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Ultrastructural Studies on the Differentiation of the Sperm Head in the Sand Skink *Scincus mitranus* (Anderson 1871) (Squamata, Reptilia)

Othman A. Al-Dokhi

Zoology Department, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

ABSTRACT. Several aspects of spermiogenesis in the sand skink *Scincus mitranus* (Anderson 1871) were Ultrastructuraly investigated by transmission electron microscope. The ultrastructure of early spermatid, the development of the acrosomal vesicle and of the acrosomal granules were revealed. The nuclear changes including chromatin condensation and the roles of the microtubular manchette surrounding the nucleus and of the Golgi bodies were discussed.

There are many ultrastructural studies on spermiogenesis in lizards and several aspects of the process including the development of the acrosomal vesicle and the acrosomal granules, as well as chromatin condensation and the role of both of the Golgi bodies and of the microtubular manchette surrounding the nucleus were revealed in several lizards species (Butler and Gabri 1984, Courtens and Depeiges 1985, Al-Hajj *et al.* 1987, Dehlawi and Ismail 1990, 1991, Dehlawi *et al.* 1990, 1992 and Dehlawi 1992). However, these aspects of spermiogenesis vary from one lizard species to another (Yasuzumi 1974 and Dehlawi *et al.* 1992).

These aspects are yet to be studied ,in the sand skink *Scincus mitranus* (Anderson 1871). Hence, in the present study an attempt is being made to investigate the ultrastructure of the sperm head differentiation in *S. mitranus.*

Key Words. Lizards, Spermatid, Spermiogenesis, Acrosome, Sperm head differentiation.

Materials and Methods

Five adult males of the sand skink, *S. mitranus* were collected during April and May (period of sexual activity) from Thumamah, Saudi Arabia (25° 07'N, 46° 49'E). The animals were dissected and the testes were cut into small pieces that were immediately fixed in 4% gluteraldehyde solution in 0.1M sodium cacodylate buffer at pH 7.2 for at least 4 hours at 4°C. The tissues were then washed in the buffer and were subsequently post fixed in 2% osmium tetroxide in 0.1M sodium cacodylate buffer at pH 7.2 for 2 hr., then washed in the buffer overnight. This was followed by dehydration through a graded series of ethanol before embedding in Epon 812. Ultrathin sections were stained in uranyl acetate and lead citrate and were examined by a Jeol 100 CX electron microscope operating at 80 kv.

Results

The newly formed spermatids are spherical, with centrally located, oval or round nuclei. Their finely granular chromatin is evenly distributed in the nucleoplasm. The most notable constituents of the spermatid cytoplasm is a prominent Golgi complex with a few flattened cisternae and numerous associated vesicles (Fig. 1). Groups of mitochondria with tubular cristae are found in the vicinity of the Golgi complex, and few are dispersed in the cytoplasm. Single or rosettes of ribosomes are randomly distributed throughout the cytoplasm, but some are found in association with endoplasmic reticulum profiles. Scattered lipid droplets and vesicular bodies are also found, but distinct and organised microtubules are not present at this stage.

The Golgi complex increases in size with the development of the early spermatid. Later, it develops many microvesicles that coalesce into a alrger proacrosomal vesicle anterior to the nucleus and in close association with the nuclear envelope. Few mitochondria begin to aggregate near the acrosomal vesicle and a small depression is then formed on the surface of the nucleus posterior to the acrosomal vesicle. This cup-shaped nuclear depression increases in size and surrounds the newly formed acrosomal vesicle (Figs. 2 and 3).

Following the attachment of the acrosomal vesicle to the nuclear depression, acrosomal granules appear at the posterior part of the vesicle. First two small granules appear in close association with the posterior wall of the acrosomal vesicle and later a larger one appears between them. Also small round vesicle appears in the middle of the acrosomal vesicle, either free or attached to its membrane (Figs. 2 and 3), together with a subacrosomal nuclear space filled with an electron dense material between the nuclear envelope and the acrosomal vesicle (Fig. 3).

