International Journal of Zoological Research

ISSN 1811-9778

Academic Journals Inc.

www.academicjournals.com

International Journal of Zoological Research 3 (2): 53-64, 2007
ISSN 1811-9778

Ultrastructure of Spermatozoa of the Freshwater Turtle *Mauremys caspica* (Chelonia, Reptilia)

O.A. Al-Dokhi, S.H. Al-Wasel and M. Mubarak
Department of Zoology, College of Science, King Saud University,
P.O. Box 2455, Riyadh 11451, Saudi Arabia

Abstract: Sperm ultrastructure of the freshwater turtle *Mauremys caspica* (*M. caspica*) has not yet been published. Therefore, the present study was carried out to elucidate the ultrastructural details of the sperm of this species. The spermatozoa of *M. caspica* are filiform in shape with a curved head measuring 7.9 μm. Acrosome complex (acrosomal cap and subacrosomal material) measures 3.7 μm, nucleus is 6.5 μm (2.3 μm acrosomal region, 4.2 μm post-acrosomal region) in length and midpiece and tail (principal and end pieces) are maximally of 4.2 μm and 35.8-38.6 μm length, successively. Diameter of the different tail pieces is gradually diminished from midpiece (1.2 μm) toward principal (0.5 μm) and end (0.3 μm) pieces. The anterior tapered nuclear tip is caped with the conical sheath formed by the acrosome and the subacrosomal material. Endonuclear canals (2-3) extend from the nuclear rostrum deep into the nucleus. Midpiece encompasses a mitochondrial sheath (total of 40 mitochondria) encircling the axonemal complex but lacks a fibrous sheath. Mitochondria are rounded having concentric cristae and central dense bodies. Annulus is present as a dense ring marking the distal end of midpiece. The principal tail piece consists of a typical axoneme (9+2 pattern of microtubular arrangement) surrounded by the fibrous sheath and plasma membrane. The axoneme is surrounded only by the plasma membrane in the end piece which lacks the fibrous sheath. The present findings were discussed and compared with that reported in other reptilian species.

Key words: Turtles, spermatozoon, ultrastructure, chelonia, reptilia

INTRODUCTION

Spermatogenesis and spermiogenesis were extensively investigated in mammalian species. Spermiogenesis and sperm ultrastructure of reptiles have relatively received a limited attention. Many reptile families including turtle species still await adequate description.

Ultrastructure of spermiogenesis and mature sperm of turtle species was the focus of some previous studies (Yasuzumi and Yasuda, 1968; Upadhyay and Guraya, 1970; Callard *et al*., 1976; Mahmoud *et al*., 1985; Sprando and Russell, 1988; Hale *et al*., 1989; Hess *et al*., 1991).

Our previous reports (Al-Dokhi and Al-Wasel, 2001a, b; 2002) were the first to describe the process of spermiogenesis in the fresh water turtle *Mauremys caspica* (*M. caspica*). We are not aware of any study describing the ultrastructure of *M. caspica* mature spermatozoon. Hence, the present study was undertaken to elucidate the ultrastructural morpholoyy of the mature sperm of this turtle species. The morphological comparative aspects that characterize spermiogenesis of *M. caspica* from other reptiles are also indicated.

Corresponding Author: Dr. O.A. Al-Dokhi, Department of Zoology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia Tel: 009661-4675923 Fax: 009661-4678514

MATERIALS AND METHODS

Ten adult males of the freshwater turtle *M. caspica* were collected during the period of sexual activity (April-June, -----), from Al-Hassa Oasis (25 30' N, 49 40' E), east province of Saudi Arabia. After dissection, the testes were removed, diced into small pieces then immediately fixed in Karnovsky's fixative (Karnovsky, 1965) in 0.1 M sodium cacodylate buffer (pH 7.2) for at least 4 h at 4°C. Tissues were then washed in the same buffer and subsequently post-fixed in 2% osmium tetroxide in 0.1 M sodium cacodylate buffer (pH 7.2) for 2 h. This was followed by dehydration, clearing and then embedding in Agar100 epoxy resin. Ultrathin sections were double stained by uranyl acetate and lead citrate and examined in a transmission electron microscope (JEOL 100 CX) operating at 60-80 kV.

