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Immunohistochemical Studies on Human Testicular Peritubular Matrix — Comparative Data

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The importance of testicular peritubular matrix is well established but only few data have been reported on significance of basic matrix components and interstitial microvasulature as well as the relationship between small blood vessels and Lyedig cells. In this respect we aimed: 1) to study morphological changes in basal membrane of seminiferous tubules under pathological conditions; 2) to investigate comparatively immunohistochemical localization and distribution of basic matrix proteins. Biopsical materials from 16 men were processed for immunohistochemistry according to classical technique and monoclonal antibodies against collagen type IV, laminin and fibronectin were used. The results showed strong immunoreactivity of matrix proteins that revealed specific localization and distribution. The intensity of immune reaction corresponds to the degree of testicular lesions. In conclusion, our results indicate that the peritubular matrix with its basic components and functional complex Sertoli cells — peritubular myofibroblasts are of great importance for the normal process of testicular morphogenesis.

Key words: peritubular matrix, immunohistochemistry, matrix proteins.

Introduction

Different forms of disturbance in spermatogenesis are accompanied with thickening of testicular lamina propria. It is composed of basal membrane (2-6 layers elongated myofibroblasts), that support the seminiferous epithelium and layers of connective tissue fibres and amorphous substance as well [1, 3]. Alterations in lamina propria as a result of pathological processes were reported by S a l o m o n e and H a d i n g e r [7]. In men with disturbances in fertility some adverse changes in testicular extracellular matrix have been seen. To what extent myofibroblasts and fibroblasts are involved in process of formation of lamina propria in normal and under pathological conditions was investigated immunohistochemically by D a v i d o f f et al. [2]. An increase in intercellular components that include collagen and "basal membrane-material" surrounding myofibroblasts were observed [1] and abnormally thickened basal membrane was demonstrated [4, 5].

In infertile men increased peritubular matrix has been seen not only in peritubular tissue, but also in interstitial tissue, around Leydig cells and small blood

vessels so called testicular microvasculature [2]. Leydig cells are surrounded by more or less increased amount of peritubular matrix components. In these cases spermatogenesis was arrested at stage of primary spermatocytes and in some cases at spermatogonial stage [6]. It is supposed that male infertility is connected with abnormal thickening of peritubular tissue — lamina propria. [2, 3].

In men testicular interstitial and peritubular tissues interact in intertubular space and the basic components of this space are: ✓small blood vessels (microvasculature); ✓Leydig cells;✓macrophages and ✓peripheric nerves.

The present study focused on structural and functional integrity between the elements of peritubular tissue and testicular interstitium that is interrupted under pathological conditions. Abnormally thickened peritubular matrix is a real obstacle for transport not only of nutritions, but also of regulatory substances.The aim of the present study was to study morphological changes in basal membranes under pathological conditions and immunohistochemical localization and distribution of collagen type IV, laminin and fibronectin.

Material and Methods

Material was obtained from testicular biopsies of 16 infertile men. Data obtained from semen analysis showed that in 5 of them oligoastenozoospermia II-III degree was observed and in 3 — azoospermia. In two of the patients normospermia was established and they served as controls.

Biopsical materials for routine light microscopy and immunohistochemistry were fixed in Bouin's and classical immunohistochemical technique was used. Paraffin sections (7 μm) was prepared and on some of them adhesive technique with Poly-L lysine was applied. For the purposes of immunohistochemistry avidin-biotin-peroxidase method was used. Sections were deparaffinized and incubated with monoclonal antibodies against collagen type IV (Clone COL-94), laminin (Clone Lam-89) and fibronectin (Clone Fn-3E2). After the incubation with second biotinylated antibody the avidin-biotin-peroxidase complex was applied and aminoethyl carbasole (AEC) was used as substrate. Histostain kit from DAKO was used.

Results

Results from present study showed morphological alterations in seminiferous tubules that are expressed with: cessation of germ cell maturation at stage of primary spermatocytes (in patients with oligoastenozoospermia II-III dg.) and in cases with azoosprmia — at spermatogonial stage. Basal membrane is abnormally thickened, increased production of peritubular matrix and initial fibrosis are observed. Small blood vessels in interstitium are with thickened wall and process of fibrosis is evident. Some seminiferous tubules are with diminished lumen or lost their lumen. Dislocation and proliferation of myofibroblasts are observed (Fig. 1, Fig. 2 and Fig. 3)

The results obtained from immunohistochemical examination showed positive immune reaction for collagen type IV and its distribution in peritubular matrix (Fig.4). It was localized in basal membranes. Positive immune reaction is observed not only in peritubular connective tissue, but also in interstitial tissue — around Leydig cells and small blood vessels (Fig. 5 and Fig. 6).