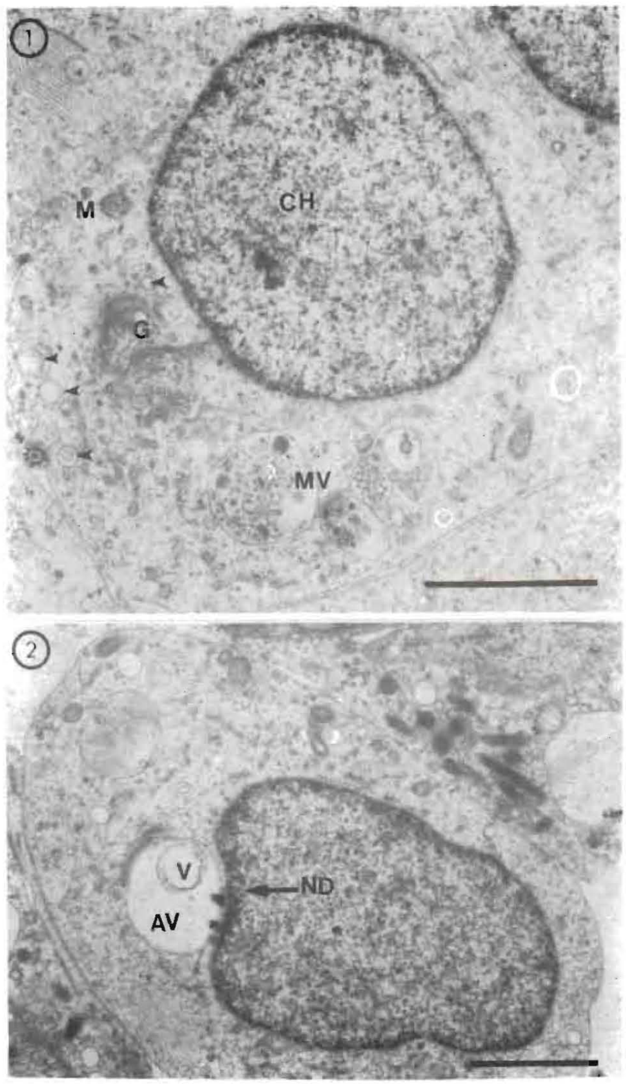
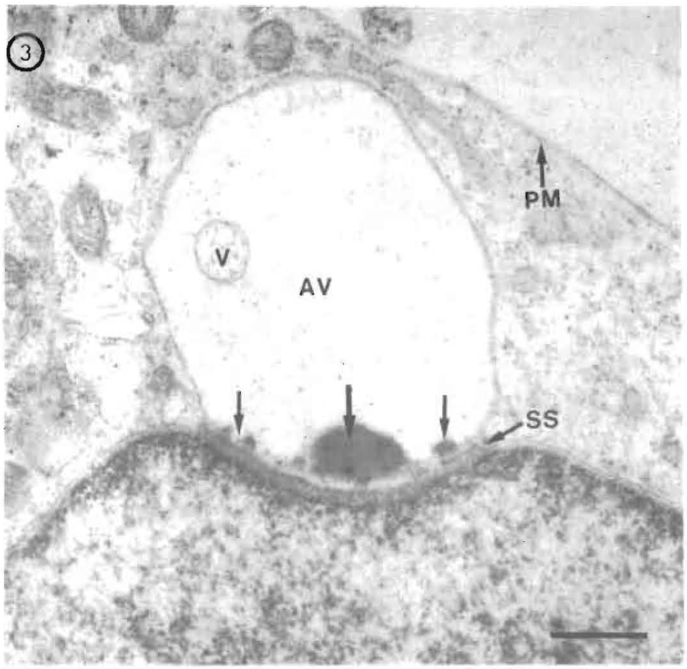


Fig. 1. A section through an early spermatid showing the nucleus with evenly distributed chromatin (CH), mitochondria (M) and multivesicular bodies (MV). Note the extensive Golgi body (G) and different sizes of vesicles (arrows heads) in close proximity to the nucleus and the prospective nuclear depression. Scale bar = 2μm.

Fig. 2. A section through an early spermatid showing the acrosomal veiscle (AV) and a round smaller vesicle (V). Note the formation of nuclear depression (ND) to lodge the acrosomal vesicle. Scale bar = 2μm.



