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Immunoexpression of Atrial Natriuretic Peptide (ANP) in Testes of Infertile Men

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Recently ANP was established in different cell types in rat testis and possible participation in germ cell development and steroidogenesis was proposed. Localization of ANP in human testis has been insufficiently examined as data are lacking for the immunoexpression of ANP in testes with disturbances in spermatogenesis. In the current study we aimed to investigate immunohistochemically the localization and distribution of ANP in testes of infertile men. Material for immunohistochemical analysis was received from testicular biopsies of 10 patients with disturbances in spermatogenesis and low levels of testosterone. Results showed positive immune reaction for ANP in seminiferous tubules in cytoplasm of spermatogonia, primary spermatocytes and Sertoli cells. Strong immunoreactivity was found in basal membrane, myofibroblasts, Leydig cell cytoplasm and in blood vessels in testicular interstitium. Localization of ANP in testes with disturbed spermatogenesis suggests a possible local production independent of testicular lesions and low levels of testosterone.

Key words: ANP, testis, infertile men, immunohistochemistry.

Introduction

It was established that atrial natriuretic peptide (ANP) is synthesized in cardiomyocytes, but its gene and receptor are widely expressed in other extracardiac organs including female and male gonads. By means of immunohistochemical methods it is possible to establish ANP localization as to elucidate the role of ANP in regulation of capillary transport, local regulation in the testis and at last but not least its participation in the processes of spermatogenesis.

ANP immunoreactivity was reported in frog spermatozoa [12]. The presence of ANP receptors in mouse germ cells was reported [10]. According to data published by M ü l l e r and M i d d e n d o r f f [9] basic place of ANP immunoexpression in mouse testes are the seminiferous tubules and cytoplasm of Leydig cells, as well. ANP is established in the cytoplasm of Sertoli cells that are known [15] to play a key role in the control of spermatogenesis. The presence of natriuretic peptide in seminiferous tubules even in the absence of Leydig cells showes that possibly Sertoli cells synthesized ANP and this fact itself emphasized that ANP is synthesized independently of the production

of testosterone and this is supported by several authors [8, 11, 13]. The mechanism of the influence of ANP under testosterone production is receptor-assisted increasing the levels of cyclic guanosine monophosphate in target cells. ANP stimulates the membrane bound form of guanilate cyclase and in the same time inhibits adenylate cyclase and thus it increases the accumulation of intracellular cyclic guanosine monophosphate and decreases the levels of cyclic adenosine monophosphate. The authors supposed that Leydig cells are target cells because of the presence of a large number of specific granules in them. These data are supported by the results of Panday and Ogrebin-Crist [10]. Localization of ANP in the acrosoma and flagellum of human spermatozoa was described by [1] and they supposed its role in the capacitation, motility and development of spermatozoa, as induction of hemotaxis – all these promote for normal fertile abilities. Receptors for natriuretic peptides in seminiferous tubules and possible participation of ANP in the processes of germinal differentiation are established [4] and its participation in the regulation of tubular transport, peristaltic activity and cell metabolism was suggested.

Widely examination of distribution of ANP in different cell types in rat and mouse testes is based on its possible participation in the processes of germ cell differentiation and steroidogenesis. Localization of ANP in human testis is still not enough investigated and the data are lacking for its immunoexpression in testes of men with disturbances in spermatogenesis and fertile disorders. That's why in the current study we aimed to examine the localization and distribution of ANP in testes of infertile men and to established correlation between ANP immunoreactivity and degree of testicular lesions.

Material and Methods

Material for immunohistochemical study was received from testicular biopsies of ten patients with disturbances in spermatogenesis and low levels of testosterone production. Results received from semen analysis showed in three of the patients oligoasthenozoospermia III dg., in five azoospermia. Two of the patients were with oligoasthenozoospermia I dg. without any morphological alterations. Paraffin sections were cut (7 μm) for morphological and immunohoistochemical studies. Sections were deparaffinized and processed according to the classical immunohistochemical technique using mouse monoclonal antibody 6C3 raised against human ANP that was generated and characterized in the Institute of Biology and Immunology of Reproduction – BAS [7]. Sections were incubated with anti-ANP antibody for 12 h. After washing with PBS, the sections were incubated with second biotinylated rabit anti-mouse antibody (1:200). Then avidin-biotin-peroxydase complex was applied and DAB was used for visualization of immune reaction.

