ORIGINAL ARTICLE

EVALUATION OF BASEMENT MEMBRANE THICKNESS OF SOMNIFEROUS TUBULES IN CRYPTORCHID TESTS

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ABSTRACT: Basement Membrane [BM] is a thin layer of specialized extracellular matrix that lies sandwiched between connective tissue and various tissues supported by it. Somniferous tubules are the sites of germination, maturation and transportation of the spermatic cells within the male testes. Somniferous tubule differentiation has been seen to be related to occurrence of germ cell neoplasia in men who have been treated surgically for cryptorchidism during childhood. Impairment of normal BM thickness of Somniferous tubules in crypt orchid testes results in alteration of diameter of Somniferous tubules and increased intertubular spaces. Retardation of Somniferous tubule differentiation results in inhibited spermatogenesis, presence of tubules with immature Sertoli cells, decreased tubular diameter, increased thickness of BM and enlarged intertubular spaces. Spermatogenic dysfunction may result from thickening of BM of Somniferous tubules and tubular sclerosis.
KEYWORDS: Basement Membrane, Crypt orchid Testes, Dysfunction, Somniferous Tubule.

INTRODUCTION: Basement Membranes are sheet like arrangements of extracellular matrix proteins which act as an interface between the support tissues and parenchymal cells.[1] The functional roles of BM are to bond cells to the underlying surrounding connective tissue and to provide these cells with flexible support. These functions are particularly evident in the lens capsule where a highly developed BM not only supports the cells but also changes shape during accommodation reflex. BM is freely permeable to substances of low molecular weight but impede the passage of macromolecules.

This property of BM is of particular advantage in glomerular filtration in the kidneys. A more speculative role of BM is that they act as substrates capable of directing cell growth and migration during morphogenesis, regeneration and repair.[2] The testes develop in the abdomen and descend into the scrotum during foetal life. The thin walled scrotum has a considerable surface area which enables the testes to remain at a temperature slightly lower than the body temperature. This lower temperature is an important prerequisite for the production of adequate numbers of spermatozoa. The spermatozoa are produced within a mass of tortuous looped Somniferous tubules that collectively fill most of the interior of each testis.[3]

Somniferous tubule basement membrane [STBM] plays an important role in spermatogenesis.[4] Somniferous epithelium undergoes cyclical changes in its composition. The turn over time of the whole epithelium is considerably longer than the length of the cycle because only a small proportion of the cells become spermatozoa and leave the epithelium in each cycle.[5] The cycle of Somniferous epithelium is a description of what happens during the course of time at each individual site in a Somniferous tubule.

A succession of changes in the cellular composition of each patch of Somniferous epithelium is followed by return of this patch of epithelium to its original cellular composition. The time taken for entire sequence of changes to occur presents the length of the cycle of Somniferous epithelium. Each Somniferous tubule is about 200µ in diameter.[6] The BM of Somniferous tubular epithelium is normally 0.1 to 0.2 µm thick.[7] Severe damage in formation of spermatogonia in undescended testis at 6 years[8] has been observed.

MATERIALS AND METHODS: One normal testis was removed from a cadaver of about 40 years of age and four orchidectomized museum specimens of cryptorchid testis were used examined histologically. The museum specimens were in the age group of 30 to 40 years. They were labeled as C, UT_1, UT_2, UT_3 & UT_4, where C and UT denote Control and Undescended Testis respectively. The specimens were already in fixed in Bouin's fluid.

Paraffin wax embedded tissue blocks were prepared and sections of thickness 6µm were obtained using rotary microtome. The slides prepared were stained using PAS (periodic acid Schiff) technique. The stained sections were observed under Carl Zeiss Observer Z1 microscope and thickness of BM in control and all samples were recorded by measuring randomly selected 20 somniferous tubules across each slide.

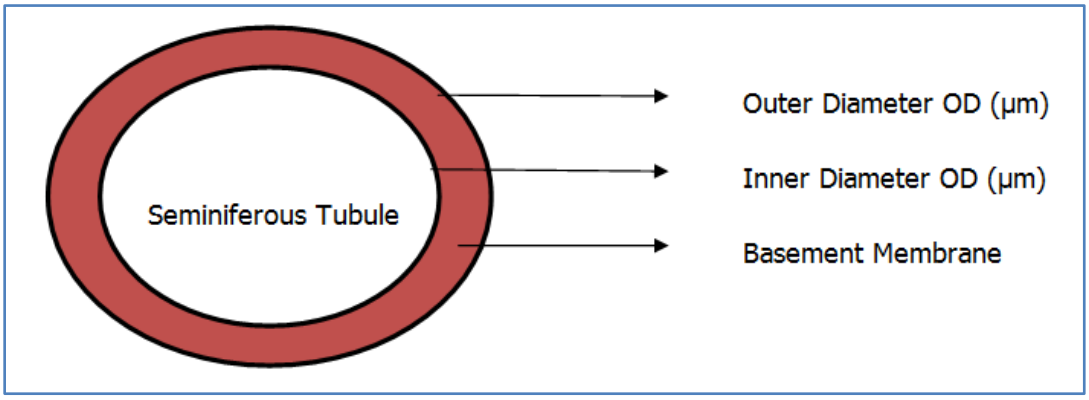

Fig. 1: STBM thickness in (µ) = OD – ID

OBSERVATIONS:

ST	C		UT_1		UT_2		UT_3		UT_4	
Serial No.	**OD**	**ID**	**OD**	**ID**	**OD**	**ID**	**OD**	**ID**	**OD**	**ID**
ST-01	225.3	225.1	61.3	49.9	51.3	40.1	62.9	50.6	61.7	48.6
ST-02	222.1	221.9	68.6	57.3	63.2	50.9	58.1	45.8	63.1	50.2
ST-03	219.7	219.6	57.3	41.2	60.7	47.6	59.6	46.9	62.3	49.9
ST-04	214.3	214.1	53.3	40.7	52.1	40.8	62.7	49.5	59.8	47.1
ST-05	211.6	211.3	49.1	37.3	57.1	45.9	48.4	35.7	42.1	29.4
ST-06	228.8	228.5	82.3	69.2	49.3	37.1	46.7	33.8	51.2	37.9
ST-07	213.9	213.7	52.7	40.6	71.7	57.8	39.3	26.2	46.7	33.5

ST-08	212.1	211.9	41.8	30.3	68.9	56.8	55.3	43.2	56.8	44.5
ST-09	206.7	206.4	71.6	59.2	75.7	64.5	59.1	46.4	55.7	43.3
ST-10	213.6	213.5	95.3	82.6	77.1	65.9	70.3	56.7	65.6	53.5
ST-11	227.5	227.4	91.2	78.9	62.1	49.9	70.7	57.8	67.1	54.2
ST-12	230.1	229.9	61.9	50.6	63.3	51.2	68.1	55.5	67.7	54.5
ST-13	214.8	214.5	58.1	45.8	61.9	49.2	68.7	56.2	60.9	48.8
ST-14	203.3	203.1	55.7	43.1	71.3	58.7	59.2	47.1	68.3	55.1
ST-15	219.2	219.1	49.3	37.2	73.2	60.9	55.6	42.9	52.1	38.6
ST-16	222.7	227.4	73.7	62.2	59.9	48.6	51.3	39.2	59.3	46.7
ST-17	226.3	226.1	72.1	59.5	60.3	47.9	55.9	42.8	55.1	42.5
ST-18	217.7	217.5	46.3	35.2	61.6	50.1	58.2	46.1	71.1	58.5
ST-19	215.9	215.7	41.2	30.1	69.7	57.3	61.1	48.8	71.7	58.8
ST-20	219.1	218.9	62.5	50.4	69.3	58.2	69.2	55.9	66.7	53.4

Table 1

Table 1: Showing the Outer Diameters (OD) and Inner Diameters (ID) of 20 random Somniferous Tubules [ST] of C, T_1, T_2, T_3 & T_4 in microns (µ).

Testes	Mean **OD**	Mean **ID**	**STBM**
C	218.24	218.28	0.21
T_1	62.27	50.07	12.20
T_2	63.99	51.97	12.02
T_3	59.02	46.36	12.66
T_4	60.25	47.45	12.80

Table 2

Table 2: Showing Mean Thickness of [STBM] of "C, UT_1, UT_2, UT_3 & UT_4" in microns (µ).

The following features were also observed during histological examination. There was reduction in the number of somniferous tubules in cryptorchid testes. The remaining somniferous tubules were atrophic in nature and distorted in shape. Most of them were either elliptical or oval. The smaller ones appeared to be hyalinized. The larger ones contained Sertoli cells only. STBM was greatly thickened. Extensive peritubular sclerosis was observed. The interstitial cells of Leydig were vacuolated. There were no identifiable spermatogonia and no evidence of spermatogenesis.