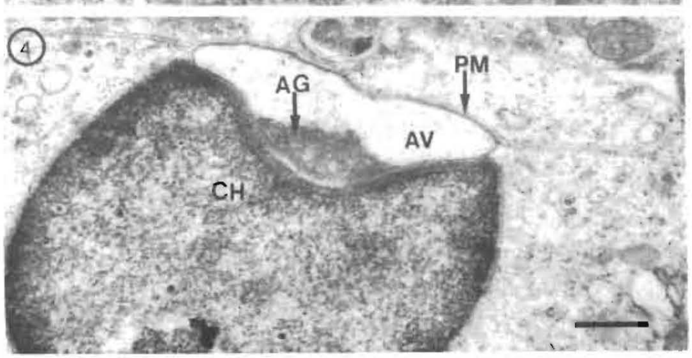


Fig. 3. A section through an early spermatid showing the acrosomal vesicle (AV) containing dense acrosomal granules of various sizes (arrows) and a round small vesicle (V). Note the subacrosomal space (SS) containing electron dense material and the acrosomal vesicle becomes closely opposed to the spermatid plasma membrane (PM). Scale bar = 2μm.

Fig. 4. A section through an early spermatid showing the anterior surface of the acrosome vesicle (AV) directly apposed to the spermatid plasma membrane (PM). Note the larger acrosomal granule (AG) and the coarse chromatin fibres (CH). Scale bar = 2μm.

As differentiation proceeds, the nucleus and the acrosomal vesicle move forward till the membrane of the vesicle and the plasma membrane of the spermatid become closely opposed (Fig. 4). Then the nucleus elongates pushing the acrosomal vesicle into the plasma membrane, which then extends and rest on the nucleus forming a cap with two lateral arms. The subacrosomal nuclear space and its opaque material become cap-shaped over the nuclear prolongation forming a subacrosomal nuclear cap (SNC) (Fig. 5). Both of the acrosomal cap (AC) and the SNC become gradually embedded in the Sertoli cell and surrounded by three membranes, that of the Sertoli cell, of the psermatid and of the acrosomal vesicle. The three membranes form the nuclear shelf (Figs. 8 and 9).

Numerous microtubules then appear around the nucleus in an anterior-posterior orientation, and eventually become associated with the nuclear membrane. Subsequently the nucleus starts to elongate and its chromatin appears as long coarse threads in an interior-posterior orientation. These threads become thicker and closely packed as an interconnected meshwork that eventually become completely condensed (Figs. 6, 7 and 9).

In late spermiogenesis, the sperm head appears as a curved structure with an AC surrounding the SNC and the nuclear prolongation. The elongate nucleus constitutes the main body of the cylindrically shaped head. It is completely opaque with no apparent substructures. The posterior end of the nucleus is concave, forming the prospective neck of the tail (Figs. 9, 10 and 11).

In the final stages of sperm head differentiation the microtubules manchette around the nucleus decrease markedly and eventually disappear.

Discussion

The developing early spermatids of *S. mitranus* are spherical cells with round nuclei and no cytoplasmic bridges. Similar observations were made by Dehlawi and Ismail (1991) in the lizard *Stenodactylus selvini*. However, cytoplasmic bridges connecting the developing spermatids were observed in some other lizard species (Da Cruz-Landim and Da Cruz-Hofling 1977, Al-Hajj *et al.* 1987, Dehlawi *et al.* 1990).

The cup-shaped depression of the nucleus of the spermatid observed in the present study is a characteristic of the Squamata (Clark 1967, Butler and Gabri 1984, Courtens and Depeiges 1985, Dehlawi and Ismail 1990, 1991 and Dehlawi 1992) and is absent from mammals (Fawcett 1975, 1991) and birds (Nagano 1962).