Results

Results received from the current morphological examination showed disturbances in spermatogenesis that is expressed with cessation of the maturation at stage of primary spermatocytes and in severe cases such as azoospermia – at spermatogonial stage. At some places myofibroblasts were missng (Fig.1). Some of the seminiferous tubules were with destructive tubular wall and mainly spermatogonia remained in the tubules (Fig.1). Basal membrane was folded and thickened. Desorganization of germ epithelium was evident (Figs 2 and 3).

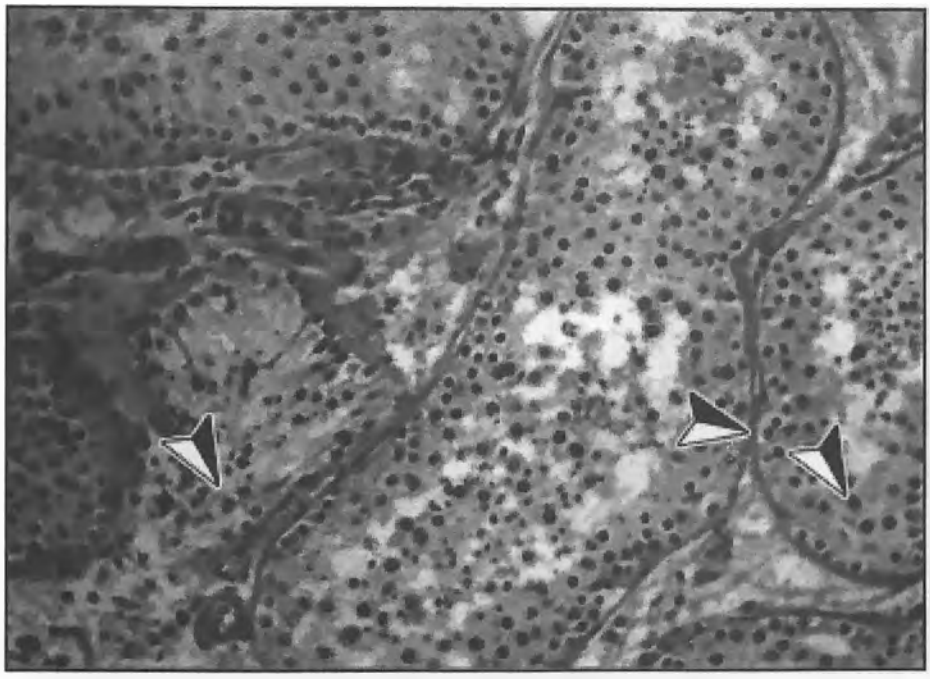

Fig. 1. Testicular biopsy. Oligoastenozoospermia I dg. Disturbed spermatogenesis expressed with cessation of maturation at stage of primary spermatocytes and spermatogonia (right arrow). At some places myofibroblasts are missing (middle arow). Seminiferous tubule with destructive wall and mainly spermatogonia are seen (left arrow). Haemotoxyline- eosine staining (Microphoto × 200)

