Morphological Study on Pigmented Cells in the Horse Testis

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ABSTRACT. One of the most attractive characteristics of a horse testis is the change of the weight during development. As the testicular weight changes and the number of Leydig cells decreases, pigments appear in interstitial tissues. In the present study, the characteristics of the pigments found in the interstitial tissues were examined histochemically and ultrastructurally. Specific stainings indicated that the pigmented granules showed almost all of the histological and histochemical characteristics of ceroid or ceroid-like pigment. The cells showed positive reaction for acid phosphatase while the pigmented cells contained a lot of lysosomes ultrastructurally. These results suggest that macrophages might phagocytize Leydig cells, and store their digested materials as ceroid-like pigment. — KEY WORDS: ceroid, equine, testis.

One of the morphological characteristics of horse testes is the change of their weights at the fetal stage. Cole et al. [3] first found that the testicular weight of the fetus reached the maximum weight (approximately 80 g) at 215 days of pregnancy. Thereafter, the weight decreased to 10–20 g in newborn horses. This change appears to result from a marked hyperplasia and hypertrophy of the interstitium. It is suggested that the initiation of these hyperplasia and hypertrophy was caused by high concentration of pregnant mare serum gonadotropin (PMSG) in the mare [6]. Cole et al. [3] also reported that the evidence of break-down of the interstitial cells in the fetal testis was seen in the cytoplasmic deposition of lipochromes and most of the interstitial cells degenerated completely at the later fetal stage. In the relation between interstitial cells and lipochromes, Nishikawa [12] mentioned that the period of cell formation and cell degeneration are not the same to each other. Aureli and Lauria [2] reported that “xanthochrome” cells (pigmented cells) had a nature of lipofuscin. However, they examined the pigmented cells histochemically only by periodic acid and Schiff (PAS) reaction, Sudan stains and autofluorescence. These histochemical methods are not sufficient to identify these pigments as lipofuscin.

In the present study, the characteristics of the pigmented cells were examined using various histochemical and ultrastructural techniques. Testes containing pigmented cells from 21 horses in age from 280-day-fetus to 2-year-old were used in this study. Fetal testes were obtained from fetuses accidentally aborted, while the testes at postnatal stages were collected by castration under anesthesia. All of the samples were fixed in Bouin’s fluid or 10% phosphate-buffered formalin. For light microscopic observations, the samples were dehydrated through a graded series of ethanol, infiltrated in propylene oxide, and embedded in paraffin. Sections were cut at 4 µm in thickness. Deparaffinized sections were stained by the following methods to establish the characterization of pigments; PAS reaction, Sudan III stain, Sudan black B stain, Schmorl reaction, Ziehl-Neelsen stain (ZN), chrome alum hematoxylin stain (CH), 0.02% Nile blue sulfate (pH 3.0) stain (NB), Gomori’s aldehyde fuchsin stain (AF), and leuco-malachite green stain (LMG). Non-stained sections were observed with a fluorescent microscope (Nikon micro-photo-SA, Nikon, Tokyo, Japan). To detect lipid materials, cryostat sections cut at 10 µm were stained with Sudan III and Sudan black B. Naphthyl phosphate-hexazonium pararosanilin method was used to detect acid phosphatase in frozen sections. For electron microscopic observations, the sample was obtained from a 9-month-old horse, fixed in 5% glutaraldehyde and postfixed in 1% osmium tetroxide with 1.5% potassium ferrocyanide [15]. The sample was then dehydrated through a graded series of ethanol, infiltrated in propylene oxide, and embedded in Epon 812 resin. Ultrathin sections of 90 nm in thickness were stained with uranyl acetate and lead citrate. Ultrastructural observations were made with a transmission electron microscope (Hitachi H-7500, Hitachi, Tokyo, Japan).

There were many large cells stained yellow by hematoxylin and eosin (HE) (Fig. 1). The granules inside the large cell were positive for PAS reaction (Fig. 2). The pigmented granules in both paraffin and frozen sections were stained light red by Sudan III and black by Sudan black B (Figs. 3, 4). With ZN, the pigmented cells were filled with many granules stained dark red (Fig. 5). The granules were stained deep blue by Schmorl reaction (Fig. 6). With CH, these pigmented granules were not stained (Fig. 7). There were many granules stained blue with NB (Fig. 8). AF showed violet granules in their cytoplasm (Fig. 9) but these granules were not stained by LMG (Fig. 10). The pigmented granules showed the clear deep red reaction with the acid phosphatase stain (Fig. 11). The granules emitted white autofluorescence by ultra-violet (UV) (360 nm) under a fluorescence microscope (Fig. 12).

Ultrastructurally, the pigmented cells were characterized by the presence of abundant residual bodies consisting of...
Fig. 1. All of these figures including electron micrographs were taken from a testis of 9-month-old sample. HE stain. There are many large cells stained yellow (arrow) in their own color. × 220.

