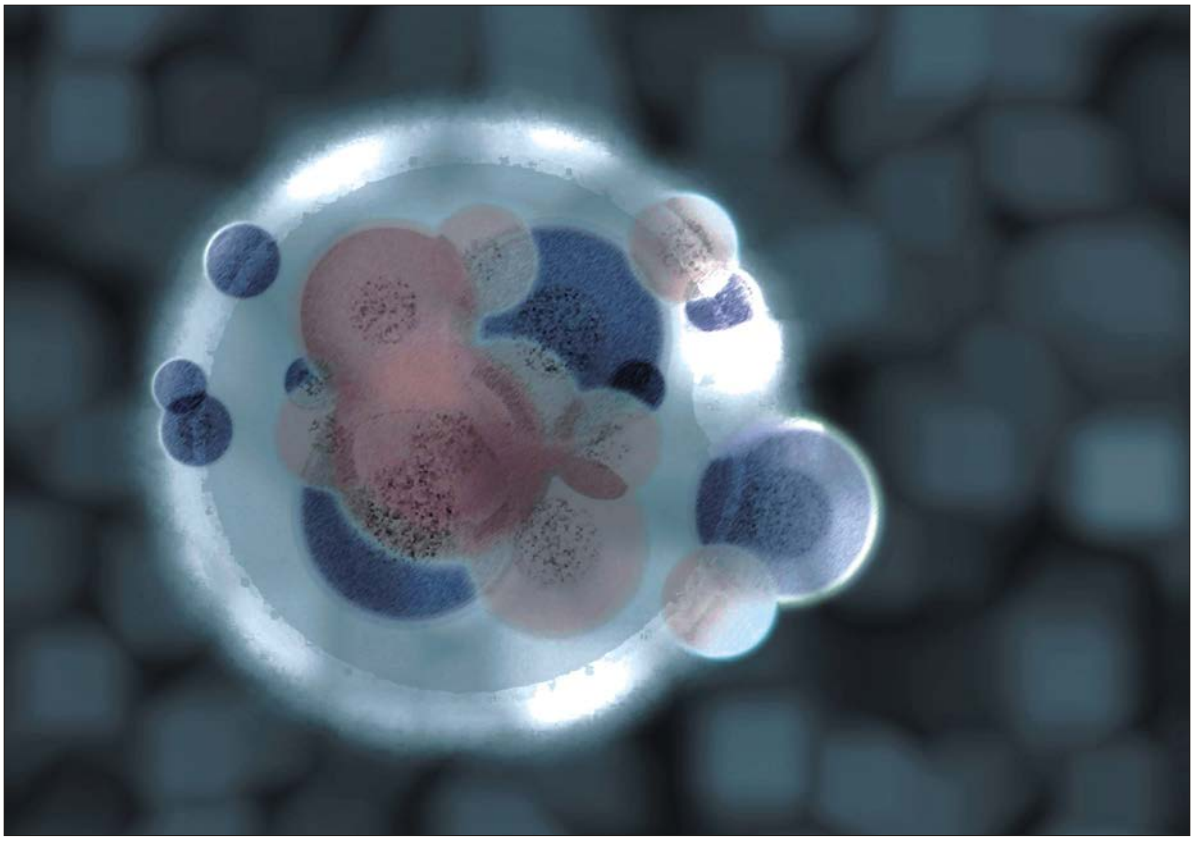

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Light and Electron Microscopy of the Testicular Tissue of the Snake *Eryx jayakari* (Squamata, Reptilia) with a Reference to the Dividing Germ Cells

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Abstract: The histomorphological as well as the ultrastructural features of the testicular tissue of the snake *Eryx jayakari* (*E. jayakari*) were described. Also, the spermatogenic cells, including spermatogonia and primary and secondary spematocytes were illustrated. The non-spermatogenic cells represented by the Sertoli cells were also demonstrated. The interstitial tissue, most importantly the Leydig cells and the seminiferous tubular lamina propria have been identified. The testicular cellular changes relevant to the period of sexual activity in this snake species were recorded. The present study is an original article on the structural morphology of the testicular tissue of the snake *E. jayakari*.