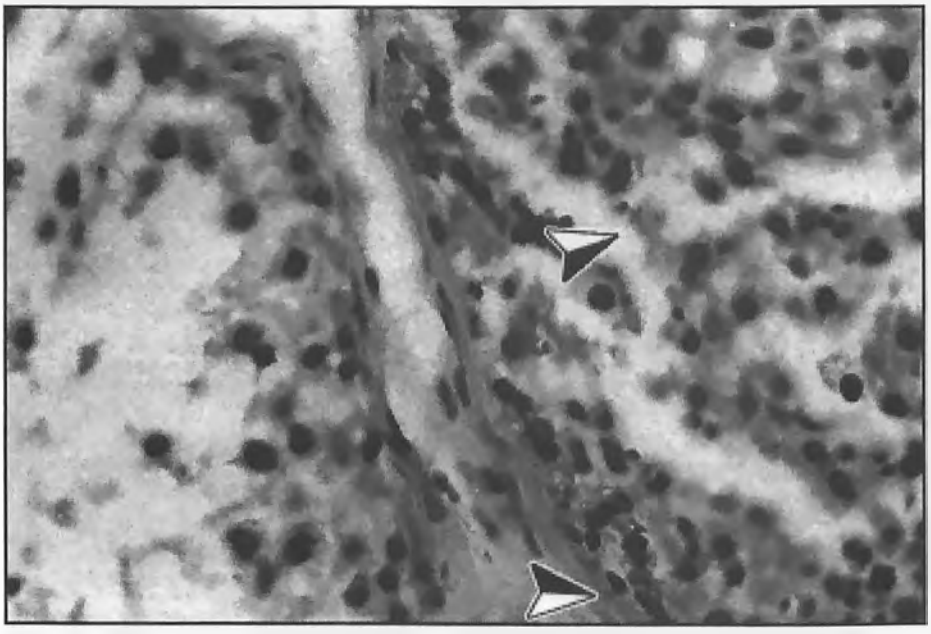

Fig. 2. Testicular biopsy. Azoospermia. Thickened basal membrane (bottom arrow) and disorganization of germinal epithelium (middle arrow). Haematoxyline-eosine staining (Microphoto × 400)

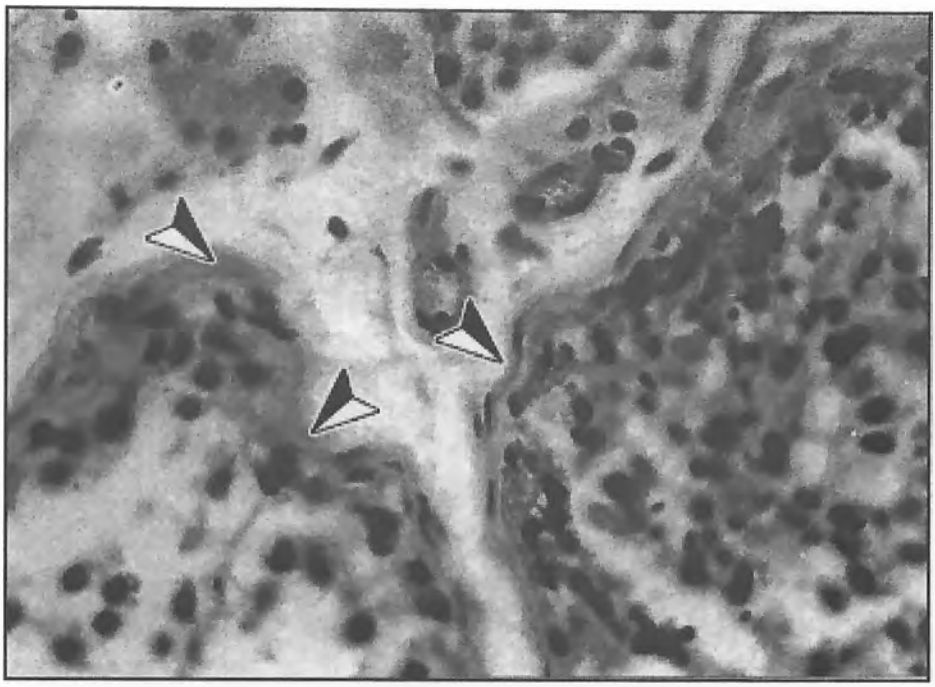

Fig. 3. Testicular biopsy. Azoospermia. Thikened and folded basal membrane (arrows). Desorganization of germinal epithelium. Myofibroblasts are missing. Haematoxyline- eosine staining (Microphoto × 400)

