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The changes of stage distribution of seminiferous epithelium cycle and its correlations with Leydig cell stereological parameters in aging men

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ABSTRACT

Purpose: To evaluate the changes of stage distribution of seminiferous epithelium cycle and its correlations with Leydig cell stereological parameters in aging men.
Methods: Point counting method was used to analyze the stereological parameters of Leydig cells. The stage number of seminiferous epithelium cycle was calculated in the same testicular tissue samples which were used for Leydig cell stereological analysis.
Results: The aging group had shown more severe pathological changes as well as higher pathologic scores than the young group. Compared with the control group, the volume density (V_V) and surface density (N_A) of Leydig cells in the aging group were increased significantly. The stage number of seminiferous epithelium cycle in the aging group was decreased coincidently compared to the young group. Leydig cell V_v in the young group has a positive relationship with stages I, II, III, V and VI of seminiferous epithelium cycle, and Leydig cell N_A and numerical density (N_V) were positively related to stage IV. However, only the correlation between N_V and stage II was found in the aging group.
Conclusions: The stage number of seminiferous epithelium cycle was decreased in aging testes. Changes in the stage distribution in aging testes were related to the Leydig cell stereological parameters which presented as a sign of morphological changes.

Introduction

Age-associated endocrine deficiencies in males are increasing in parallel to the aging population nowadays. It is generally accepted that testosterone is an important hormone in males which falls progressively with age [1,2]. A large number of males over 60 years old who have serum testosterone levels that were below the lower limits of young adult men are accompanied with a series of metabolic disease [1,3–5]. Meanwhile, the age-related testosterone deficiency syndrome or late-onset hypogonadism (LOH) may result in significant detriment in the quality of life and adversely affect the function of multiple organ systems [6].

Leydig cells which situated in the testicular interstitium can regulate spermatogenesis, maintain the accessory sex organs and erectile function through producing and secreting testosterone [7]. The testosterone secreted by Leydig cells also required in other organ systems for their proper functioning including brain, skin, muscle, liver, synovial tissue, bone, bone marrow, and kidney [7–9]. Therefore, the abnormal changes of Leydig cells may lead to decline of testosterone synthesis and play negative effect on the health of males [10].

The seminiferous epithelium cycle of human testes which involved in spermatogenesis can be divided into six stages according to the typical cellular association, and several stages (three on the average) could be seen in a single tubular cross-section [11]. Besides, the regulation of spermatogenesis is mediated by endocrine and testicular autocrine/paracrine factors via Leydig cells and Sertoli cells [12]. Different functional status of Leydig cells can modulate the process of spermatogenesis which reflects in the stages of seminiferous cycle [13]. Moreover, the progressive testicular involution with advancing age in men is a common phenomenon, and the degenerated changes such as molecular or ultrastructural abnormality of Leydig cells causing by aging

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may contribute to the decrease of testosterone production [14,15]. However, whether the morphological alterations of Leydig cells are responsible for the stage distribution changes of seminiferous epithelium cycle is remained unclear. It is important to find out that if the changes of stage distribution could suggest the functional status of Leydig cells. The present study was carried out to evaluate the changes of stage distribution of seminiferous epithelium cycle and its correlations with Leydig cell stereological parameters in aging men, aiming to expand the understanding of the pathological events occurred in the aging testes.

Materials and methods

Materials

Forty-nine testicular tissue samples obtained by orchiectomy from aging men with prostatic cancer (age 68–82 years, mean 76.8 ± 4.7 years) were served as aging group. These patients had one or more children. Control group comprised 16 testicular tissue samples obtained from young men with obstructive azoospermia underwent assisted reproductive treatment (age 25–30 years, mean 27.3 ± 2.5 years). Samples from patients receiving steroid treatment and chemotherapy within the last 6 months before surgery were excluded from this study. Moreover, patients with Sertoli cell only syndrome and testicular feminization were also excluded in this study. The patients were informed consent, and the experiment was approved by the ethics committee of First Affiliated Hospital of Jinan University.

Histological analysis

Testicular tissues were fixed by perfusion with Bouin's solution for 48 h at room temperature and then embedded in paraffin. Five-μm-thick paraffin sections were cut and stained with hematoxylin and eosin for histological analysis.