Key words: Snake, testis, histology, germ cells, ultrastructure

INTRODUCTION

Histomorphological features of the testis and process of spermatogenesis of mammalian species have been studied extensively[1-9].

Most of the available studies on reptiles have been directed to the process of spermiogenesis and little attention has been paid for the study of testicular germinal epithelium. For instance, the histology of the reptilian seminiferous epithelium and the ultrastructure of testicular germ cells were the focus of only few studies[10,11]. Thus, there is a paucity of data on this subject in reptiles and considerable number of reptilian species, especially snakes still await accurate descriptive studies. So far, no relevant study was reported on the snake *Eryx jayakari* (*E. jayakari*). Therefore, the present study was intended to provide original data on the histo-and ultrastructural morphology of the testicular tissue, including the seminiferous dividing germ cells, of the snake *E. jayakari*.

MATERIALS AND METHODS

Snakes: Ten adult males of the snake *E. jayakari* were collected during the period of sexual activity (from March to May) 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 testes were removed.

Light microscopy: Proper-sized tissue samples from the testes of the snake *E. jayakari* were fixed in Bouin's fluid for 24 h. Tissue specimens were then processed routinely for paraffin embedding technique. Thereafter, tissue sections (4-5 μm) were prepared and stained with hematoxylin and eosin (HE) stain. Selected tissue sections were also stained with Mallorys stain.

Electron microscopy: Testicular tissue samples were diced into the proper 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 testicular 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 transferred to epoxy resin (Epon/Araldite mixture) via propylene oxide. Thin sections were cut with a diamond knife on an ultramicrotome (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

Testicular capsule: The testicle of *E. jayakari* is ensheathed by a capsule (Fig. 1) which is recognized into two layers, the inner and outer ones. The outer layer (tunica vaginalis) is thin and composed of a single layer of simple epithelium having elongated nuclei and resting on a basement membrane separating it from the inner layer. The inner layer (tunica albuginea) is dominating and composed of dense connective tissue (CT) rich in

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fibrocytes and collagen fibers which are stained pink by HE (Fig. 2a) and green by Mallory's (Fig. 2b). Tunica albuginea is invested by large blood vessels and extended into the testicular tissue proper.

Tubular basement membrane: The tubular basement membrane (basal lamina) under the electron microscope is found to be a part of the tubular lamina propria which is built-up of three layers (successively from inside to outside); the internal noncellular layer, intermediate cellular layer and the external cellular layer (Fig. 3a). The internal noncellular layer (basal lamina) is thin and reveals abundance of collagen fibers. Considerable number of fibrocytes and myoid cells on a background of elastic connective tissue (CT) is the composition of the intermediate cellular layer (Fig. 3b). The external noncellular layer is identical in structure to the internal one.

Lobuli testis: The extension of tunica albuginea into the testicular tissue proper in the form of septa (septula testis) divides it into lobules (lobuli testis), each lobule contains one or more seminiferous tubules.

Interstitial tissue: Seminiferous tubules are connected by the interlobular CT (interstitial tissue) which contains ramified blood vessels, small lymphatics and interstitial cells (Fig. 4). The details of Leydig (interstitial) cells are best clarified by electron microscope. They are polygonal or elongated in shape and their varied-shaped nuclei (Fig. 5) have noticeably clumped chromatin and evident nuclear pores. Large number of lipid droplets of varying size and density are seen in Leydig cell cytoplasm which also contains considerable number of mitochondria.

Seminiferous tubules: The highly convoluted seminiferous tubules form about 80% of the testicular tissue. Diameter of the seminiferous tubules in the adult male during the early period of sexual activity approximates 150 μm and increases during the season of sexual activity to approach 190 μm. Each seminiferous tubule has a basement membrane lined with several raws of germ cells arranged in successive layers representing the different stages of cell division (spermatogenesis and meiosis) and differentiation (spermiogenesis). Five types of germ cells (spermatogenic lineage) can be identified (successively from the basement membrane to tubular lumen); spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa (Fig. 6). Sertoli cells (non-spermatogenic supporting cells) are tall extending from the tubular basement membrane to the tubular lumen. By light microscope the outline and details of Sertoli cells are barely identified. The tubular lumen during the season of sexual activity contains free sperms and also sperms embedded by their heads in the apical recesses of Sertoli cells. Diameter of the tubular lumena ranges from 80 to 100 μm during the period of sexual activity.

