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## Electron Microscopic Study of Sperm Head Differentiation in the Arabian Horned Viper *Cerastes cerastes* (Squamata, Reptilia)

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**Abstract:** In the present study some aspects of the ultrastructure of sperm head differentiation in the Arabian horned viper *Cerastes cerastes*, were described. These include the development of the acrosomal vesicle, aggregation of the acrosomal granules, formation of the subacrosomal nuclear space, nuclear elongation and the role of microtubules manchette. The manchette participates in nuclear shaping and organelle movement. During head differentiation the fine granular chromatin of early spermatid is gradually replaced by highly condensed contents. The chromatin material condenses into a curved elongated head of the full-differentiated sperm.

**Key words:** Head, spermatid, spermiogenesis, squamata, sperm differentiation

### INTRODUCTION

Spermiogenesis is the process of sperm differentiation. Only a few literatures available on spermiogenesis of the Arabian reptiles<sup>[1-15]</sup>. Reviewing of these literatures revealed variations in the process of spermiogenesis among Arabian reptiles species. Apparently, there is no published work on the spermiogenesis of the Arabian horned viper *Cerastes cerastes*. Therefore, the present study is intended to provide new data on sperm head differentiation in the Arabian horned viper *Cerastes cerastes*.

### MATERIALS AND METHODS

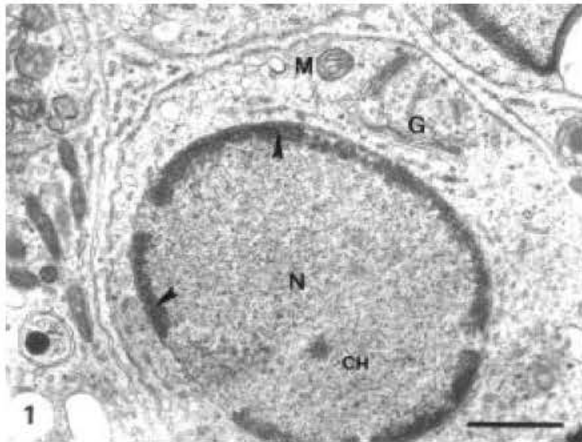
Four adult males of the Arabian horned viper *Cerastes cerastes* were collected during April and May (period of sexual activity) 2002, from Althumamah region (25° 10' N, 46° 50' E), north-east of the city of Riyadh, Saudi Arabia. After decapitation, the snakes were dissected and their testes were removed and chopped into appropriate small pieces that were immediately fixed by immersion in 3% buffered glutaraldehyde (0.1 M sodium cacodylate buffer at pH 7.2) for at least 4 h at 4°C. The fixed tissue specimens were washed in the same buffer and then were post-fixed in 1% osmium tetroxide (OsO<sub>4</sub>) in 0.1 M sodium cacodylate buffer (pH 7.2) for 2 h and then were washed in buffer and kept in the same buffer overnight. Subsequent dehydration of the fixed tissues was done in ascending grades of ethanol before final

embedding in epon/araldite mixture. Thin sections were double stained with uranyl acetate and lead citrate and were examined by a transmission electron microscope (Jeol, 100 CX) operating at 80 kv.

### RESULTS

Early spermatids were spherical with centrally located oval or round nuclei which had evenly distributed fine granular euchromatin and occasional clumped heterochromatin. Golgi complex is well-developed and composed of flattened cisternae and many associated round vesicles. Mitochondria with linear cristae are distributed randomly in the cytoplasm, some are present in the vicinity of Golgi complex. Free ribosomes and lipid droplets were randomly distributed throughout cytoplasm (Fig. 1).

Many microvesicles were originate from Golgi complex which later form proacrosomal vesicle. Subsequently larger acrosomal vesicle is formed in close proximity to spermatid nucleus. With growing of the acrosomal vesicle, a cup shaped proximal nuclear depression was resulted to lodge the vesicle. Some microvesicles were occasionally seen inside the acrosomal vesicle. The subacrosomal nuclear space with dense material nature, was developed between the nucleus and the acrosomal vesicle (Fig. 2). As differentiation progressed the nucleus and the attached acrosomal vesicle were displaced forward till the acrosomal vesicle membrane became opposed to the spermatid plasma membrane.