RESULTS

Spermatozoa of *M. caspica* are filiform (Fig. 1A and B) and approximately of 50 μm (mean of ten) length. Head is curved and measures 7.9 μm. The acrosome complex is 3.7 μm long, nucleus is 6.5 μm in length (2.3 μm acrosomal region, 4.2 μm post-acrosomal region) and midpiece is of 4.2 μm lenghth. Flagellum behind the midpiece (from light microscopy) measures 35.8-38.6 μm. The spermatozoon is circular in cross section throughout its length with a maximum diameter, at the midpiece of approximately 1.2 μm. Diameters of principal piece and midpiece approximate 0.5 and 0.3 μm, successively.

Acrosomal Complex

The acrosomal complex is located in the anterior most region of the head, it consists of a conical elongated membrane-bound vesicle. The acrosome is curved and envelops the tapered anterior end of the nucleus (Fig. 2A and B). The acrosomal vesicle has a homogeneous and moderately opaque matrix (Fig. 3). The base of the acrosomal complex rests on a widening region of the nucleus as distinct shoulder-like shape. The subacrosomal cone is a thin electron lucent layer underlying the acrosome and investing the tapered anterior end of the nucleus. The subacrosomal cone lacks a membrane of its own forming a thick relatively pale layer. The acrosome is circular at its base and becomes increasingly depressed in transverse section near the apical tip (Fig. 4A-F).

Nucleus

Nucleus is elongate, slightly curved and circular in cross section. It tapers to a point anteriorly within the acrosomal complex (Fig. 5A and B). Two to three narrow endonuclear canals are present, they are twisted helically around each other and lined by the nuclear envelope (Fig. 6). The chromatin is condensed and highly electron dense and is invested by nuclear and plasma membrane. The cross section of the nucleus is circular throughout. Basely the nucleus has a compact shallow depression, the nuclear fossa that houses the proximal centriole (Fig. 7).

Neck Region and Centrioles

The neck region connects the tail with the sperm head. The neck region is the junction between the nucleus and the midpiece; it contains the proximal centriole, which consists of nine triplets with two central singlets. This region consists of two centrioles and an extensive deposit of pericentriolar material. The proximal centriole is positioned centrally, parallel to the base of the nucleus. The distal centriole constitutes the basal body of the axoneme. The long axis of the distal centriole, which forms the basal body of the flagellum, is in the long axis of the axoneme. The distal centriole lies perpendicular to the proximal centriole. An extensive deposit of perinuclear material extends from the nuclear fossa to cover the proximal centriole.

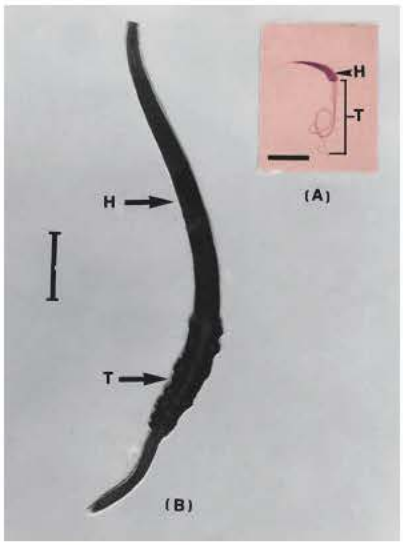

Fig. 1: A) Light micrograph demonstrating *M. caspica* mature epididymal sperm. Note the curved head (H) and the noticeably long tail (T). H and E stain. Scale bar = 20 µm. B) Transmission electron micrograph revealing the filiform shape of *M. caspica* mature epididymal spermatozoon. Head (H) is curved and tapered anteriorly. The tail (T) (midpiece and part of principal piece) is seen extending from the posterior nuclear extremity. Scale bar = 5 µm