Spermatogonia: Spermotogonia cells are located at the basal portion of the germinal epithelium in contact with the tubular basement membrane. They are the largest germ cell and 3 types of them are recognized; spermatogonia type A, intermediate spematogonia and spematogonia type B. Type A cells are the largest and their broad base is obviously attached to the tubular basement membrane and their nuclei are rounded, centrally located and nuclear chromatin is evenly distributed (Fig. 7a). Intermediate spermatogonia are the smallest and their attachment to the basement membrane is comparatively of lesser degree. Nuclei of these cells are also rounded and central but have one or two nucleoli and more dense chromatin (Fig. 7b). Spematogonia type B cells show the less degree of attachment to the basement membrane. These cells are elongated and possess apical rounded nuclei which have chromatin of similar density to that of the intermediate cells. At the ultrastructural level, many rounded or rod-shaped mitochondna are distributed throughout the spermatogonia cytoplasm which also contains amorphous vacuoles (Fig. 7c). Neither connections between spermatogonia and tubular basement membrane nor bridges interconnecting the neighboring spermatogonia are detected by electron microscopy.

Primary spermatocytes: These cells are located at higher level to spermatogonia cells and usually found as aggregates of rounded cells. Their nuclei are rounded and central and have chromatin of more density than that of spematogonia cells. Cytoplasm of primary spematocytes is clear and the cell membrane is distinct and well-identified (Fig. 8a). Electron microscopy of these cells shows clumped nuclear chromatin, indistinct nucleoli, dispersed mitochondria and well-recognized Golgi complex (Fig. 8b).

Secondary spermatocytes: These cells are the least in number and smaller in size compared to spermatogonia and primary spermatocytes. They are found singly or gathered in small groups (Fig. 9a). At the ultrastructural level, secondary spermatocytes are the least encountered germ cells and as the case in primary spermatocytes, the rounded nucleus is large occupying most of cytoplasm. Nuclear chromatin is marginally clumped and there are 2-3 nucleoli (Fig. 9b).

Sertoli cells: Sertoli cells are the largest intratubular cell, by light microscope their shape and outline are poorly

Fig. 1: A section through the whole testicular tissue of the snake *E. jayakari*. The testicular tissue proper (*), including seminiferous tubules and interstitium, is ensheathed by the capsule (C). Epididymis (E) is attached to the testicular capsule. HE. Scale bar=400 μm

Fig. 2 a: Higher magnification for the testicular capsule showing the thin outer layer, tunica vaginalis (TV) and the thick inner layer, tunica albuginea (TA). The earlier is composed of a layer of simple epithelium and the latter is built-up of dense CT which is stained pink with HE stain. Testicular tissue proper (*). HE. Scale bar=50 μm.

b: The inner thick and dense tunica albuginea (TA) is stained green with Mallory's stain indicating its richness in fibrous elements. Scale bar=50 μm

Fig. 3a: The tubular lamina propria showing the three composing layers; the internal noncellular layer (1), the intermediate cellular layer (2) and the external noncellular layer (3). Note the nucleus (N) of a fibrocyte and part of Sertoli cell (SR) cytoplasm. Scale bar=0.5 μm

b: Higher magnification for the intermediate cellular layer showing nuclei of fibrocyte and myoid cells (MN, FN) Note the deposited collagen fibrils (arrows) in the vicinity of the fibrocyte. Scale bar=0.2 μm

Fig. 4: Seminiferous tubules (T) form the major proportion of the testicular tissue and connected by the inertubular (interstitial) tissue (*) which contains Blood Vessels (BV) and small Lymphatics (L). HE. Scale bar=250 μm

Fig. 5: Leydig (interstitial) cell having irregular-shaped nucleus (N) which shows clumped chromatin. Note the varied-sized lipid droplets (LP) in Leydig cell cytoplasm. Scale bar=0.5 μm