Fig. 2. PAS reaction. The granules show a positive reaction (arrow). × 220.
Fig. 3. Sudan III stain. The pigmented granules are stained light red (arrow). × 220.

Fig. 4. Sudan Black B stain. The cytoplasm of pigmented cell is filled with granules stained black (arrow). × 220

Fig. 5. Ziehl-Neelsen stain (acid-fast stain). The granules stained red are deposited in the cytoplasm of the pigmented cells (arrow). × 220.

Fig. 6. Schmorl reaction. The pigmented granules are stained blue (arrow). × 220.

Fig. 7. Chrome alum hematoxylin stain. In the pigmented cells, nuclei are stained blue and unstained granules are observed (arrow). × 220.

Fig. 8. 0.02% Nile blue sulfate stain. There are many granules stained blue (arrow). × 220.

Fig. 9. Gomori’s aldehyde fuchs in stain. The pigmented granules are stained violet while the nuclei are stained yellow (arrow). × 220.

Fig. 10. Leuco-malachite green stain. The pigments are not stained (arrow). × 220.

Fig. 11. Acid phosphatase stain counterstained by hematoxylin. The pigmented granules show a positive reaction (arrow). × 220.

Fig. 12. Non-stained preparation. The pigmented cells emit white autofluorosence exerted by UV (360 nm) (arrow). × 220.

Fig. 13 (a). Electron micrograph of the pigmented cell. Abundant materials with various electron densities are observed in the cytoplasm. Nucleus is irregular in shape and located near the plasma membrane (arrow). Bar = 10 µm. × 1,200. (b). The residual bodies (arrows) are found in the secondary lysosomes isolated from other intracellular components by unit membrane (arrowheads). Bar = 0.1 µm × 70,000.
secondary lysosomes having variable internal structures and a nucleus located eccentrically in the cytoplasm (Fig. 13a). Large and small residual bodies contained granular materials showing various sizes and electron densities (Fig. 13b). These bodies were enclosed by a limiting membrane.

From the results of the specific stains, it was suggested that the pigmented granules found within the pigmented cells have some characteristics of ceroid as shown in the following stains or reactions: Sudan III, Sudan black B, PAS, Schmorl, CH, NB, AF, ZN and autofluorescence. However, these pigmented granules were unstained by LMG which distinguishes ceroid from lipofuscin.

Lillie et al. [10] first found some yellow globular pigments in the cirrhotic liver of rats by feeding them a low protein diet, and called the pigments as ceroid. In later studies [8, 9, 13, 16], these pigments were observed under different circumstances and in various tissues of animals. Lee [8] stated that ceroid might be a mixture of variable substances whose characteristics were altered by a combination of various other substances. The present results may also indicate the various natures of ceroid. Generally, it is known that ceroid is formed by phagocytosis of macrophages under physiologically abnormal conditions like necrosis, cirrhosis and tumors. However, there were several reports that ceroid or ceroid-like pigments, such as in the adrenal gland and ovary, are seen under physiologically normal conditions [14, 17]. In the horse testis, the appearance of the pigmented cells might indicate that macrophages phagocytise some materials and store them as ceroid-like pigments in their body.

In electron microscopic observations, the pigmented cells contained a lot of residual bodies (secondary lysosomes) enclosed by a limiting membrane. The presence of abundant secondary lysosomes indicates that intracellular organelles or extracellular substances were digested by the lysosomes [4]. Considering the positive reaction for acid phosphatase at light microscopic level, the present results indicate that the pigmented cells digested some materials vigorously. Although the present results suggest a possible origin of the pigmented cells from macrophage, further detailed studies will be required to clarify this problem.

It is known that testes of fetal horse start to decrease in size and weight from mid-pregnancy to the neonatal period, and the number of Leydig cells also decreases during the same period [3, 5]. Almahbobi et al. [1] reported that the fetal type of Leydig cells disappeared completely and were replaced by the post-pubertal or adult type of Leydig cells as the horse grows up.

In our previous study, we examined the morphogenesis of horse testis by morphometric analysis from fetuses to adult horses [7]. We found that the period of appearance of the pigmented cells coincided with the period of the reduction of interstitial regions at 3 days of age, and as the abrupt reduction of the interstitial regions stopped, the pigmented cells almost disappeared at 2 years of age [7]. It has been suggested that the pigmented cells were closely related to the reduction of interstitial regions.

As a whole, we concluded that the macrophages phagocytize the degenerating fetal type of Leydig cells, and they gradually increase in volume by storing digested materials as ceroid-like pigment. This might suggest that the abrupt appearance and disappearance of pigmented cells did not reflect abnormal conditions of horse testis. Moreover, such cells were not found in the testes of other species [11]. The appearance of these pigmented cells only in horses remained to be determined. Further investigation will be required to clarify this problem.

REFERENCES