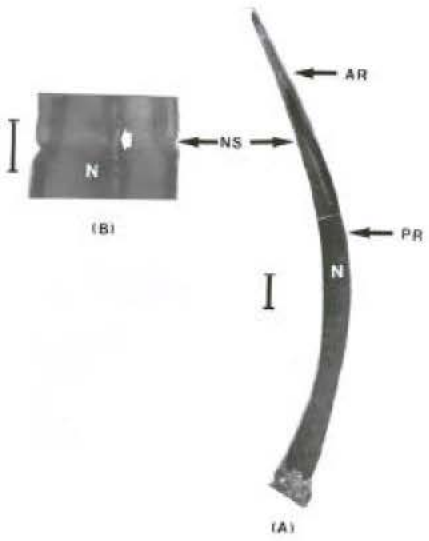

Fig. 2: A) Acrosomal complex at the anterior head region. The acrosome is curved and envelops the tapered nuclear rostrum (acrosomal region, AR) and the rest of the nucleus (N) has no acrosomal envelope (post-acrosomal region, PR). Scale bar = 2 µm. B) The junction between the acrosomal and post-acrosomal regions at the nuclear shelf (NS) in the epididymal sperm of *M. caspica*. Arrow indicates an endonuclear canal runing into the nucleus (N). Scale bar = 0.3 µm

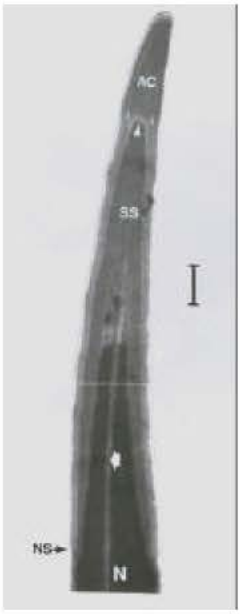

Fig. 3: Acrosomal complex (AC) containing a homogenous matrix of a moderate electron density. Acrosome forms a cap over the nucleus (N). Thin arrows indicate the lateral arms of the acrosomal vesicle which terminally rest on the nuclear shelf (NS). Note the endonuclear canal (thick arrow). Subacrosomal space (SS). Scale bar = 0.2 µm

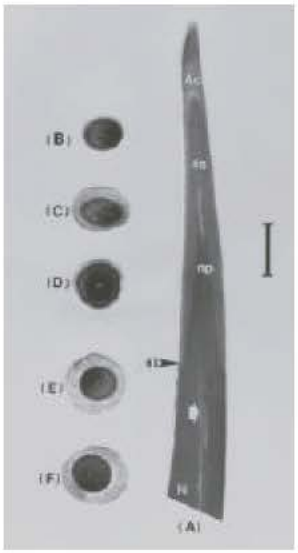

Fig. 4: A) A longitudinal section through the anterior head region of *M. caspica* mature sperm. Note the acrosomal complex (AC), subacrosomal space (SS), nuclear prolongate (nP), nucleus (N) and nuclear shelf (NS). Arrow indicates the endonuclear canals. Scale bar = 0.5 µm. B-F) Transeverse sections through the acrosomal complex and nucleus. Note the double tinny electron lucent spots in each transeverse section which represent the endonuclear canals. Scale bar = 0.5 µm

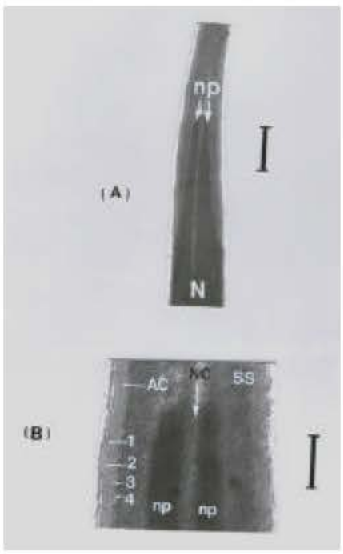

Fig. 5: A) Nucleus (N) is tapered anteriorly within the acrosomal complex to form the nuclear prolongate (np). Scale bar = 0.5 µm. B) Nuclear prolongate (np) is extended into the acrosomal complex (AC). Note the successive membranes ensheathing the nuclear prolongate (from outside to inside): (1) Plasma membrane, (2) Outer acrosomal membrane, (3) Inner acrosomal membrane, (4) Nuclear membrane. Note the nuclear canal (NC) and the subacrosomal space (SS). Scale bar = 0.1 µm