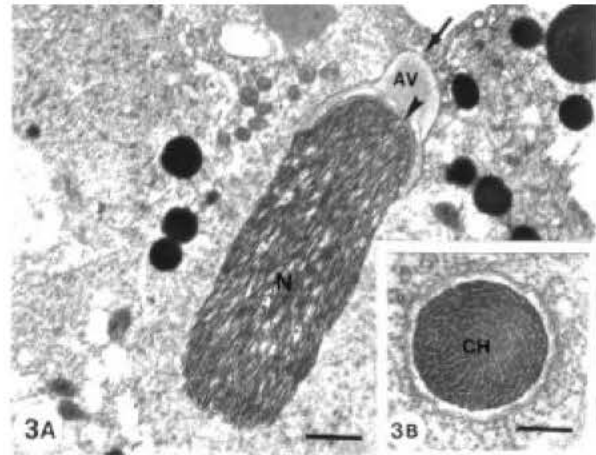


**Fig. 1:** Transverse section through an early spermatid showing the centrally located round nucleus (N), the evenly distributed fine euchromatin (CH) with dense patches of heterochromatin (arrows heads). Note the well developed Golgi body (G) in close proximity to the nucleus (N) and the prospective nuclear depression. Note also the well developed mitochondria (M). (Scale bar= 0.5  $\mu$ m)



**Fig. 2:** A section through an early spermatid showing a large acrosomal vesicle (AV) formed as a result of microvesicles coalescence. The cup shaped proximal nuclear depression lodges the newly formed acrosomal vesicle (AV). Vesicles with electron-dense granules (arrow) are occasionally seen inside the acrosomal vesicle. (Scale bar= 0.5  $\mu$ m)

As differentiation proceeds, the chromatin becomes first denser at the periphery of the nucleus, this marks the start of chromatin condensation. The nucleus commences



**Fig. 3A:** A longitudinal section of spermatid. The lodged acrosomal vesicle is obviously pushed against the spermatid plasma membrane (arrow). Note the acrosomal vesicle (AV) which overlies a well developed subacrosomal nuclear space (arrow head). At the beginning of condensation, chromatin is seen as closely packed coarse filaments which form an interconnected meshwork at the elongating nucleus (N). (Scale bar= 0.5  $\mu$ m)

**B:** A transverse section through a middle stage spermatid. Note the filamentous chromatin (CH) which form an interconnected meshwork. (Scale bar= 0.5  $\mu$ m)

elongation while its chromatin differentiates into granular chromatin. The fine chromatin are gradually converted to granular consistency in an anterior-posterior direction. As a consequence of the nuclear condensation, the chromatin appeared as a compact homogenous dense mass (Fig. 3A and B).

The microtubules manchette appear in the spermatid cytoplasm, just prior the start of chromatin granulation. With further differentiation numerous microtubules are increasingly present around the lateral sides of the nucleus and parallel to the longitudinal axis of the nucleus. Both longitudinal and circular microtubular manchettes were detected (Fig. 4A and B).

As differentiation proceeds the acrosomal vesicle was progressively flattened over the nuclear sides to form the acrosomal cap which covered almost the anterior two thirds of the spermatid nucleus. Thus, in the late spermatid, the highly condensed elongated cylindrical nucleus constituted the main portion of the head region and had well-organised acrosomal cap and subacrosomal nuclear cap (Fig. 5A,B and Fig. 6).

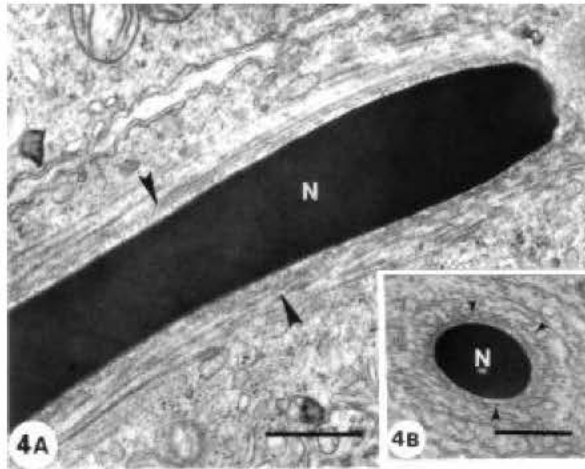


Fig. 4A: A longitudinal section of the head of late spermatid. The elongated nucleus (N) appears with completely condensed chromatin. Note also the longitudinal microtubules manchette (arrows heads) surrounding the nucleus. (Scale bar=0.5  $\mu\text{m}$ )

B: A transverse section through the head of the sperm. Note the condensed nucleus (N) and the microtubules manchette in cross sections (arrows heads). (Scale bar= 1  $\mu\text{m}$ )

An exceptional fine feature in the head region of late spermatids, was the presence of electron-dense bodies encircling spermatid head. These bodies were located external to nuclear envelope and acrosomal cap and subjacent to spermatid plasmalemma (Fig. 7).