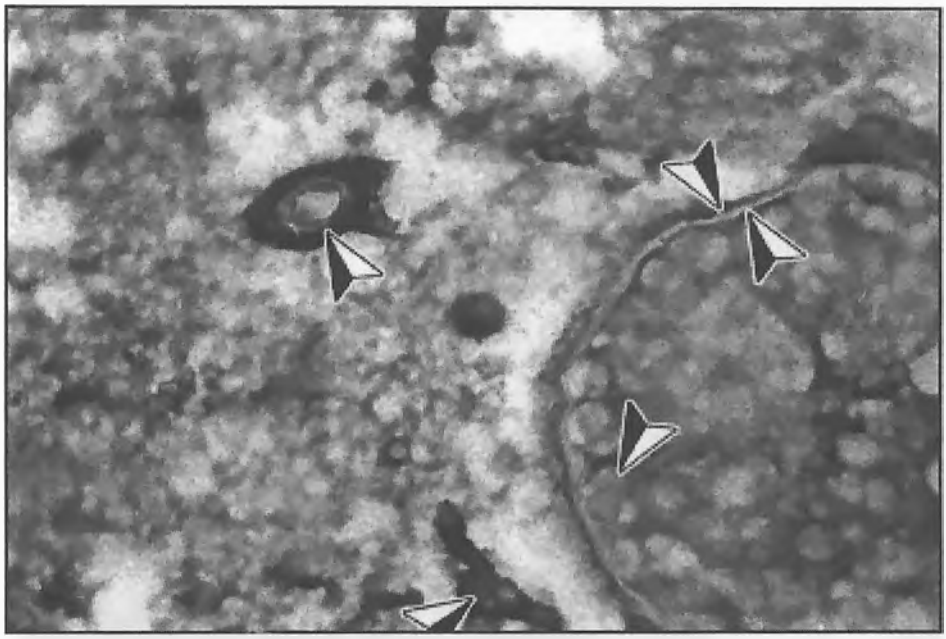

Fig. 4. Testicular biopsy. Oligoasthenozoospermia III dg. Immunoexpression of ANP in inner and outer layer of basal membrane (right arrows), in cytoplasm of germ and Leydig cells (bottom arrows), in the wall of blood vessels (left top arrow). Monoclonal anti-ANP antibody (Microphoto × 200)

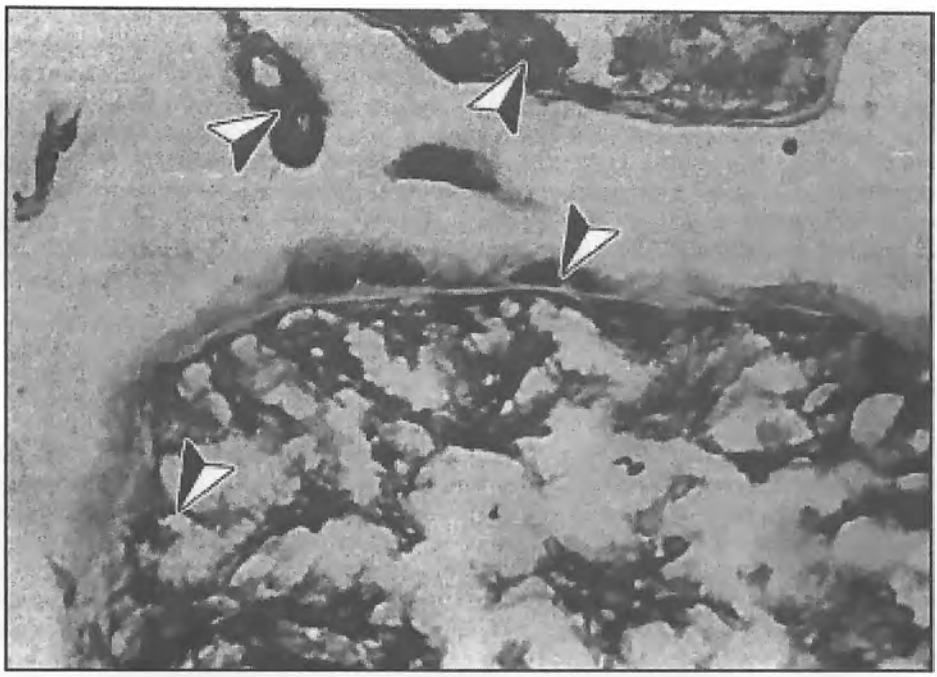

Fig. 5. Testicular biopsy. Azoospermia. Immunoexpression of ANP in germ and Sertoli cells (middle top arrow), in blood vessels (left top arrow) and in the cytoplasm of myofibroblasts (central arrow). Monoclonal anti-ANP antibody (Microphoto × 400)