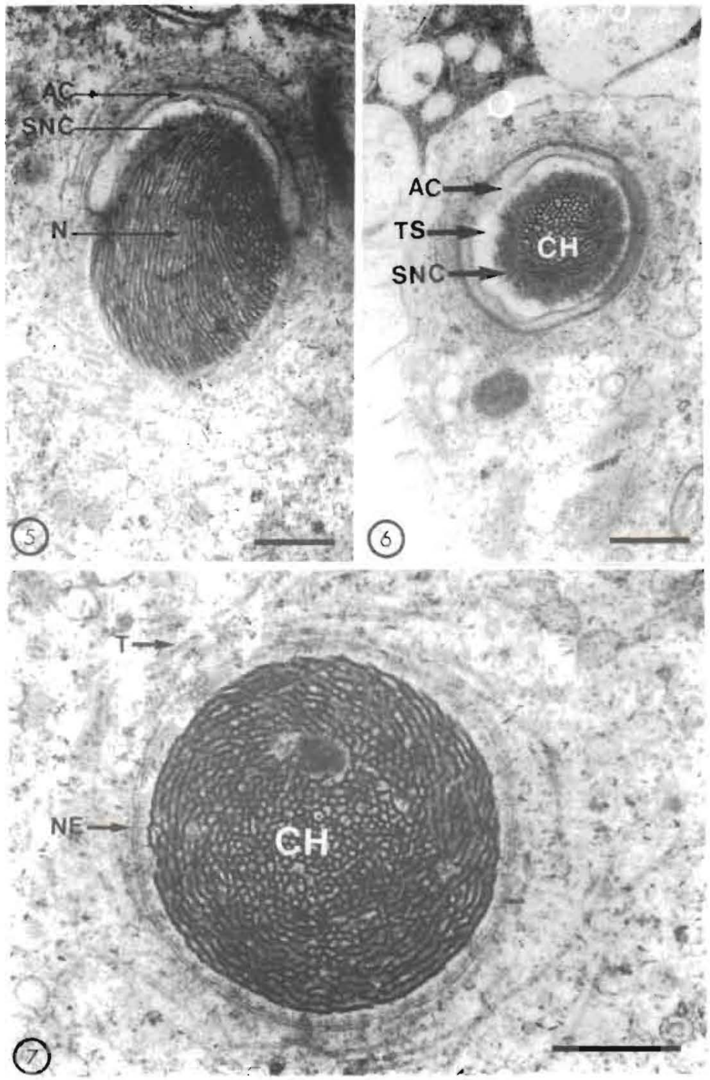


Fig. 5. A longitudinal section through an intermediate spermatid showing the nucleus (N), the anterior-posteriorly chromatin threads, the acrosomal cap (AC) and the subacrosomal nuclear cap (SNC) over the nucleus. Scale bar = 2μm.

Fig. 6. A transverse section through the acrosomal region of an intermediate spermatid showing the meshwork appearance of the chromatin threads (CH). Note the acrosome cap (AC) and the subacrosomal nuclear cap (SNC) separated by an electron transparent space (TS). Scale bar = 2μm.

Fig. 7. A transverse section through the nucleus of an intermediate spermatid showing the interconnected meshwork appearance of chromatin threads (CH). Note also the nuclear envelope (NE) and the dispersed microtubules (T) around the nucleus. Scale bar = 2μm.