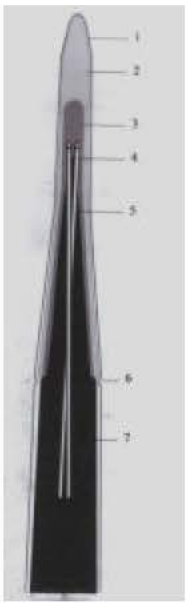

Fig. 6: Diagrammatic representation of the endonuclear canal (4) extending from the subacrosomal space (3) and directed posteriorly deep into the nucleus (7). 1.plasma membrane, (2) Acrosome, (5) Nuclear prolongate, (6) Nuclear shelf

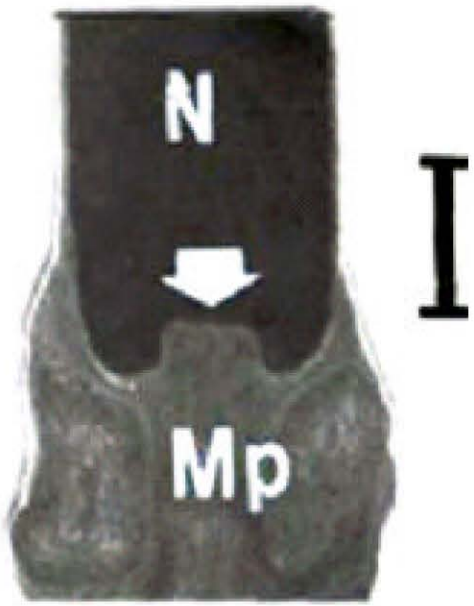

Fig. 7: Nuclear fossa (arrow) appears as a shallow depression at the base of the nucleus (N). Proximal part of the midpiece (Mp). Scale bar = 1 μm

Midpiece

Midpiece consists of the neck region and the axoneme, surrounded by the mitochondria which are arranged in eight rows of five crowns, in a total of 40 mitochondria (Fig. 8 and 9). It is much shorter than the head and terminates posteriorly at a distinct annulus.

The flagellum is formed by the axoneme, extending throughout the remaining length of the spermatozoon. It is organized in the usual 9+2 microtubules pattern, surrounded by the peripheral fibers.

At the distal extremity of the midpiece, a small dense ring, the annulus, with circular to oval shape in cross section, define the terminus of the midpiece. It is closely applied to the inner surface of the plasma membrane.

Principal Piece

The initial portion of the principal piece can be identified by a reduction in the diameter of the spermatozoon. It begins immediately behind the annulus, This is the longest portion of the flagellum, consisting of the axoneme surrounded by the fibrous sheath, cytoplasm and plasma membrane (Fig. 10A and B). In this region the axoneme presents the 9+2 microtubules pattern. The diameter of the principal piece is gradually diminishes, as a result of decreasing cytoplasm between the fibrous sheath and the plasma membrane and a reduction in the width of the fibrous sheath.

End Piece

The axoneme extends behind the fibrous sheath as an end piece. The end piece is referred to the very slender tail of the spermatozoon. It consists of the axoneme and the plasma membrane (Fig. 11A and B). The fibrous sheath is, by definition, absent from the end piece. The pattern of the microtubules is maintained although their diameter is very reduced.

Basing on the aforementioned data, complete schematic diagramm of *M. caspica* mature spermatozoon was visualized (Fig. 12).