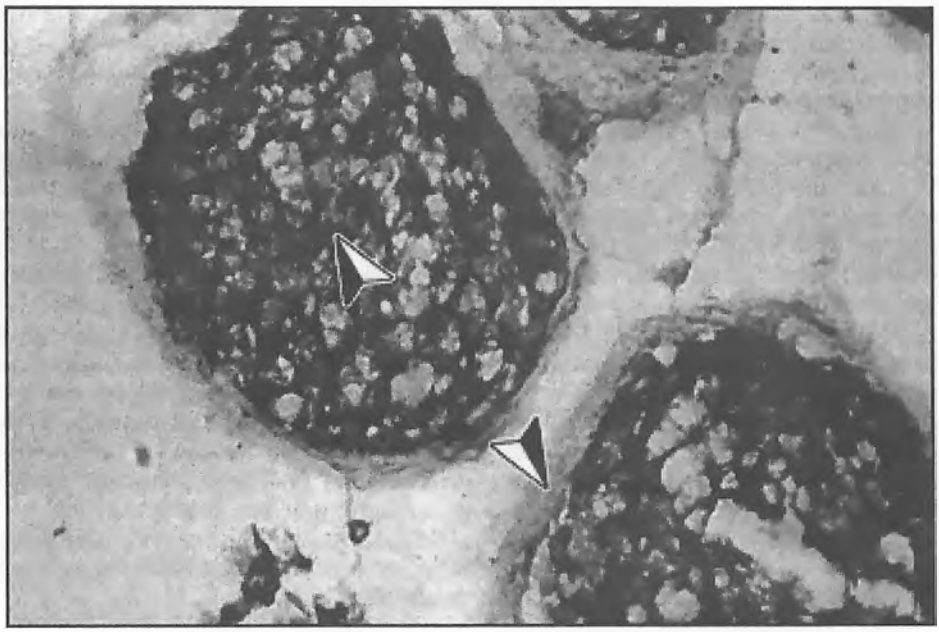

Fig. 6. Testicular biopsy. Azoospermia. Negative immune reaction in basal membrane (right arrow). Very strong immune reaction in cytoplasm of germ cells (left arrow). Monoclonal anti-ANP antibody (Microphoto × 200)

Results obtained from immunohistochemical study showed localization of ANP in inner and outer layers of basal membrane. Strong immunoreactivity was observed in the wall of small blood vessels (Fig. 4). Intensive immunostaining for ANP was observed in cytoplasm of germ cells (spermatogonia) and in cytoplasm of myofibroblasts (Fig. 5). Strong immune reaction was seen in the walls of small blood vessels. At some places where basal membrane was destructive or where myofibroblasts were missing the immune reaction for ANP was negative (Fig. 6, 7, 8, 9, 10). In cases of azoospermia thickened blood vessels wall was observed and initial process of hyalinization was evident and immune reaction was weak or negative, as well (Fig. 8). Leydig cells looked small in number and the immunoexpression of ANP in their cytoplasm was well pronounced (Fig. 10).

Discussion

Our results revealed that ANP was widely distributed in testes of infertile men. The immune reaction in seminiferous tubules was observed in cytoplasm of spermatogonia, primary spermatocytes and Sertoli cells and these findings supported data presented by [2, 9]. So far, data for immunoexpression of ANP in myofibroblasts have not been presented and our results from immunohistochemical study showed strong reaction in inner layer of basal membrane and as well as an intensive immunostaining in cytoplasm of myofibroblasts. We also found immuonexpression of ANP in cytoplasm of Leydig cells that supported the data reported by many authors [5, 6, 8, 11,13].

In relation to testicular lesions, on the one hand, we established that disorganization of germ epithelium, folded and destructive basal membrane were associated with reduction or lack of immunostaining for ANP in the membrane. On the other hand, the immune reaction was strong in the cytoplasm of germ and Leydig cells which looked small in number, corresponding to low levels of testosterone. The presence of ANP in seminiferous tubules with germ cell depletion we found even in the absence of Leydig cells suggested possible local synthesis of ANP by Sertoli cells that it is independent on germ cell degeneration and low testosterone production.

Therefore locally synthesized ANP might has autocrine and/or paracrine actions and is supported by B e n t o n et al. [3]. It has been considered by M ü l l e r and M i d d e n d o r f f [9] that basic place of expression of ANP – receptor in testes are germ cells which put up a question whether Leydig cells or seminiferous epithelium are dominant in ANP action. Seminiferous tubules, particularly Sertoli cells produce factors which stimulates Leydig cells differentiation and function and as ANP is synthesized in seminiferous tubules it may has a local stimulating effect on Leydig cells [14].