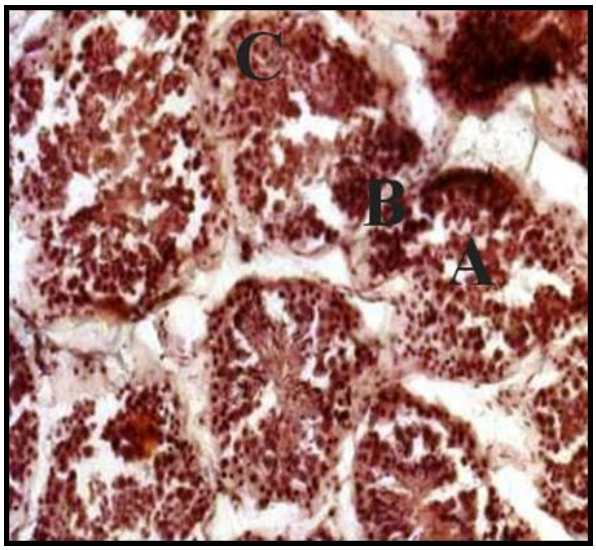

Fig. 2: Slide of normal testis obtained from cadaver showing

(A) Normal Somniferous Tubules
(B) Normal Basement Membrane
(C) Presence of Spermatogonia within the Somniferous Tubules

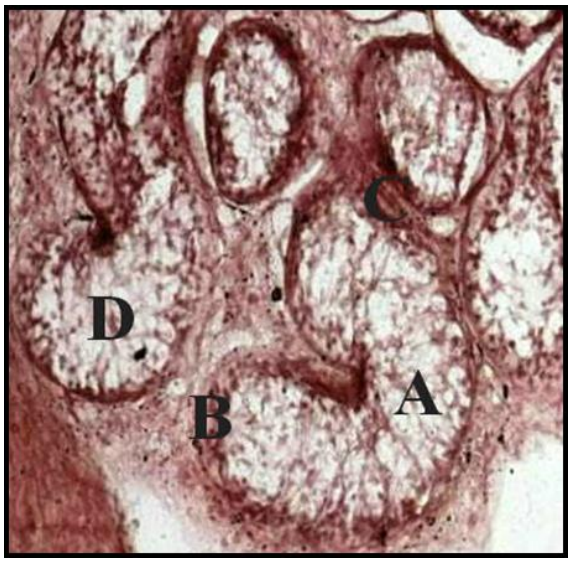

Fig. 3: Slide of undescended testis showing

(A) Distorted Somniferous Tubule
(B) Thickened Basement Membrane
(C) Peritubular Sclerosis
(D) Absence of Spermatogonia

DISCUSSION: The basement membrane is the thin supporting layer placed between basal surface of epithelium and underlying connective tissue. This membrane is not seen very clearly in most of the organs in H & E (haematoxylin and eosin) preparations. However when stained with PAS technique it appears as a well-defined magenta (pink) layer. This colour reaction is due to presence of carbohydrates in the basement membrane.[9] The term basement membrane is mostly used in light microscopy and basal lamina in electron microscopy.[9]

The lamina propria of the human Somniferous tubules consist of five to seven layers of flattened cells separated by laminae of extracellular connective tissue components consisting of connective tissue fibres and amorphous ground substance.[10] The inner three to four cellular layers consist of myofibroblasts (myoid cells) and the outer one to two layers of fibroblasts.[7] The Somniferous tubular epithelium is separated from rest of the lamina propria by the basement membrane. The basement membrane is an acellular, amorphous layer of extracellular matrix and is normally 0.1 to 0.2µm thick.[11]

In conditions producing alteration in spermatogenesis, the lamina propria, inclusive of the basement membrane, shows a considerable increase in thickness. This thickening is predominantly attributed to an increase in the extracellular matrix between the cellular layers. The inner layers of myofibroblasts lose their myoid quantities and are transformed into fibroblasts.[12] The newly formed fibroblasts also participate in secretion of extracellular matrix components, further contributing to the increased thickness of the lamina propria.[7] In our study considerable degree of thickening of BM was observed in all specimens of cryptorchid testes. Ultra structurally the basement membrane consist of three layers, lamina lucida, lamina densa and lamina reticularis.[13]

Sertoli cells are the only non-germinal components of the Somniferous tubule and have an important secretory function, producing Somniferous tubule fluid and several peptides, proteins and steroids.[14] The thickness of STBM in in undescended testis was 2.2µm at 6years and increased with age to 9.9µm at 24 years.[8] In our study the STBM in cryptorchid testis in the age group 30-40 years was recorded to range from 12.02-12.80µm.

The synthesis and secretion of extracellular matrix components by peritubular cells are involved in the formation of the STBM and are important in maintaining the structural integrity of Somniferous tubules and in promoting structural differentiation of the cells and spermatogenesis.[15] Vast majority of Somniferous tubules with normal lamina propria display intact spermatogenesis whereas a regular lamina propria is rarely found in Somniferous tubules with impaired spermatogenesis[16]

CONCLUSION: Thickening of STBM coupled with peritubular sclerosis may result in spermatogenic dysfunction as BM also provides metabolic support to the underlying supporting tissue.

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