Fig. 6: Seminiferous tubule showing the sequence of the spermatogenic cells, successively from the tubular basement membrane to lumen (L); Spermatogonia (1), primary spermatocytes (2), spermatids (3) and spermatozoa (4). The irregular spaces (*) represent site of Sertoli cell cytoplasm. Note the Leydig (interstitial) cells (arrow) in the intertubular tissue. HE. Scale bar=50 μm

Fig. 7a: Spermatogonia type A (SA) with a broad base attached to the tubular basement membrane (arrow). Nucleus (N) is rounded, centrally located and has evenly distributed chromatin. HE. Scale bar=10 μm

b: Intermediate spermatogonia (IS) showing a lesser attachment to the tubular basement membrane (arrow). Nucleus (N) is also rounded and central but has more dense chromatin. HE. Scale bar=10 μm.

c: Transmission electron micrograph showing spermatogonia cell (S) which has a broad base and rounded central nucleus (N) with even fine chromatin. Note that a part of Sertoli cell cytoplasm (*) separates the base of spermatogonia from the tubular basement membrane (arrows). Scale bar=1 μm

Fig. 8a: Primary spermatocytes (PS) having rounded central nuclei (N) with dense chromatin. These cells have distinct cell membrane. HE. Scale bar=10 μm

b: Transmission electron micrograph showing a primary spermatocyte which possesses a rounded central nucleus (N) having dense clumped chromatin. Cytoplasm of this cell contains a Golgi complex (GO) and dispersed mitochondria (M). Scale bar=1 μm

Fig. 9a: Secondary spermatocyte (SS) having a rounded nucleus with peripherally clumped chromatin. HE. Scale bar=10 μm

b: Transmission electron micrograph revealing the relatively smaller nucleus (N) of a secondary spermatocyte. Nuclear chromatin is clumped and there are two nucleoli (arrows). Scale bar=0.5 μm

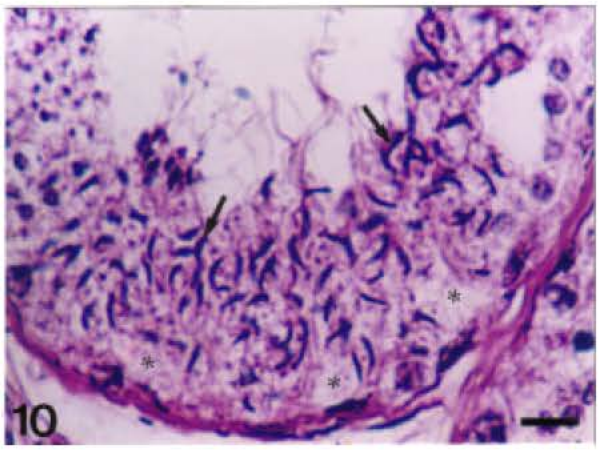

Fig. 10: Differentiated spermatids (arrows) embedded by their heads in the apical processes of Sertoli cells. In this HE-stained tissue section, the outlines of Sertoli cells are not identified and the irregular spaces (*) marks the site of their unstained cytoplasm. HE. Scale bar=25 μm

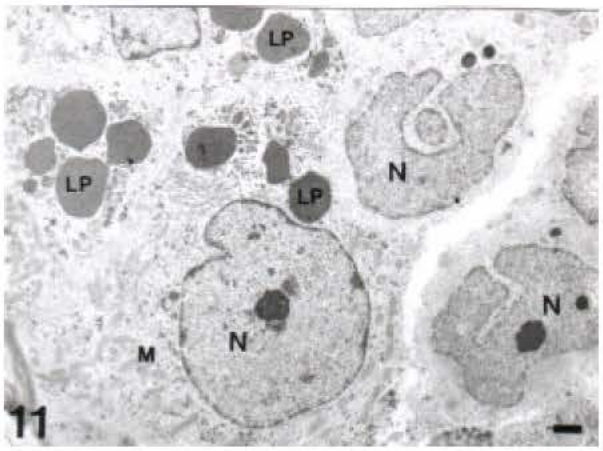

Fig. 11: Transmission electron micrograph showing the remarkably varied shaped nuclei (N) of Sertoli cells. Lipid droplets (LP) are numerous in cytoplasm of Sertoli cells and there is abundance of mitochondria (M). Scale bar=1 μm