Ten round or nearly round seminiferous tubule profiles were selected and assessed by using Olympus IX51 microscope (Olympus Biosystem, Munich, Germany; magnification 100×). Eight items (tubule diameter, cell differentiation, cell density, lamina propria, cell necrosis, interstitial and vascular fibrosis, interstitial cell degeneration and interstitial edema) which scores from 1 to 5 were chosen to evaluate the testes pathologic phenomenon [16]. Total scores of each sub-scale was range from 1 to 40. The higher scores might suggest the more severe pathological changes.

Stereological procedures

Fifteen to twenty fields of view from each section were selected randomly in this study. Digital images were uniformly acquired and snapped at 200× or 400× magnification by Olympus IX51 equipped with a DP72 device camera. The diameter, area and perimeter of the seminiferous tubules were calculated by using image analysis software (Image Pro Plus6.0, Media Cybernetics, Silver Spring, MD, USA). Parameters of Leydig cells including volume density (V_V), surface density (N_A) numerical density (N_V) and the ratio of nucleus and cytoplasm (R_{np}) were measured by using point counting method based on the stereological theory [17].

Stage number score

Ten fields of light microscope from each cross section were chosen at random. The entire surface of the seminiferous tubules in these fields were classified and counted according to the different stages of the cycle observed [11]. The stages of seminiferous epithelium cycle were calculated in the same testicular tissues which used for Leydig cell stereological analysis.

Statistical analysis

All statistical analyses were carried out by using SPSS software (SPSS 19.0, Chicago, IL, USA). Data were expressed as mean ± standard deviation (SD), and *t*-test was used. Spearman's rho test was performed to analyze the correlation coefficients of the stereological parameters and the stages of seminiferous epithelium cycle. Differences were considered as statistically significant for $p < 0.05$.

Results

Histological characteristics and pathological assignment score

In contrast to the well-rounded seminiferous tubules and normal spermatogenesis in young group (Fig. 1a), seminiferous tubule atrophy, epithelium vacuolation and spermatogenic cell deficiency were frequently observed in aging group (Fig. 1b). Meanwhile, Leydig cell degenerations were also commonly existed in aging group (Fig. 1b).

The seminiferous tubular sections were divided into separate stages according to six typical cellular associations corresponding to six stages of the seminiferous epithelium cycle (Fig. 2). However,

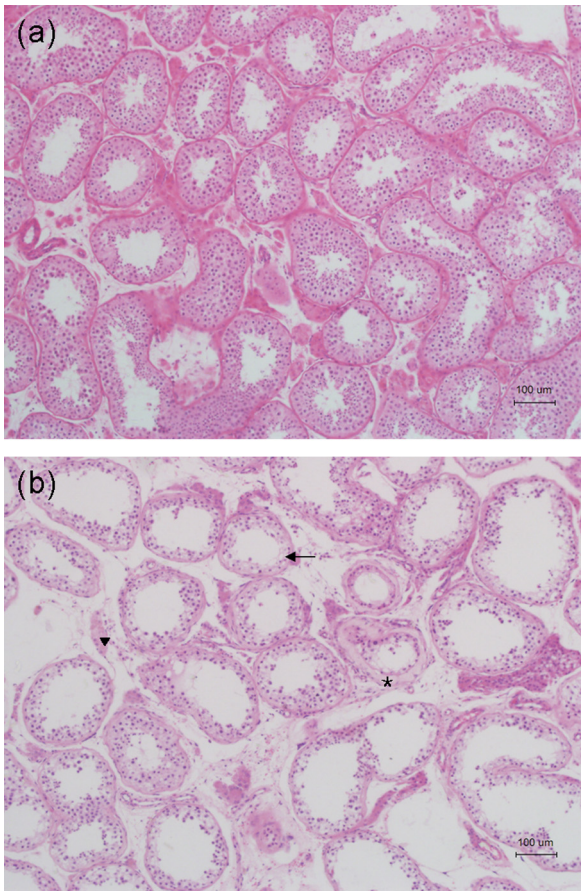

Fig. 1. Histology of testes of young and aging men. Hematoxylin–eosin staining. (a) Testicular compartments with normal structure are visible in young testes. Regular arrangement of seminiferous epithelium in seminiferous tubules (ST). Well-developed interstitial tissue (IT) with Leydig cells. (b) Atrophic seminiferous tubules with epithelium vacuolation (*arrow*) and thickened basal membrane (*asterisk*) in aging testes. Leydig cells are loosely located in the interstitial space of tubules (*triangle*). Magnification 100×