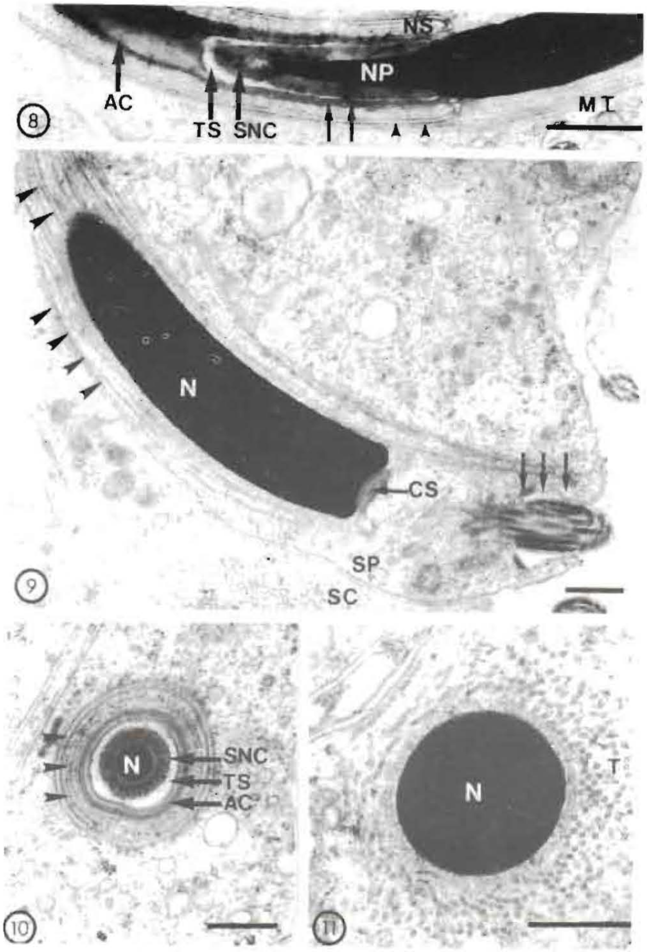


Fig. 8. A longitudinal section through a late spermatid showing the tip of the head. Note the acrosomal cap (AC), the subacrosomal nuclear cap (SNC), the transparent space (TS), the longitudinally oriented microtubules manchette (MT) surrounding both nucleus and Sertoli cell processes, nuclear prolongation (NP), nuclear shelf (NS), spermatid membranes (arrows) and Sertoli cell membranes (arrows heads). Scale bar = 2μm.

Fig. 9. A longitudinal section through a late spermatid (SP) showing elongated nucleus (N) with completely condensed chromatin and closely surrounded by microtubules manchette in a parallel fashion (arrows heads). Note the concave shaped posterior end of the nucleus (CS), the beginning of tail formation (arrows) and Sertoli cell (SC). Scale bar = 2μm.

Fig. 10. A transverse section through the acrosomal region of a late spermatid shwoing the nucleus (N) with completely condensed chromatin and surrounded by subacrosomal nuclear cap (SNC), electron transparent space (TS), acrosomal cap (AC) and the spermatid and Sertoli cell membranes (arrows heads). Scale bar = 2μm.

Fig. 11. A transverse section through the nucleus (N) of a late spermatid showing the completely condensed chromatin and microtubules (T) directly associated with it. Scale bar = 2μm.

Following the formation of the acrosomal vesicle, electron dense acrosomal granules appear apart from each other in the posterior wall of the acrosomal vesicle in *S. mitranus.* In *Agama adramitana,* however, the granules are attached to each other (Dehlawi *et al.* 1992) and in *Chalcides ocellatus,* only a single centrally located granule was observed (Dehlawi 1992). Similar to other lizards (Courtens and Depeiges 1985, Dehlawi and Ismail 1990, 1991 and Dehlawi 1992) the nuclear changes during spermiogenesis in *S. mitranus* include both chromatin condensation and nuclear elongation. The chromatin condensation allows for major reduction in nuclear volume to streamline the cell and to help in locomotion. Moreover, the tight packing of the DNA might help in the preservation from physical damage or mutation during storage and transport (Browder 1984).

It has been suggested that the microtubules manchette which surround the nucleus of the spermatid might be involved in nuclear elongation due to a progressive reorientation from circular to longitudinal manchette, which later determines the nuclear shape (Courtens and Depeiges 1985). A similar role might also be attributed to those microtubules manchette seen in *S. mitranus,* which might also be supported by the disappearance of these microtubules at the completion of the nuclear changes. Moreover, the manchette may also be involved in the development of the acrosome, due to the intimate association between the acrosome and the manchette in late spermiogenesis.

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دراسة التركيب الدقيق لتمايز رأس الحيوان المنوي للسحلية الرملية الصقنقور *Scincus mitranus* (Anderson 1871)

عثمان بن عبد الله الدوخي

قسم علم الحيوان - كلية العلوم - جامعة الملك سعود

ص .ب (٢٤٥٥) - الرياض ١١٤٥١ - المملكة العربية السعودية

لدراسة التركيب الدقيق لتمايز الحيوان المنوي تم جمع خمسة ذكور من سحلية الصقنقور *Scincus mitranus* خلال فترة النشاط الجنسي (ابريل - مايو) وذلك من منطقة الثمامة ، شمال شرق مدينة الرياض .

