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Morphological Alterations in Rat Testis during Aging

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The present study aimed to investigate dynamic of the changes in rat aging testis using specific immunohistochemical markers. Aging of the testis is manifested by germ cells (GCs) loss, reduced responsiveness of Sertoli cells (SCs) to androgens and suppression of steroidogenesis in Leydig cells (LCs). Thickened basal lamina and vessel wall proved by immunohistochemistry for α-smooth muscle actin are indicative for disturbed communication between seminiferous epithelium (SE) and interstitium as well as altered testicular trophic during ading.

Key words: Sertoli cells, Leydig cells, spermatogenesis, aging testis.

Introduction

The functions and histology of the testis during development and in the adults are well described but the effects of aging on the testis have not been as intensively studied. Aging of the testis is accompanied by loss of germ cells (GCs) leading to a decline sperm production [3]. Studies by C h e n et al. [1] have shown that the production of testosterone (T) per Leydig cells (LCs) is reduced with aging, but the numbers of LCs per testis do not change, suggesting that changes to individual cells account for reduced serum T levels. The abnormalities and loss of SCs seen in aging testis are probably involved in disruption of Sertoli cells—germ cells communications. Aging of seminiferous epithelium (SE) is associated with thickening of basal lamina that created an additional barrier for nutrient and regulatory factors form the interstitium [4]. The precise mechanisms underlain the alterations in aging testis remain unknown yet. In this respect **the objective** of the present study was to investigate dynamic of the changes in rat testis using specific immunohistochemical markers and to characterize relationships between different testicular cell types during aging.

Materials and Methods

Testes from Lewis rats were sampled at different ages (2, 7, 12, 15, 18 and 21 months), fixed in Bouin's, embedded in paraffin and cult into 5 μ. Immunohistochemistry was performed for: 1) α-smooth muscle actin using mouse Mo Ab (Sigma, UK) in dilution 1:5000); 2) 3β-hydroxy steroid dehydrogenase (3β-HSD) using rabbit Poly Ab (1:1000) kindly provided by Professor I. Mason, Edinburgh University; 3) Androgen receptor (AR) using rabbit Poly Ab (Santa Cruz Biotechnology, USA) diluted 1:200. Measurement of seminiferous tubule (ST) diameter was carried out.

Results

The seminiferous epithelium of the rats of 2 to 12 months of age exhibited intact spermatogenesis with all the steps of germ cell development. By 18 months of age, both normal and altered tubules were observed (not shown). At 21 months tubules with regression of SE were predominantly present. Some germ cells sloughed off into the lumen. The altered tubules were smaller in size, with a thickened basement membrane and a disrupted spermatogenesis. Quantitative analysis of ST diameter demonstrated significant and progressive age-related reduction by 15-30% (Fig. 1).

Between 2-6 months, the basal lamina of the ST was visualized as a thin layer. At the age of 12-18 months, the basal lamina is thicker, with irregular contour. Immunohistochemical study showed positive reaction for β-smooth muscle actin in basal membrane of the ST and peritubular myofibroblasts, as well (Fig. 2A). Strong

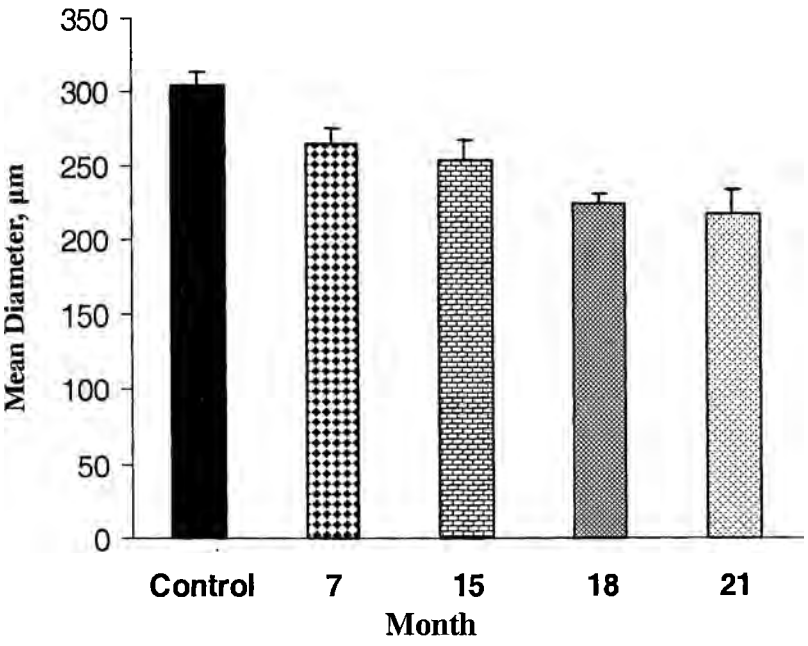

Fig. 1. Progressive age-related reduction in mean diameter of seminiferous tubules (μm). Data represent mean value ± SE. All data are significant compared to control