Presence of ANP in endothelial cells [4] and its inhibitory function on contractility of smooth muscle cells was reported by S u e n o b u [16]. Our results on infertile men revealed strong immune reaction for ANP in the wall of interstitial blood vessels in patients with oligoastenozoospermia II dg, as in severe cases with oligoastenozoospermia III dg. In azoospermic patients we observed thickened blood vessels wall and weak or negative immune reaction for ANP. These results are new finding for immunoexpression and localization of ANP in testicular microvasculature in testes of infertile men.

In conclusion, our data that ANP is widely expressed in the testis of infertile men suggest its key role for human spermatogenesis and provide new understanding for the importance of ANP for testicular function and fertility.

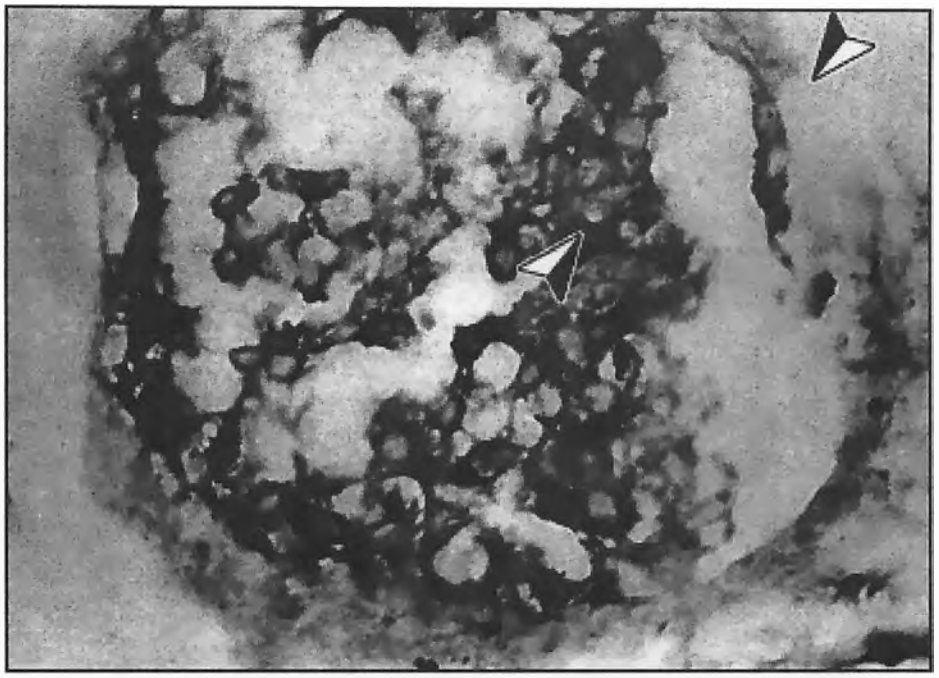

Fig. 7. Testicular biopsy. Azoospermia. At some places where myofibroblasts are missing and basal membrane is destructive and folded, the immune reaction for ANP is weak or negative (left arrow). Positive immune reaction in germ cells (central arrow). Monoclonal anti-ANP antibody (Microphoto × 400)

Fig. 8. Testicular biopsy. Oligoastenozoospermia III dg. Immunoexpression of ANP in germ cells. Immune reaction is weak in basal membrane (bottom arrows), but it is strong in interstitium, in Leydig cells and moderate to weak in the thickened wall of blood vessels (central arrow). Monoclonal anti-ANP antibody (Microphoto × 200)