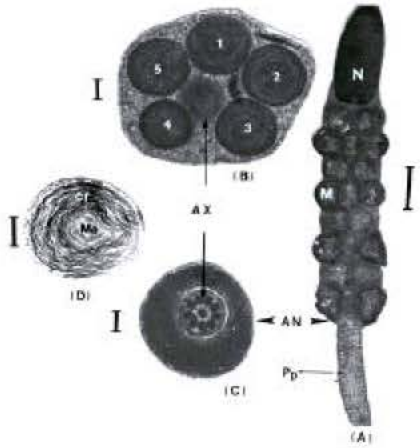

Fig. 8: A) Longitudinal section through the midpiece of *M. caspica* epididymal sperm. Midpiece encompasses the axoneme which is ensheathed by mitochondria (Mi). Principal piece (PP). Scale bar = 1 µm. B) Transeverse section through the proximal portion of midpiece. Five mitochondria (1-5) in each crown encircle the axoneme (AX). Scale bar = 0.1 µm. C) Transeverse section through the most distal portion of midpiece at the level of the annulus (AN) which encicle the axoneme (AX). Scale bar = 0.1 µm. D) Higher magnification of a rounded mitochondrion in the midpiece showing the concentric cristae (Cr) (lamellated appearance). Note the intramitochondrial central dense material (Ma). Scale bar = 0.1 µm

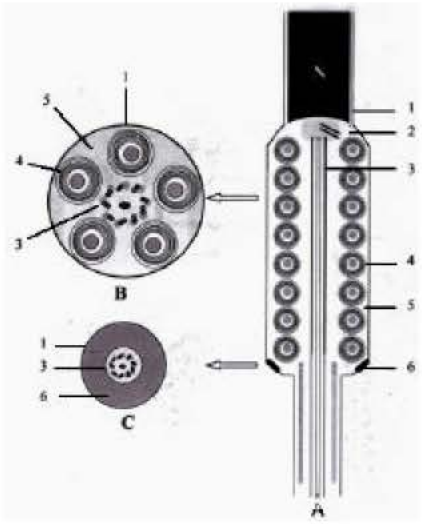

Fig. 9: A) Diagrammatic representation of a longitudinal section in the midpiece. Note the eight raws of mitochondria (4). (1) Plasma membrane, (2) Centriole, (3) Axoneme, (5) Cytoplasmic droplet and (6) Terminal ring (annulus). B) Diagrammatic representation of a transverse section in the midpiece Note the five mitochondria (4) ensheathing the axoneme (3) in each crown. (1) Plasma membrane and (5) Cytoplasmic droplet. C) Diagrammatic representation of a transverse section in the most distal part of the midpiece The dense terminal ring (annulus) (6) defines the terminus of the midpiece and encricling the axoneme (3)

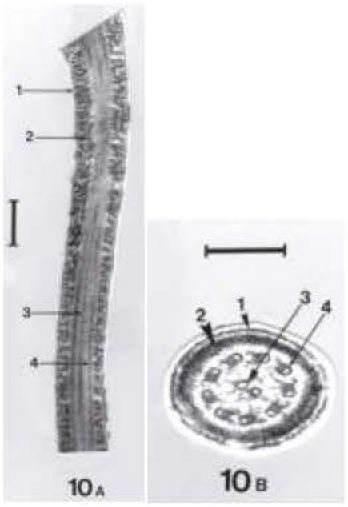

Fig. 10: A) A longitudinal section through the principal piece of the mature epididymal sperm. Principal piece consists of axoneme which involves central microtubules (3) and peripheral microtubules (4). The fibrous sheath (2) and plasma membrane (1) surround the axoneme. Scale bar = 0.1 μm. B) A transerverse section in the principal piece. The axoneme consists of two central singlets (3) and nine peripheral doublets (4) (9+2 pattern). Fibrous sheath (2) and plasma membrane (1) surround the axonemal structure. Scale bar = 0.1 μm