### DISCUSSION

In general, sperm head differentiation in the Arabian horned viper *C. cerastes* would appear to be similar to that described in other reptilian species<sup>[16,24,6,17,8,9,11,13,14,18]</sup>

In some reptiles early spermatids are connected by cytoplasmic bridges. These intercellular bridges were not observed in the present study, however, they are not a consistent structure in all reptiles, since they were not reported in some species such as the sand skink *Scincus mitranus*<sup>[11]</sup> and the lizard *Senodactylus selvini*<sup>[2]</sup>. However, these bridges were described in some other species<sup>[9,20,34,18]</sup>. These bridges are an indication to incomplete meiotic divisions which result in formation of clusters of interconnected haploid spermatids<sup>[21,22]</sup>.

Mitochondria in *C. cerastes* early spermatids, were arranged randomly in the cytoplasm. Some reptiles show aggregations of mitochondria<sup>[8,9]</sup> and others reveal dispersed mitochondria<sup>[13]</sup> or at the periphery of cytoplasm as in *B. tuberculatus*<sup>[18]</sup>.

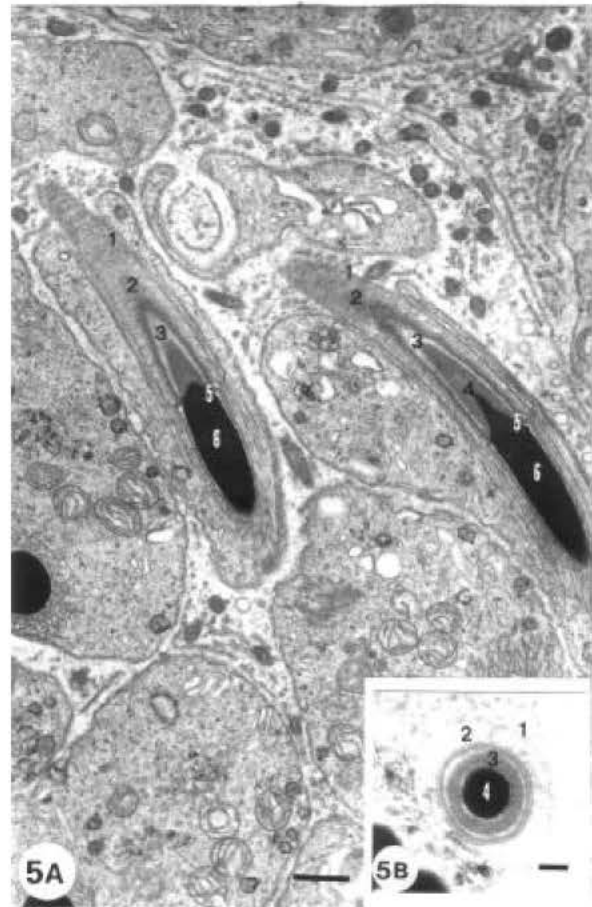


Fig. 5A: A longitudinal and transverse sections through the head of mature spermatozoon. The acrosome (2) is resting on the subacrosomal nuclear space (3). 1- plasma membrane, 2- acrosome, 3- the subacrosomal nuclear space, 4- the nucleus tip, 5- nuclear shelf, 6- nucleus. (Scale bar = 1  $\mu\text{m}$ )

B: A transverse section in the head of mature spermatozoon. 1- plasma membrane, 2- acrosome, 3- the subacrosomal nuclear space, 4- the nucleus. (Scale bar=0.5  $\mu\text{m}$ )

The anterior nuclear cup-shaped depression which partially housed the acrosomal vesicle is considered as a characteristic feature of the spermiogenesis in squamata<sup>[23,24,16,1,24,18]</sup>. This nuclear depression was not reported in the developing spermatids of mammals and birds<sup>[25,16,217,8,10]</sup>.

Chromatin condensation and nuclear elongation described in the present study are identical to that of squamates which follows a complex fibrous-lamellar