بعد إستئصال الخصي تم تقطيعها إلى قطع صغيرة جداً ، ثم تثبيتها في ٤٪ جلوترألدهيد ثم في ٢٪ رابع أوكسيد الاوزميوم ، بعد ذلك تم تجفيفها ثم طمرها في EPON 812 . تم أخذ قطاعات رقيقة وصبغها ثم فحصها بالمجهر الالكتروني النفاذ .

الطليعة المنوية حديثة التكوين تبدو دائرية الشكل مع نواة بيضاوية أو دائرية تتمركز في وسط الخلية . كما يبدو الكروماتين كحبيبات دقيقة جداً متوزعة بشكل متساوي في النواة . يوجد في السيتوبلازم أجسام جولجي بشكل واضح مع حويصلات كثيرة تحيط بها . كذلك توجد الاجسام السبحية (الميتوكوندريا) قريبة من أجسام جولجي وفي أنحاء أخرى من السيتوبلازم . الرايبوسومات موزعة بشكل عشوائي أو مرتبطة مع الشبكة الاندوبلازمية ، كما يوجد أيضاً حبيبات دهنية . لم يلاحظ وجود أنيبيبات دقيقة منظمة في هذه المرحلة .

مع نمو الطليعة المنوية تكبر أجسام جولجي في الحجم وتظهر حويصلات صغيرة حولها ، تتجمع بعد ذلك هذه الحويصلات الصغيرة في حويصلة كبيرة (حويصلة الاكروسوم) أمام النواة وقريبة من الغلاف النووي . يتزامن مع هذا تكون إنغماد في النواة في الجهة القريبة لحويصلة الاكروسوم ، هذا الانغماد يأخذ شكل كأس ويزداد في الحجم محيطاً بحويصلة الاكروسوم ، بعد عملية الالتصاق بين حويصلة الاكروسوم والانغماد النووي ، تبدأ حبيبات الاكروسوم في التجمع والظهور ملاصقة للجدار الخلفي لحويصلة الاكروسوم .

مع إستمرار عملية تمايز الطليعة المنوية نجد أن النواة وحويصلة الاكروسوم تتحرك حتى تلامس الغشاء البلازمي ، ثم تتطاول النواة ضاغطة حويصلة الاكروسوم على الغشاء البلازمي والتي تتمدد بعد ذلك وتأخذ شكل غطاء بذراعين جانبيين على النواة .

العديد من الانيبيبات المنوية تبدأ في الظهور في هذه المرحلة ، حول النواة مترتبة على المحور الامامي الخلفي لها ، وتصبح في النهاية قريبة جداً من الغلاف النووي . تبدأ بعد ذلك النواة بالاستطالة ويبدأ الكروماتين بالتحول إلى خيوط سميكة على شكل شبكة مترابطة .

في نهاية عملية التمايز يظهر رأس الحيوان المنوي كتركيب منحني إسطواني محاطاً بحويصلة الاكروسوم على شكل غطاء ، كما يظهر الكروماتين كتركيب مصمت تماماً . النهاية الخلفية للنواة تبدو مقعرة ، مكونةً الاساس لمنطقة العنق للحيوان المنوي . كما أن حزم الانيبيبات الدقيقة تتناقص بشكل سريع ثم تختفي في النهاية .

مراحل تمايز الطليعة المنوية في سحلية الصقنقور *Scincus m.* تشبه إلى حد كبير ما تم وصفه في الابحاث السابقة عن السحالي . إن عملية إستطالة النواة وتكثف الكروماتين تساهم في إختزال حجم النواة وتكوين الشكل الانسيابي لرأس

الحيوان المنوي ، كما أنها تحافظ على المادة الوراثية أثناء عملية التخزين أو الانتقال إلى أن يحدث الاخصاب .

الانيبيبات الدقيقة المحيطة بالنواة تساهم بشكل واضح في توجيه عملية إستطالة النواة وإعطائها الشكل المحدد ، ويؤيد هذا إحاطتها بالنواة أثناء عملية الاستطالة ثم إختفائها بعد إكتمال تكوين رأس الحيوان المنوي .