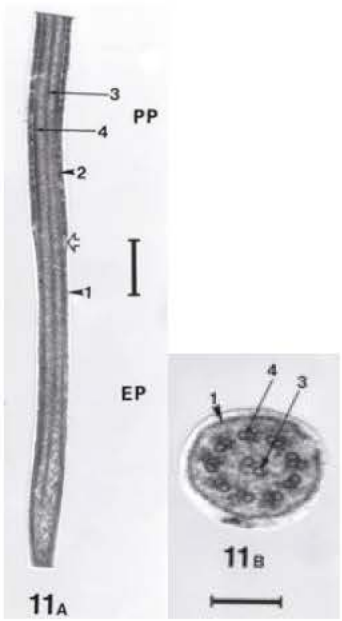

Fig. 11: A) A longitudinal section through the terminal part of the principal piece (PP) and proximal part of the end piece (EP) indicated by the arrow. (1) plasma membrane, (2) fibrous sheath, (3) central microtubules and (4) peripheral microtubules. Scale bar = 0.1 μm. B) A transerverse section in the end piece consisting of the axoneme (central microtubules, 3 and peripheral microtubules, 4) and surrounded only the plasma membrane (1). Scale bar = 0.1 μm

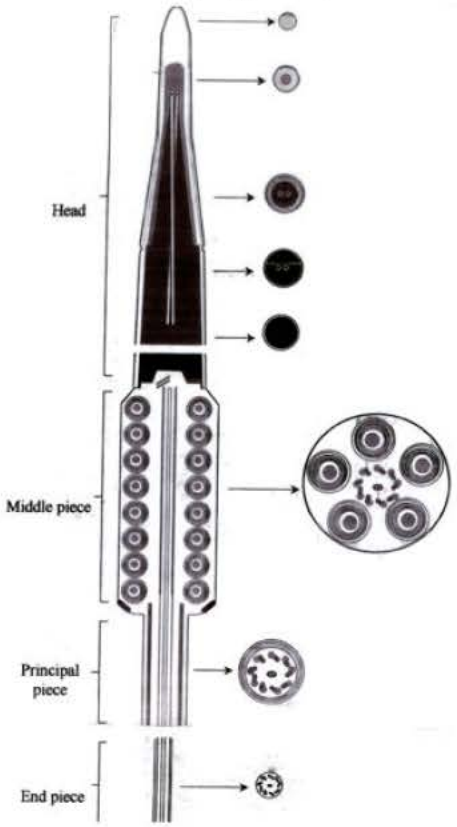

Fig. 12: Diagrammatic representation of the general ultrastructure of *M. caspica* mature spermatozoon

DISCUSSION

Mature spermatozoa of the turtle *M. caspica* are filiform in shape with a curved head. Conforming with the general sperm head features of turtles (Furieri, 1970; Hess *et al*., 1991; Jamieson, 1995), *M. caspica* sperm head exhibites a pointed form of acrosome, subacrosomal cone and a taperred nuclear tip of a cylindroid elongate nucleus, which lies mostly within this cone. The head of mature sperm of *M. caspica* is typically cylindrical in shape as evidenced by the regular rounded appearance of the transverse successive sections. This sperm head configuration is exhibited by some reptile species, such as the lizard *Lacerta vivipara* (Courtens and Depeiges, 1985), the snake *Boiga irregularis* (Oliver *et al*., 1996) and the Australian sphenodon, *Sphenodon punctatus* (Healy and Jamieson, 1994). Also, flattening and attenuation of the terminal part of the sperm head is a feature of some other reptilian species, including the lizard *Progona barbata* and *Varanus gouldii* (Oliver *et al*., 1996) and the scincus *Nangura spinosa* (Jamieson and Scheltinga, 1993). Nucleus in the sperm head is elongate and bent towards its anterior end to assume a similar nuclear shape to that reported in the lizard *Lacerta taurca* (Butler and Gabri, 1984), the scincus *Scincus mitranus* (Al-Dokhi, 1996), *Agama sinsita* (Ismail and Dehlawi, 1994), the crocodile *Crocodylus johnstoni* (Jamieson *et al*., 1997) and the Australian sphenodon *S. punctatus* (Healy and Jamieson, 1994). The rounded nuclear shoulders and the paracrystalline substructure of the subacrosomal cone which are basic features of squamates sperm head (Oliver *et al*., 1996) are lacking in *M. caspica* sperm.

