RABBIT SEMINIFEROUS TUBULES
MORPHOLOGICAL OBSERVATIONS ON THE BOUNDARY TISSUE

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ABSTRACT... Objective: The aim of this article was to study the ultrastructure of the boundary tissue of rabbit seminiferous tubule. **Design:** Experimental study. **Period:** During July 2010 to July 2011. **Material:** Eight sexually mature male rabbits (Oryctolagus cuniculus) were used in this study. Rabbit maturity was determined by age and semen samples. **Method:** Ultrastructure observations by electron microscope transmission of normal rabbit testes. **Setting:** Samples processing were done in both university of Khartoum (Sudan) and then Assiut university (Egypt). **Results:** The boundary tissue is formed of three lamellae, inner fibrous, inner and outer cellular lamellae. The inner fibrous lamella consists of three strata; internal and external homogenous enclosing a middle stratum of collagenous fibers. The internal homogenous stratum of the inner fibrous lamella is subdivided into four layers of moderate electron density. **Conclusions:** The general structure of the seminiferous tubule of rabbit is similar to that of hamster and mouse, but the internal homogenous stratum is formed of four layers.

Key words: Rabbit. Seminiferous tubule. Ultrastructure. Boundary tissue. Internal homogenous layer.

INTRODUCTION

The boundary tissue (BT) of the seminiferous tubule (ST) is assumed to have many functions such as, mechanical support, discharge of spermatozoa and as a physiological barrier regulating material transport across it. The interactions between both interstitial cells and Sertoli cells affect the BT[1-5].

The mammalian[6] STs are enclosed by one or more layers of adventitial cells derived from primitive tissue elements of the interstitium. The ST wall was described by some authors as being formed of two distinct concentric layers: basement membrane (BM) and lamina propria (LP)[7-11].

Others however, consider the BM as a component of the LP, being its innermost layer, in apposition with the seminiferous epithelium[12,13]. Moniem et al[14] described the BT being constituted of four basic components; a homogenous matrix, collagenous fibres, elongated contractile (Myoid) cells and fibroblasts. These components invest the STs in the form of concentric layers, displaying certain species differences[15,16]. Three types of BT have been described[17] in different species.

This paper aimed at a detailed description of the ultra-structure of the boundary tissue of rabbit seminiferous tubule hitherto not reported.

MATERIAL AND METHODS

Eight sexually mature male rabbits (Oryctolagus cuniculus) were used in this study. Animals breed and sample collection done in the department of anatomy, Faculty of veterinary medicine, university of Khartoum (Sudan). Study duration was from July 2010 to July 2011.

Testes were removed after animals were anaesthetized intramuscularly (Diazepam 1 ml (5 mg) /Kg "Shanghai Pharmaceutical Co. Ltd. China" and 1 ml (50 mg) /Kg of Ketamine Hydrochloride "Rotexmedica. Tritiall. Germany") and slaughtered.

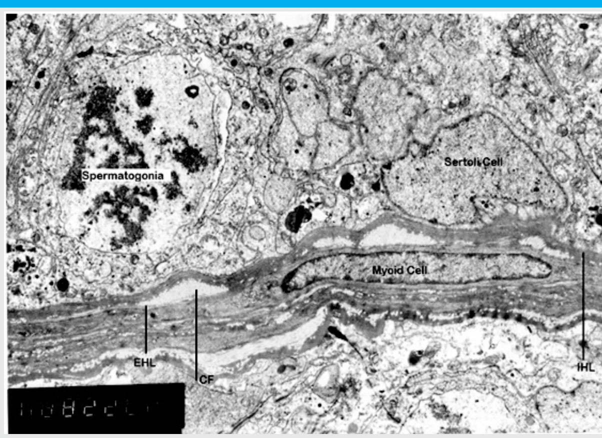
For the ultrastructural study, small pieces of testis of both sides of the eight rabbits (about 1mm thick) were obtained and rapidly fixed in cold 5% Glutaraldehyde, washed in phosphate buffer (pH 7.2) four times for about 20 minutes each. The samples were post-fixed in osmium tetroxide (O4S4) for two hours, washed again in the same buffer four times for about 20 minutes each, dehydrated in ascending grades of alcohol (30- 50- 70- 90%) for 30 minutes each and in 100% alcohol two times for two hours and then embedded in Epon araldite mixture.

Semithin sections of 0.5.μm thick were cut in LKB ultramicrotome. Desirable parts were then selected and ultrathin sections (500-700Å) were cut in Leica AG Ultractus microtome, stained with Uranyl acetate and Lead citrate and examined in Jeol TEM 100 C XII electron microscope. Ultrastructural study was done in the electron microscope unit, university of Assiut (Egypt).

RESULTS

The BT of the ST consisted of three lamellae; inner fibrous, inner and outer cellular one's Diagram 1(Hand drawing representing the summary of the ultrastructural findings in all samples) and Figure 1.

Fig-1. Boundary tissue of rabbit seminiferous tubule. IHL, Internal homogenous layer. EHL, External homogenous layer. CF, Collagen fibers layer.

