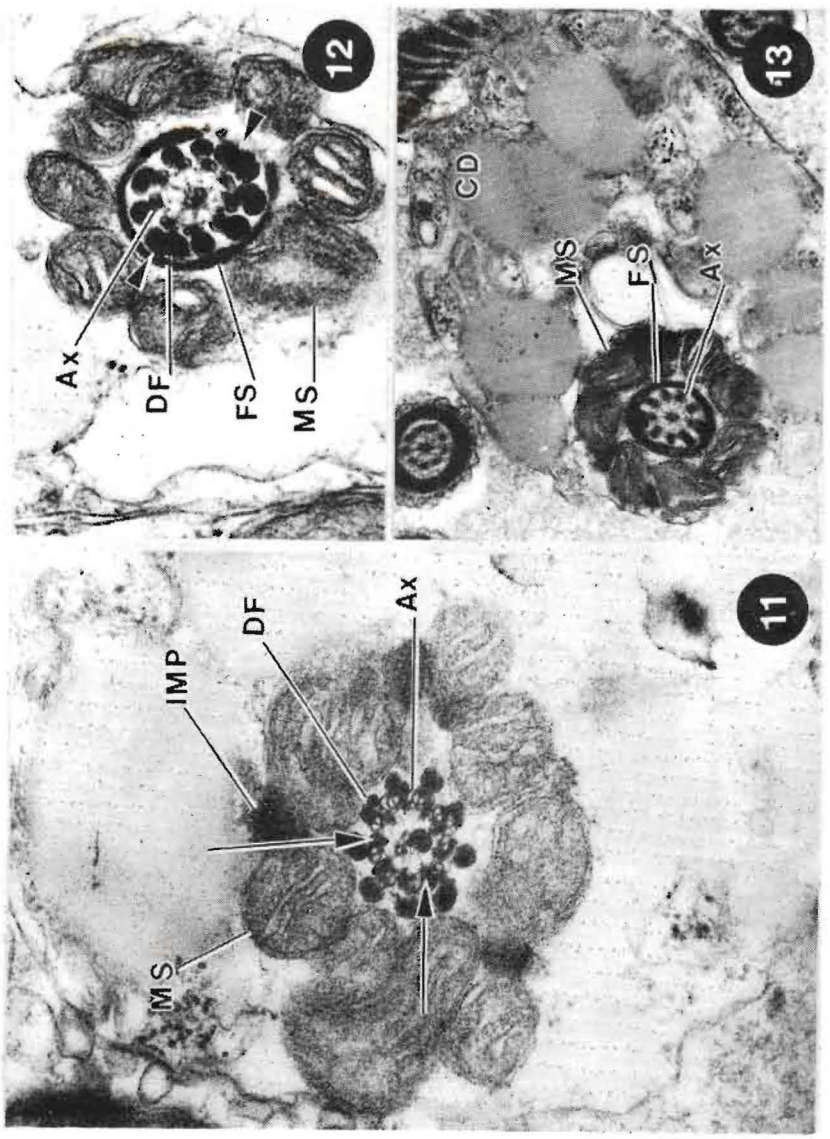
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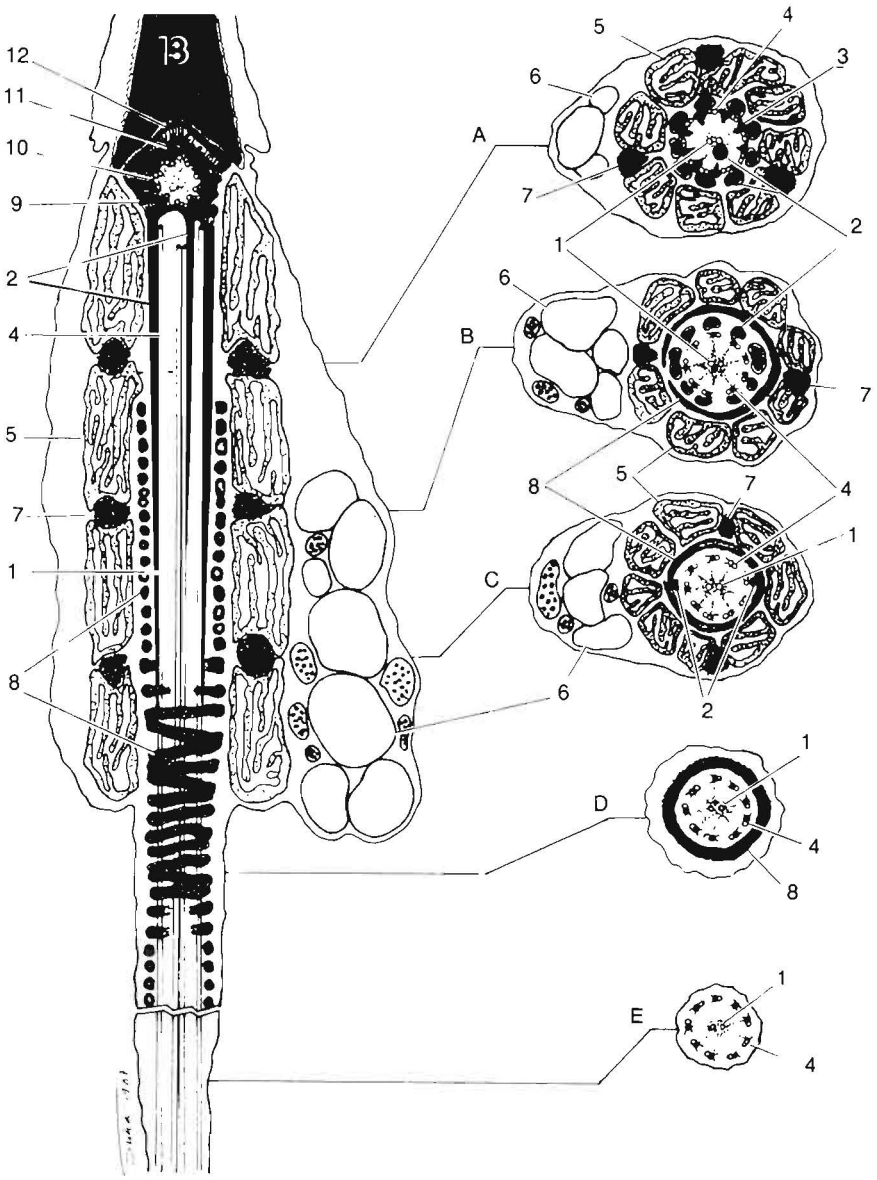