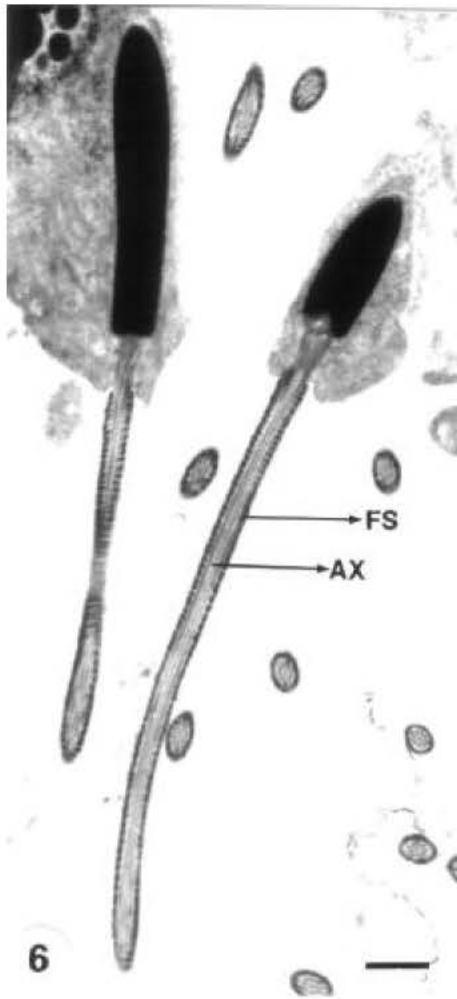


Fig. 6: A longitudinal section of the late formed sperm showing part of the head and the tail. The principal piece of the tail composed of the fibrous sheath (FS) and the axoneme (AX). (Scale bar= 1  $\mu$ m)

pattern<sup>[23,26,11,27,28,18]</sup>. Chromatin condensation may contribute in determining the nuclear shape through a specific pattern of aggregation of the nucleohistones and nucleoplasm proteins<sup>[29]</sup>. Other important roles of chromatin condensation is the substantial reduction of nuclear volume to streamline the cell and to preserve the genome<sup>[22]</sup>.

Both circular and longitudinal microtubules manchettes were observed in the developing spermatids of *C. cerastes*. The circular manchette was usually associated with nuclei having a filamentous chromatin and the longitudinal one with those having a condensed chromatin. Therefore, we postulate that the longitudinal

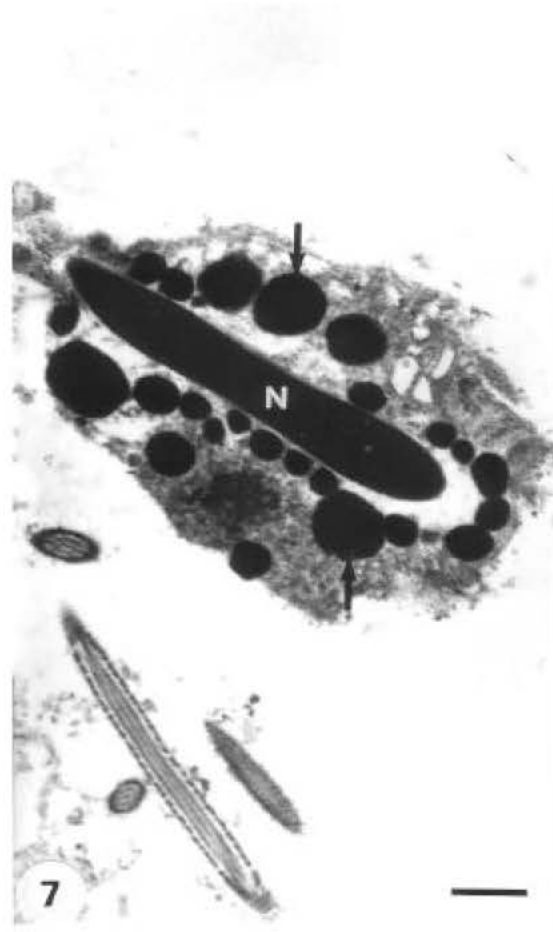


Fig. 7: A longitudinal section of the head of late formed sperm. The elongated nucleus (N) appears with completely condensed chromatin. Note the electron-dense bodies (arrows) encircling the spermatid head. (Scale bar= 1  $\mu$ m)

manchette originates as a consequence of rearrangement of the circular manchette which probably appears earlier. Also, longitudinal manchette is presumably only commenced with the appearance of condensed chromatin. Moreover, nuclear elongation and acquirement of the peculiar nuclear shape may result as a sequel of the progressive manchette reorientation<sup>[30,22]</sup>.

A remarkable feature in *C. cerastes* late spermatid was the electron-dense droplets encircling the head region. The electron density of these structures suggests a lipoid nature as proposed by Hamilton and Fawcett<sup>[31]</sup> or similar structures having the same nature. These structures were described as a tegosomal sheet in *Python sebae*<sup>[32]</sup>, *Lacerta vivipara*<sup>[16]</sup>, *Chalcides ocellatus*<sup>[4]</sup> and in *B. tuberculatus*<sup>[18]</sup>.

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