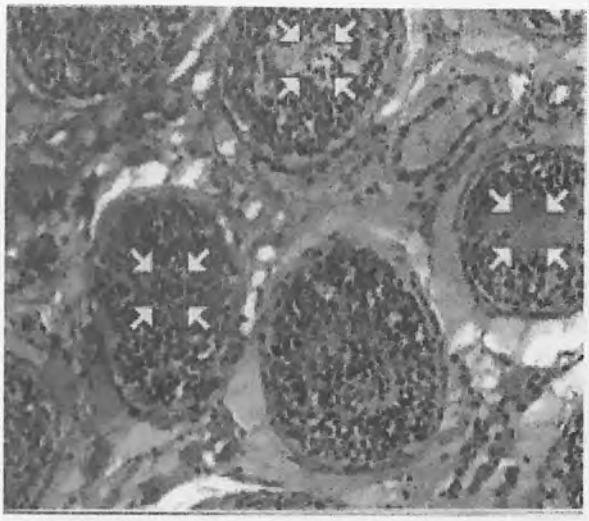

Fig. 1. Testicular biopsy. Azoospermia. Increasing production of peritubular matrix. The seminiferous tubules are with diminished lumen or lost completely their lumen (white arrows). Haematoxyline- Eosine staining (× 200)

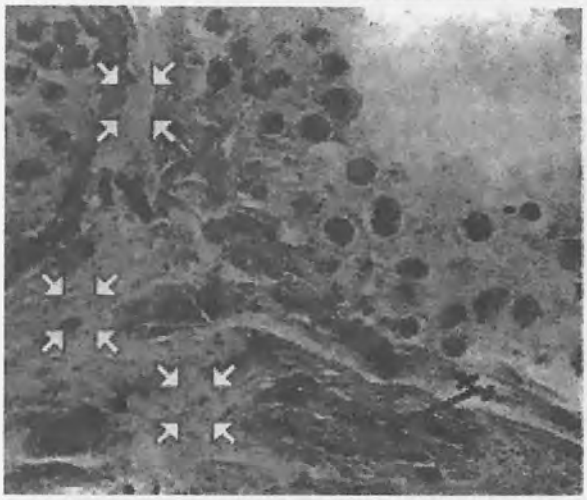

Fig. 2. Testicular biopsy. Oligoastenozoospermia III dg. Dislocation of myofibroblasts and increased production of collagen in peritubular matrix, basal membrane is fragmented and folded (arrows). Haematoxyline-Eosine staining (× 400)

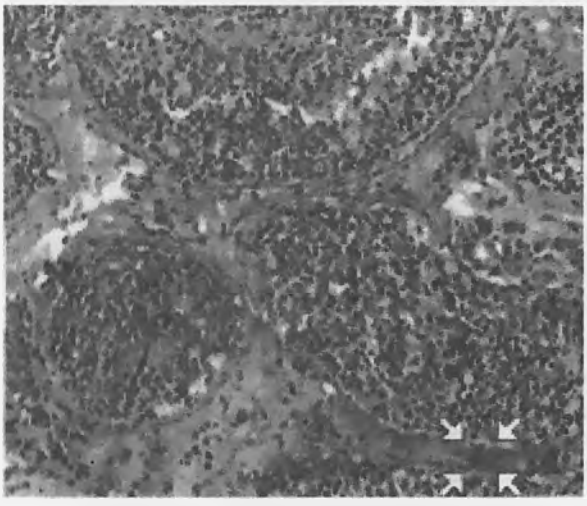

Fig. 3. Testicular biopsy. Azoospermia. Disorganization of germinal cells with intertubular cessation of maturation. The basal membrane is thickened (arrows). Haematoxyline-Eosine staining (× 200)

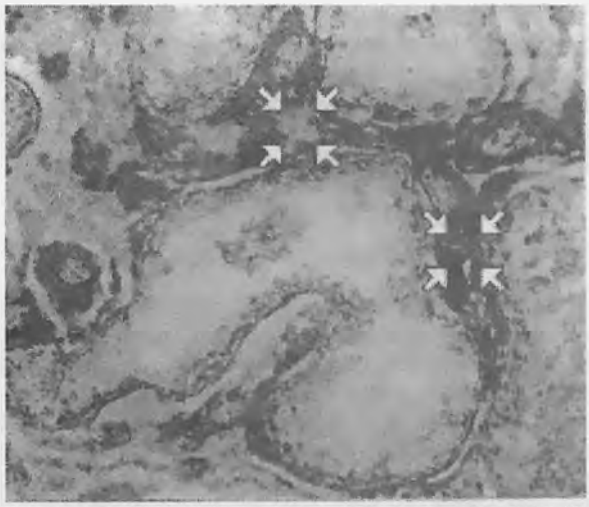

Fig. 4. Testicular biopsy. Azoospermia. Complete disorganization and massive loss of germ cells. Possitive immune reaction showing localization of collagen type IV in peritubular tissue, interstitium and around blood vessels and Leydig cells (arrows) (× 400)