Similar to *Chelonia* species, crocodilians and squamates (Jamieson *et al*., 1997), nucleus of *M. caspica* sperm is noticeably elongate. Endonuclear canals in *M. caspica* sperm originate near the area

separating the acrosome and nucleus and extend posteriorly deep in the nucleus. Like other turtles and also sphendon (Healy and Jamieson, 1992, 1994; Jamieson and Healy, 1992; Jamieson, 1995) the endonuclear canals of *M. caspica* sperm form a loose spiral around each other. Such enonuclear canals are missing in squamates (lizards and snakes) sperm (Healy and Jamieson, 1994; Oliver *et al*., 1996). However, its existence was reported in certain reptiles, such as *Pseudomys scripta, Caiman crocodylus*, *C. johnstoni* and *S. punctatus* (Saita *et al*., 1987; Sprando and Russel, 1988; Healy and Jamieson, 1994; Jamieson *et al*., 1997). Endonuclear canals in *M. caspica* mature sperm are multiple (2-3 in number) resembling those in some other reptilian species, e.g., *Crocodylus johnstoni* (Jamieson *et al*., 1997). On the contrary, a single endonuclear canal was reported by the latter authors in *C. crocodylus* sperm and moreover a single endonuclear canal appears to be a basic feature of amniotes sperm (Jamieson, 1995). The present variation in number of endonuclear canals from sperm to another is probably related to the plan of section through the sperm head. The function of the endonuclear canals is not well-identified, however it is supposed that it has a supporting role for the sperm nucleus which is noticeably long in *M. caspica* sperm. Also, endonuclear canals probably contain a putative perforatorial material (Jamieson *et al*., 1997).

Tail of *M. caspica* mature sperm is over 40 μm in length and considered comparatively long. Concerning the different tail components of *M. caspica* sperm, midpiece exhibits no dense bodies inbetween its motochondria, these intermitochondrial dense structures were described in some other species (Jamieson and Scheltinga, 1993; Oliver *et al*., 1996). However, only intramitochondrial dense structures, representing matrical densities, do exist and bear similarity to that observed in *C. johnstoni* sperm (Jamieson *et al*., 1997). We have managed to determine the total number of mitochondria in the midpiece of *M. caspica* sperm through observation of a large number of transverse and longitudinal sections of this tail piece. Multiplying the maximum number of mitochondria in transverse sections by the maximum number of mitochondrial rays appears in longitudinal sections led us to conclude that *M. caspica* mature sperm possesses 40 (5×8) mitochondria. As the case in other turtles (Furieri, 1970; Healy and Jamieson, 1992) mitochondria in midpiece of *M. caspica* mature spermatozoon assume a peculair morphology judged by its rounded profiles, the concentric cristae (lammellated appeaance) and the central intramitochondrial structures. This characteristic mitochondrial morphology appears to exist only in a few reptilian species, such as the Australian sphenedon *S. punctatus* (Healy and Jamieson, 1994) and the crocodile *C. johnstoni* (Jamieson *et al*., 1997). Additionally, arrangement pattern of mitochondrial cristae is regarded as a differential criterion for mature sperms, for instance that of squamates sperms are linear (Oliver *et al*., 1996).

As the condition in other turtles (Jamieson *et al*., 1997), fibrous sheath is absent in the midpiece of *M. caspica* mature sperm and its existence is only confined to the principal piece. Extension of the fibrous sheath through both midpiece and principal piece of mature sperm tail was reported in squamates (Healy and Jamieson, 1992; Jamieson and Scheltinga, 1993; Oliver *et al*., 1996). This fibrous sheath extension in squamates sperms was considered as the most significant criterion differentiating squamates from other reptilian species (Oliver *et al*., 1996). We suggest that annulus (terminal ring) is responsible for formation of the fibrous sheath material in *M. caspica* mature sperm. This suggestion is supported by two observations, the first is the location of the proximal end of the fibrous sheath in a close proximity to the annulus site. The second is the presence of a fine dense filament connecting the annulus to the fibrous sheath which probably indicate that the latter is an accumulation of products produced by the annulus.

Principal and end tail pieces of *M. caspica* mature sperm follow the typical structure reported in other turtles and also squamates (Jamieson and Scheltinga, 1993; Jamieson, 1995; Jamieson *et al*., 1997). The principal piece is built-up of a 9+2 axoneme surrounded by a fibrous sheath and plasma membrane and extended as the end piece which lacks the fibrous sheath.

