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MORPHOLOGICAL, LECTIN-HISTOCHEMICAL AND PCNA-IMMUNO HISTOCHEMICAL COMPARISONS OF THE TESTICULAR TISSUES BETWEEN THE FIRST WAVE AND THE REINITIATION OF SPERMATOGENESIS AFTER EXPOSURE TO A SHORT PHOTOPERIOD

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Introduction

Golden hamsters (*Mesocricetus auratus*), a seasonal breeder, show a marked testicular regression in a short photoperiod condition 1. They have been used as a model for the study of reproductive seasonality 2. After exposure to a short day cycle, the testis weight and the diameter of seminiferous tubules gradually decreased and reached the minimum at 13 weeks. At that time, spermatogenesis was severely disrupted. After 13 weeks, the testis gradually recovered its weight and function. Thereafter, spermatogenesis was apparently reinitiated at 23 weeks 3. Although the first wave (occurrence of spermatogenesis after birth) and the reinitiation of spermatogenesis after exposure to a short photoperiod seem to be similar with each other in morphological and hormonal aspects, no comparative studies on these two processes are available at present. In the present study, morphological, lectin-histochemical and PCNA-immunohistochemical comparisons of the testicular tissues between the first wave and the reinitiation of spermatogenesis after exposure to a short photoperiod were carried out to find out the differences between the two processes.

Methods and Materials

Animals:

Male ACN golden hamsters used in this study were maintained as a closed colony in our laboratory. From birth to 8 weeks, they were kept on a long day cycle (LD; 14 hr of light, 10 hr of dark). Four animals were sacrificed every one week. Some of sexually mature hamsters (8 weeks) were exposured to a short day cycle (SD; 6hr of light, 18 hr of dark). From 13 to 23 weeks after

exposure, 4 animals were sacrificed every 2 weeks. Some of the animals exposured to the SD for 13 weeks were transferred to the LD again. From 1 to 4 weeks after transfer to the LD, 4 animals were sacrificed every one week.

Light microscopy (LM) for comparison of morphological characteristics in testicular tissues:

Each animal was anesthetized by intraperitoneal injection of sodium pentobarbital (0.5 mg/head) and then perfused with Bouin's fixative through the left ventricle after washing briefly with 0.9% saline. The testes were surgically excised, sliced into slabs and immersed in the same fixative for 2 to 3 hr. Then, they were dehydrated in a graded series of ethanol, infiltrated in xylene, and embedded in paraffin. Sections of 4 μm in thickness were stained with periodic acid Schiff (PAS)-hematoxylin or hematoxylin-eosin (HE) and observed by LM.

Staining with lectins for comparison of the distribution of glycoconjugates in testicular tissues:

Deparaffinized sections were rehydrated and treated with 1% bovine serum albumin (BSA) in 10 mM phosphate-buffered saline (PBS), pH7.4. The sections were incubated with biotinylated lectins, *Canavalia ensiformis* (ConA), *Lens culinaris* (LCA), *Ricinus communis I* (RCA-I), *Phaseolus vulgaris* (PHA-E), *Arachis hypogaea* (PNA), *Bauhinia purpurea* (BPA), *Griffonia simplicifolia I,II* (GS-I, GS-II), *Glycine max* (SBA), *Vicia villosa* (VVL), *Solanum tuberosum* (STL), *Triticum vulgaris* (sWGA), *Datura stramonium* (DSL), *Phaseolus vulgaris* (PHA-L), *Dolichos biflorus* (DBA), *Ulex europaeus* (UEA-I), *Artocarpus integrisolia* (Jacalin, Vector Laboratories, Burlingame, CA, U.S.A; 25 _γ/ml) in 0.1% BSA-PBS for 60min, and then washed with PBS. They were incubated with avidin-biotin peroxidase complex (ABC, Vector Lab.) for 30 min. After a rinse with PBS again, they were immersed in 3,3'-diaminobenzidine (DBA, 0.2 mg/ml)-H_2O_2 (0.005%) for 5 to 10 min, and rinsed in distilled water. They were dyhydrated, mounted and observed by LM.

Staining with PCNA (proliferating cell nuclear antigen) for comparison of proliferative activity in testicular tissues:

Each animal was perfused with 4% paraformaldehyde. Deparaffinized sections were rehydrated and treated with 1% BSA in 10 mM PBS, pH7.4. The sections were incubated with anti-PCNA (Sigma, 1:200) in 0.1% BSA-PBS for 60min, and washed with PBS. They were incubated with biotinylated monoclonal anti-mouse IgG (Sigma, 1:1000) in 0.1% BSA-PBS for 60min, and washed with PBS. They were incubated with avidin-biotin peroxidase complex (ABC, Vector Lab.) for 30 min. After a rinse with PBS again, they were immersed in 3,3'-diaminobenzidine (DBA, 0.2 mg/ml)-H_2O_2 (0.005%) for 5 to 10 min, and rinsed in distilled water. They were dyhydrated, mounted and observed by LM.

Results and Discussion

At birth, the seminiferous epithelium was composed of Sertoli cells and pre-spermatogonia 4. While, at 13 weeks after exposure to the SD, the seminiferous epithelium was composed of Sertoli cells, spermatogonia and a few spermatocytes, similar to the case at 2 weeks after birth. The number of spermatocytes at 3 weeks after birth resembles that at 15 weeks after exposure and at 1 week after transfer to the LD. Round spermatids first appeared at 4 weeks after birth and at 17 weeks after exposure. Elongate spermatids first appeared at 6 weeks after birth, at 23 weeks after exposure and at 2 weeks after transfer. Spermatozoa first appeared at 8 weeks after birth, at 4 weeks after transfer. In the first wave, it took about 4 weeks from the appearance of spermatocytes to spermatozoa. However, in the SD exposure, it took more than 10 weeks. In the LD transfer, it took less than 4 weeks. Concerning the lectin-histochemistry, 2 of 17 lectins showed a different binding pattern between the first wave and the reinitiation of spermatogenesis from 13 weeks after exposure to the SD. Although RCA-I strongly bound to acrosomes at 6 weeks after birth, it bound to spermatocytes at 13 weeks after exposure. Then, the reaction in spermatocytes became weak, and it bound to spermatids at 23 weeks after exposure. DBA bound to spermatogonia at birth and its number gradually increased from birth to 4 weeks. After 6 weeks, the positive spermatogonia abruptly decreased. While, it bound to spermatogonia at 13 weeks after exposure, thereafter the number gradually decreased. At 1 week after transfer, the DBA reaction in spermatogonia was similar to that at 6 weeks after birth. Concerning the PCNA immunohistochemistry, positive spermatogonia at 3 weeks after birth were numerous, compared to those at 15 weeks after exposure to the SD, in spite of the similarity in constituted cell types. These findings suggest that the activity of the self-renewal of spermatogonia changes by expose to a short photoperiod.

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