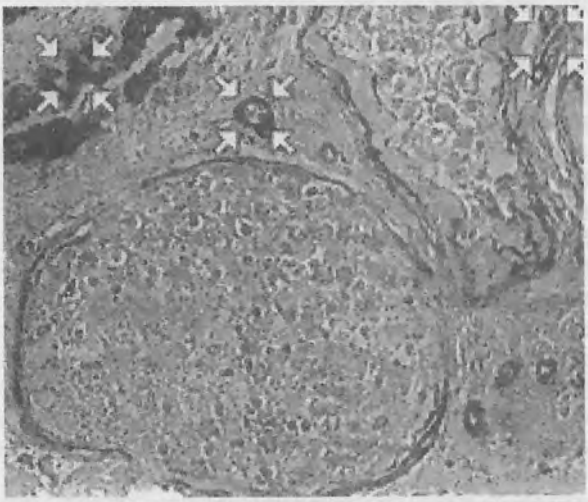

Fig. 5. Testicular biopsy. Oligoastenozoospermia III dg. Positive immune reaction showing localization of collagen type IV in basal membrane. The positive immunostaining for collagen type IV is observed in interstitium as well as around blood vessels (× 400)

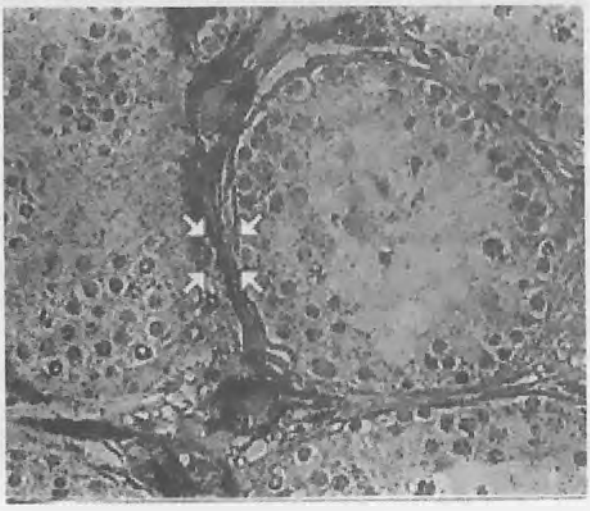

Fig. 6. Testicular biopsy. Oligoastenozoospermia III dg. Positive immune reaction for collagen type IV, showing its localization in both layers of basal membrane of seminiferous tubules and around Leydig cells in the interstititum (arrows) (× 400)

Positive immunostaining for fibronectin was observed in peritubular tissue. It was localized in fibroblasts, myofibroblasts and in Sertoli cells of the seminiferous tubules (Fig. 7 and Fig. 8). A strong immune reacion for laminin was found in basal membrane of seminiferous tubules, especially in inner layer of basal membrane (Fig. 9). Positive immunostaining is also observed in interstitial tissue and around blood vessels.

Discussion

The increased peritubular matrix is an obstacle for transport of nutritious and of regulatory substances as well. As an element of haematotesticular barrier the basal membrane is vulnerable in pathological processes. Our results clearly indicate fragmentation and folding of basal membrane in patients with oligoasthenozoospermia III degree and azoospermia on the base of pseudocryptorchidism. Matrix proteins ensure normal proceeding of spermatogenesis and contribute for structural integrity, for cell-cell and cell-matrix interactions and thus they are important for testicular morphogenesis. As main structural component of basal membrane collagen type IV is involved in tissue differentiation, morphogenesis and in the process of repair. It also plays an important role in tissue structure, metabolism and cell differentiation and migration. Collagen type IV is accumulated on sheafs and is presented in all layers of basal membrane. Collagen serves for connection of myoid cells one to one and sets them firmly to elastic and collagen fibrils, presenting in basal membranes [2]. The authors showed the relation of myoid cells with the elements of connective tissue. We have established thickened and folded basal membrane associated with its invagination and initial fibrosis in patients with pseudocryptorchidism. Sertoli cells and peritubular myoid cells are in close relationship during different stages of spermatogenesis and they influence on basal membrane structure and components of extracellular matrix [7]. This hypothesis was supported by our previous data [6]. An extraordinary expansion of the intercellular layers of matrix components of the connective tissue that was observed by D a v i d o f f et al. [2] is probably responsible for the thickening of the lamina propria of the seminiferous tubules. The results of the current study demonstrate that laminin as a highly-specific, tissue-specific and cell-specific factor is localized mainly in basal membrane of the seminiferous tubules, as in capillaries of the interstitial tissue and around Leydig cells. Other earlier studies [4] pointed out the fact that laminin is a major component in all the basal membranes and it is involved in cell migration and cell differentiation. We found that laminin is localized in outer layer of basal membrane, collagen type IV is observed in both layers and most intensively laminin is accumulated in the layer of peritubular myofibroblasts. On the base of these results we postulate that laminin may play an important role in morphogenesis and structural stability of seminiferous tubules and peritubular myofibroblasts are surrounded by collagen type IV [6] and laminin. The immunohistochemical data showed the localization of fibronectin in inner layer of lamina propria. Our results suggest the possible role of fibronectin in Sertoli cell-peritubular myofibroblast complex that is essential for cell adhesion and formation of basal membrane and functional units in testicular peritubular matrix.