identified owing to the numerous invaginations and foldings in their membranes. The clarity of Sertoli cells cytoplasm is attributable to the dissolution of the cytoplasmic lipid material during tissue processing. The average length of Sertoli cells, from the basement membrane to tubular lumen, approximates 40 μm. Sertoli cells overly the spematogonia cells and partially envelope the other spematogenic cells. The differentiated spematids are embedded by their heads in the apical processes of Sertoli cells (Fig. 10). Ultrastructure of Sertoli cells reveals its low density cytoplasm and the numerous membrane folds and invaginations exhibited by them to accommodate and conform with the tubular germ cells. Nuclei of Sertoli cells are highly variable in shape and have distinct nucleoli (Fig. 11). The remarkable ultrastructural cytoplasmic feature in Sertoli cells is the presence of large number of lipid droplets. Mitochondria are scattered and the parallel cisternae of endoplasmic reticulum are basally located in cytoplasm which has microtubules at its apical portion and also Golgi complex.

DISCUSSION

As demonstrated in the present study, the testis of the snake *E. jayakari* is composed mainly of long convoluted seminiferous tubules connected by intertubular (interstitial) CT which contains Leydig cells. This basic testicular structure is identical to that of other reptilian species[12,13].

The presently described testicular lamina propria involves 3 successive layers which serve to ensheath the testicular tissue proper. In reptilian testis, the circumferential lamina propria encompasses up to 5 layers[14]. The compound structure of the lamina propria plays a crucial role in isolation of the intratubular structures from the surrounding environment[15].

Diameter of the seminiferous tubules of E. jayakari differs according to the state of sexual activity being the largest during period of reproduction. This notice was also recorded in other reptiles[13,16-19]. The capability of seminiferous tubules to increase in diameter reflects their contractibility which is said to be under nervous control[20]. On contrast, Bloom and Fawcett[3] attributed this phenomenon to the autonomous ability of the peritubular myoid cells to contract and relax. In our opinion, the dilatory capacity of the seminiferous tubules is attributable to the myoid cells activity.

Presently the morphological features of *E. jayakari* spermatogonia and primary spermatocytes were described. The ultrastructure of the reptilian spermatogonia and primary and secondary spematocytes was only described in a few studies[10,11].

The described different types of spermatogonia cells are the consequence of the high mitotic activity of these cells which divide indefinitely[9]. Spermatogonia cells represent the original source of spermatogenic cells[1,5]. Primary spermatocytes are the spermatogonia that quitted division and moved to higher position than spermatogonia[8].

The secondary spermatocytes are the least encountered cell form as they are directly shifted to the second meiotic division to give rise to haploid cells (spermatids)[21].

The reptilian Sertoli cells received relatively more attention than spermatogonia[10,11,21-23]. Sertoli cells play a principal role in the process of spermatogenesis starting from the division of spermatogonia up to the differentiation of mature sperms. Sertoli cells of *E. jayakari* show numerous folds and invaginations and also variability concerning the position of their nuclei which is either basal or apical. Similar features are reported in lizards[22], turtles[24] and snakes[11]. Additionally, the cytoplasmic lipid droplets in Sertoli cells are increased in number and size during the period of sexual activity.

The described ultrastructure of *E. jayakari* Leydig cells is that of active cells during the sexual period as evidenced by the large-sized nuclei and large cytoplasmic lipid droplets. These hormones are suspected to be stored in the cytoplasmic lipid droplets which are found consistently in Leydig cells of many reptiles[14]. Leydig cells are active throughout the year in contrast to the activity of Sertoli cells which is only confined to the period of active spermatogenesis[25].

The present study is an original one to describe the histological and ultrastructural features of testicular tissue of the snake *E. jayakari* with a reference to the dividing germ cells. Besides, this descriptive study may provide a basic knowledge for the researchers interest in the biological aspects of reptiles.

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