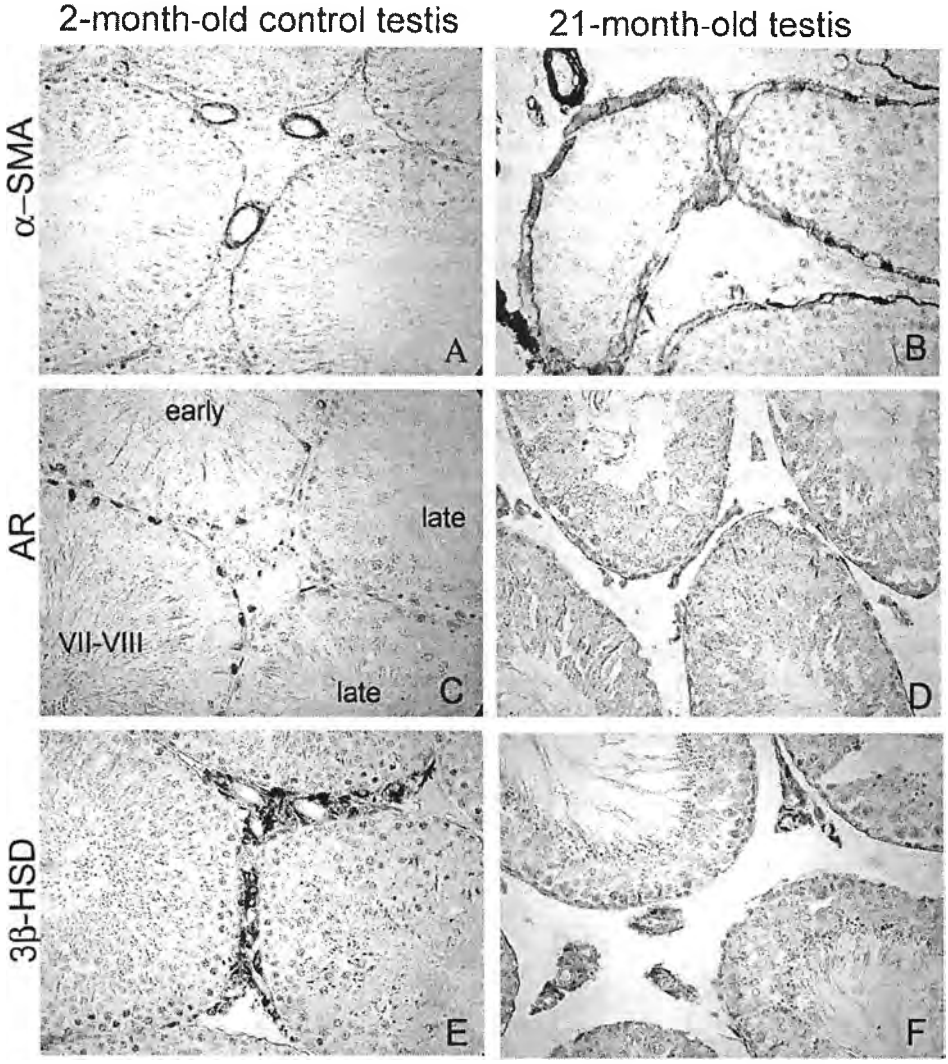

Fig. 2. Immunohistochemical visualization of α-smooth muscle actin (A, B); Androgen receptor (C, D); 3β-HSD (E, F) of 2-month-old control and 21-month-old aging rat testis. × 400

immunoreactivity was found in the testis of 21 months of age (Fig. 2B). Intense reaction was seen in the thickened wall of small blood vessels in the interstitium.

Normally, Androgen receptor (AR) is localized in the nuclei of Sertoli cells, Leydig cells, peritubular cells but not in germ cells. There was a stage specific pattern of immunoexpression of AR in SCs with maximal staining in stages VII-VIII of the spermatogenic cycle and lowest intensity in late (IX-XIV) stages (Fig. 2C). During aging we observed decrease in immunoreactivity of AR in the testicular cell types and there was lack of stage specificity in SCs at age of 21 months (Fig. 2D).

The interstitium of aging testes appeared to increase in size without any evidence for LCs hyperplasia. This appearance was simply a consequence of age-re-

lated tubular regression. Immunoexpression of 3β—HSD (marker for steroidogenic activity of LCs) was greatly reduced at age of 21 months compared to 2-month-old control (Fig. 2 E, F).

Discussion

In present study we investigated the influence of aging on the main cell types in rat testis. Our findings of a reduction of tubular diameter confirmed results by W a n g et al. [9] reporting significant age-related decrease in tubular volume, diameter and length and luminal volume. It is known that lamina propria displays pronounced changes in the period of aging that involved peritubular cells and intercellular matrix. [5, 6]. The thickening of the basement membrane in aging rats and humans was coincidental with changes in the blood-testis barrier and germ cells depletion [3, 6]. Our results for intense reaction in thickened basal lamina and vessel wall are indicative for disturbed communication between SE and interstititum as well as altered testicular trophic during aging. Androgens are especially important for maintenance of spermatogenesis in adulthood [2, 7] and their effects on GCs are mediated via androgen receptor localized in Sertoli cells. In our study we observed that the reduced AR immunoreactivity in SCs first occurred in androgen dependent stage (VII-VIII) and loss of stage specificity probably is associated with the functional alterations (decreased responsiveness to androgens) in SCs [8]. It is likely that SCs from aging testis are unable to provide adequate support for germ cells and to respond to selective signal from them. The abnormalities and loss of SCs seen in the adding testis might be responsible for disruption of the blood-testis barrier [3, 5] and germ cell depletion.

Our findings of reduced immunoexpression of 3β-HSD are indicative for suppressed steroidogenic activity of aging LCs that correlate with data for reduced serum T levels. According to Z i r k i n and C h e n [10] the reduced ability of aging LCs to produce T might be caused by events occurring outside or inside these cells that impinge upon them involving accumulation of free radicals. In rats, aging is associated with a decline in serum LH levels, suggesting that the reduced T production probably is due to chronic understimulation of LCs by LH [1]. The mechanism by which suppression of steroidogenesis results in a delay or prevention of age-related reductions of LCs ability to produce T remains uncertain [10].

In conclusion, our data suggest that altered trophic of seminiferous epithelium that occurred with aging is associated with decreased LC steroidogenesis and reduced androgen signalling in SCs.

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