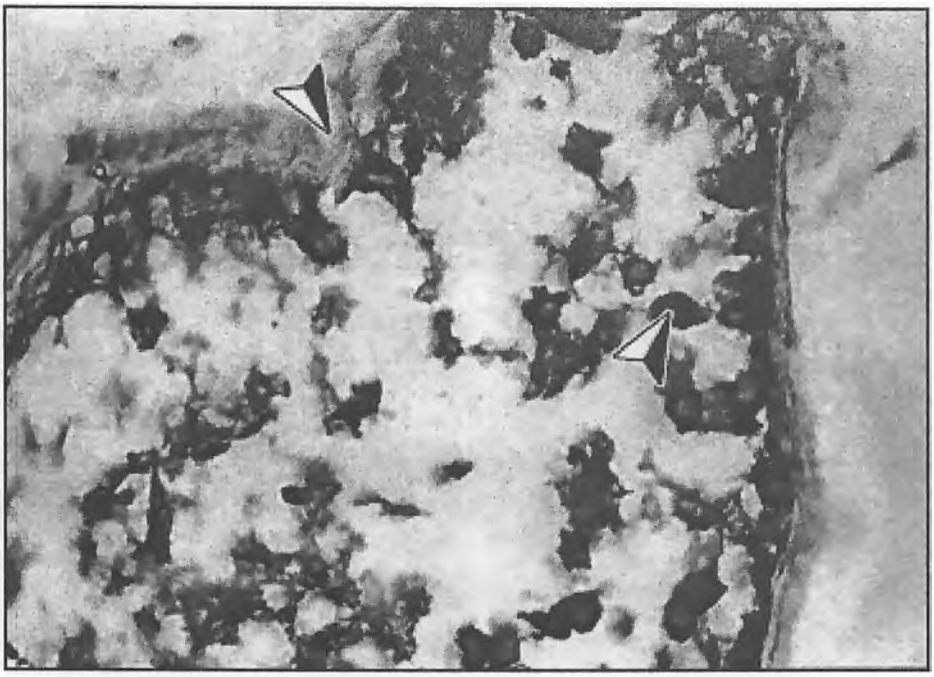

Fig. 9. Testicular biopsy. Azoospermia. Immunoexpression of ANP in germ cells. Immune reaction is weak in destructive basal membrane (left top arrow). Myofibroblasts are not seen and primary spermatocytes as well. Only spermatogonia are present and the immune reaction for ANP is very strong (right arrow). Monoclonal anti-ANP antibody (Microphoto × 400)

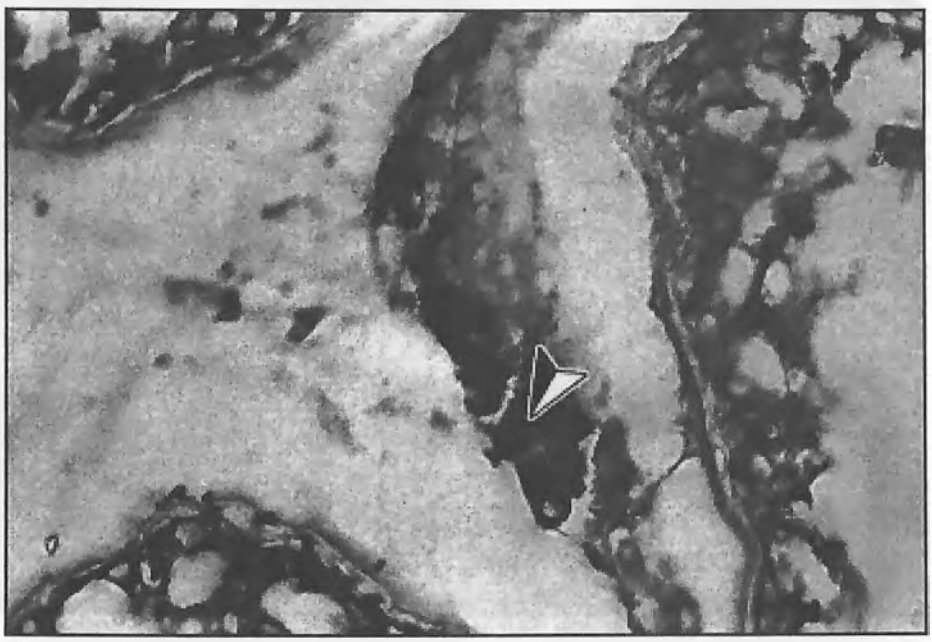

Fig. 10. Testicular biopsy. Azoospermia. Folded basal membrane and strong immune reaction in testicular interstitiuim. Immune reaction for ANP is very strong in cytoplasm of Leydig cells (arrow). Monoclonal anti-ANP antibody (Microphoto × 400)

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