In conclusion, our results indicate that the peritubular matrix with its basic components and functional complex Sertoli cells-peritubular myofibroblasts are of a great importance for the normal process of testicular morphogenesis. In patho-

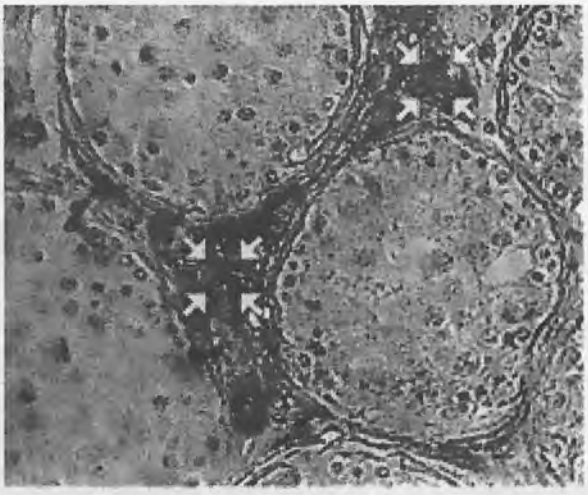

Fig. 7. Testicular biopsy. Azoospermia. Positive immune reaction for fibronectin, showing its localization in myofibroblasts and in Sertoli cells (black arrow) (× 400)

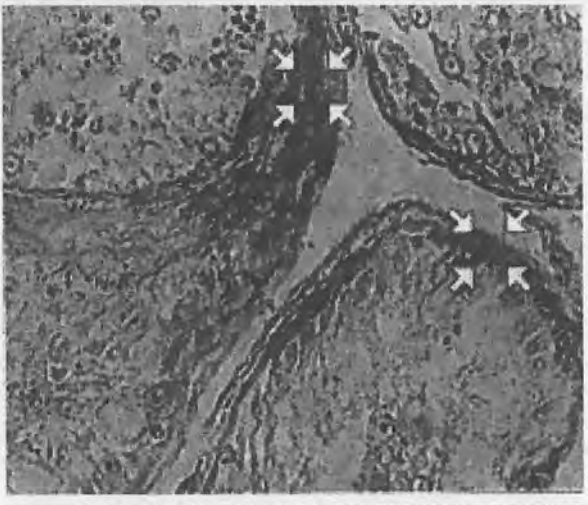

Fig. 8. Testicular biopsy. Azoospermia. Positive immune reaction for fibronectin, showing its localization in peritubular tissue (myofibroblasts and at some places in inner layer of basal membranes of seminiferous tubules) (× 400)

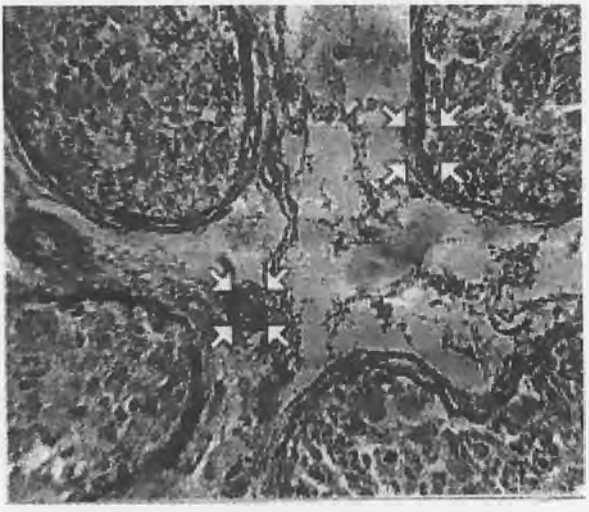

Fig. 9. Testicular biopsy. Oligoastenozoospermia III dg. Positive immune reaction for laminin. It is localized in interstitial tissue, around blood vessels. In particular, the localization of laminin is seen very well in inner layer of basal membranes of seminiferous tubules (× 400)

logical conditions this complex together with basal membrane (as one of the constituents of haemato-testicular barrier) are vulnerable to different ethiological factors and that makes worse the prognosis of male fertility.

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