ACKNOWLEDGMENTS

This study was supported by a grant from Research Center-College of Science, King Saud University, Project No. (St/ Zoo/ 1419/ 01). We gratefully acknowledge this support.

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. 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. Egyp. German Soc. Zool., 36(B): 93-106.

Al-Dokhi, O. 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., 15A: 355-366.

Al-Dokhi, O. and S. Al-Wasel, 2002. Ultrastructure of spermiogenesis in the freshwater turtle *Maurymes caspica* (Chelonia, Reptilia), III: The sperm tail formation. J. Union Arab Biol. Zool., 18A: 327-341.

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

Callard, I.P., G.V. Callard and V. Lance, 1976. Seasonal changes in testicular struture and function and the effects of gonadotropins in the freshwater turtle, *Chrysemys picta*. Gen. Comp. Endocrinol., 30: 347-356.

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

Furieri, P., 1970. Sperm Morphology in Some Reptiles, Squamata and Chelonia. In: Comparative Spermatology. Baccetti, B. (Ed.), pp: 115-143. Academic Press (New York), pp: 573.

Hale, D.W., B.G. Hanks, J.W. Bickham and I.F. Greenbaum, 1989. Centeriolar lenghth variability in testicular cells from side-necked turtles. J. Submicroscopic Cytol. Pathol., 21: 211-214.

Healy, J.M. and B.G.M. Jamieson, 1992. Ultrastructure of the spermatozoon of the tuatara (*Sphenodon punctatus*) and its relevance to the relationship of the sphenodontida. Philosophical Transactions of the Royal Society of London Series Biological Sciences, 205: 193-235.

Healy, J.M. and B.G.M. Jamieson, 1994. The ultrastructure of spermatogenesis and epididymal spermatozoa of the tutara *Sphenodon punctatus* (Sphenodontida, Amniota). Philosophical Transactions of the Royal Society of London Series Biological Sciences, B 344: 187-199.

Hess, R.A., R.G. Thurston and D.H. Gist, 1991. Ultrastructure of the turtle spermatozoon. Anatomical Record, 229: 473-481.

Ismail, M.F. and G.Y. Dehlawi, 1994. Ultrastructure of the spermiogenesis of Saudian reptiles. The sperm differentiation in *Agama sinaita*. Proceedings of the Zoological Society, Egypt, 25: 83-94.

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 Transactions of the Royal Society of London Series B, 335: 207-219.

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

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.), Memoires du Museum National dHistoire Naturelle, Paris, 166: 359-383.

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.

Karnovsky, M.J., 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J. Cell Biol., 27: 137A.

Mahmoud, I.Y., R.V. Cyrus, T.M. Bennett, M.J. Woller and D.M. Montag, 1985. Ultrastructural changes in the testis of the snapping turtle, *Chelydra serpentina* in relation to plasma testosterone, delta 5-3 beta-hydroxysteroid dehydrogenase and cholestrol. Gen. Comp. Endocrinol., 57: 454-464.

Oliver, S.C., B.G.M. Jamieson and D.M. Scheltinga, 1996. The ultrastructure of spermatozoon of squamata. II. Agamidae, Colubridae, Elapidae and Boidae (Reptilia). Herpetologica, 52: 216-241.

Saita, A., M. Comazzi and A. Perrotta, 1987. Electron microscope study of spermiogenesis in *Caiman crocodilus*. L. Boll. Zool., 4: 307-318.

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

Upadhyay, S.N. and S.S. Guraya, 1970. Histochemical observations on the acrosome formation in the turtle spermiogenesis. Acta Morphologica Neerlando-Scandinavica, 8: 79-84.

Yasuzumi, G. and M. Yasuda, 1986. Spermatogenesis in animals as revealed by electron microscopy. 18. Fine structure of developing spermatids of the japanese freshwater turtle fixed with potassium permanganate. Zeitschrift fur Zellforschung und Mikroskopische Anatomie, 1: 18-33.