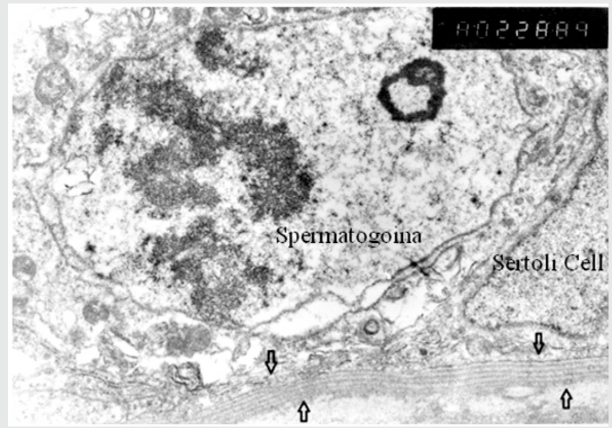


The inner fibrous lamella was subdivided into three strata; internal and external homogenous strata enclosing a thin middle one of collagenous fibers. The internal homogenous layer was further subdivided into four layers of moderate electron density packed together with apparently equal distances (Fig 2).

Both homogenous strata were of light electron density. The collagen fibers were mostly obliquely oriented.

The inner cellular layer was formed of myoid cells. These

Fig-2. Boundary tissue of seminiferous tubule. Internal homogenous layer (Note the arrows) and electron dense layers within it.



were elongate in shape with oval nuclei longitudinally oriented in the wide middle part of cell. The cells were disposed in the form of one or two strata and connected by tight junctions. The cellular cytoplasm was of low electron density and was poor in organelles (Diagram 1).

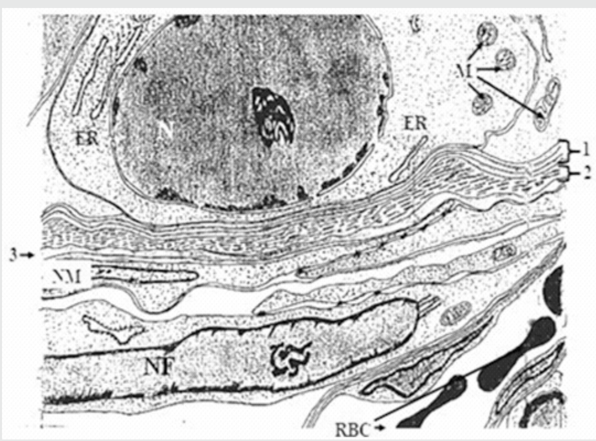
The outer cellular layer was formed of attenuated flattened fibroblasts, which tended to follow the contour of the STs. The nuclei were oval in shape and occupied the middle parts of cells. The cytoplasm was poor in organelles (Figure 1).

DISCUSSION

The ultrastructural observation of the rabbit BT showed that it is formed of three lamellae: innermost fibrous layer which in turn is subdivided into internal and external homogenous strata enclosing a third one of collagenous fibers. The internal homogenous stratum is further subdivided into four layers of moderate electron density. The inner cellular lamella was essentially formed of myoid cells, while the outer cellular lamella was constituted of fibroblasts. Both cellular lamellae are essentially similar to what has been reported in mouse[18,19] and rat[20-22]. The general organizations of BT of rabbit STs observed in the present study resemble that of hamster, mouse and rat[15,16,23].

The present observations conform with the earlier reports[15,23- 25] showing that the BT of the STs of normal rabbit consists of several layers.

Diagram (1): Hand drawing showing the ultrastructure of the boundary tissue of rabbit seminiferous tubule. The inner homogenous layer of the inner fibrous layer (1) was further subdivided into four layers of strong electron density. The fibrous layer (2) was formed of collagenous fibres with different orientations. The outer homogenous layer (3) was narrow. The nucleus of Myoid cells (NM) was elongate in shape and the cells were connected by tight junctions. Fibroblasts form the outer cellular layer and bounded by blood vessels contains (RBC). Spermatogonia cell appeared with rounded nucleus (N) surrounded by cytoplasm containing endoplasmic reticulum (ER) and Mitochondria (M).



Some authors consider the innermost fibrous layer as “lamina propria” with a morphological entity distinct from the BT[7]. However, other authors included the LP in the BT, considering it as the innermost layer, in apposition with the seminiferous epithelium[11-13].

The ultrastructure organization of the inner homogenous layer of internal fibrous lamella has not been described in the earlier reports in a number of species including rabbit[26-29]. Although the boundary tissue of testes has not been studied histochemically in details, Moniem et al[14] are of the opinion that the homogenous layers of the boundary tissue are formed of glycoprotein. Nowadays, it is well established that the fibrous lamina contains laminin, type IV collagen, heparan sulfate proteoglycans, fibronectin, and nidogen/entactin[30,31]. Laminins, prominent glycoproteins in basal lamina, are large complexes composed of a heavy α-chain, and the light β and γ-chains[29]. Both Sertoli and peritubular cells are the main sources of the different laminins[29,32,33]. Recently Rezigalla 34 studied the effect of vasectomy on rabbit testes and demonstrated an increase in the number of layers to five post unilateral or bilateral vasectomy.

The inner cellular lamella formed of Myoid cells possessed numerous pinocytotic vesicles and an ordered array of cytoplasmic filaments. Myoid cells showed a characteristic feature of contractile cell which has been adopted by many authors. The myoid cells demonstrated under stimulation[35] the ability of to secrete a number of substances including extra cellular matrix components, fibronectin and type I collagen. The myoid cells occupied one layer and this is similar to that of hamster, mouse and rat[16]. In contrast, multilayered myoid cells have been described in cat and man[17,36] ram and boar[37] domestic fowl[16,37,38] and camel[14].

Cells in the outer cellular layer are assumed to be fibroblasts[14]. The adventitial layer of seminiferous tubules consists of the last two cell layers made of fibroblasts and fibrocytes containing characteristic collagen, elastin and vimentin cytoskeletal filaments[11]. Fibroblasts form the outer most cellular lamella in the BT despite the type of the BT[17] except in fowl where they are related to the inner fibrous lamella[38].

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