Fig. 14. Schematic Drawing of Sperm Tail of *A.stellio*

دراسة مجهرية الكترونية لتمايز ذيل الحيوان المنوي في السحلية الأردنية اجاما ستيليو

حميد الحاج و سهى جنكات و فهمي محمود

قسم العلوم الحياتية ـ الجامعة الأردنية ـ عمان ـ الأردن

يتكون ذيل الحيوان المنوي في السحلية الأردنية اجاما ستيليو من القطعة الوسطى، القطعة الأساسية، والقطعة النهائية، ويتصل الذيل بالنواة بعنق قصير جداً، يتألف من عقدة وقطعة موصلة ذات عناصر مخططة، وتتكون القطعة الوسطى من الغمد الميتوكوندريوني، والغمد الليفي، وتسعة خيوط غليظة، ومحور من تسعة أنيبيبات مضاعفة، وأنيبوبين منفردين. ويوجد ما بين الميتوكوندريا، التي تنتظم بشكل طولي، صفائح داكنة. ويتألف الغمد الليفي من حلقات تمتد من الثلثين الخلفيين للقطعة الوسطى حتى نهاية القطعة الأساسية، التي تتكون بدورها من المحور، إضافة للغمد الليفي. أما القطعة النهائية، فإنها تتكون من المحور فقط. ويحيط غشاء البلازما بجميع مكونات الذيل.