Age-Related Morphological Changes in the Testis of the BDF₁ Mouse

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ABSTRACT. Age-related morphological changes in the testis of the BDF₁ mouse were studied by light and transmission electron microscopy. No apparent changes were detected until 12 months of age. After 18 months of age, vacuoles firstly appeared in the seminiferous epithelium. These vacuoles were gradually increased in number and showed a tendency to cluster with each other in accordance with age. While, germ cells were decreased in number. The sloughing of germ cells caused a thin seminiferous epithelium. In the tubule with a thin epithelium, spermatogenesis was severely interrupted. After 30 months of age, extremely thin seminiferous epithelia were observed. In these epithelia, most of spermatids and spermatocytes disappeared, and most of Sertoli cells lost their polarity to be flattened. On the other hand, in the interstitial region, PAS-positive cells (mononuclear phagocytes) tended to increase in number after 24 months of age. PAS-positive extracellular matrix newly appeared at 27 months of age. In the cytoplasm of Leydig cells, a whorl of sER was frequently found. Degeneration of testes proceeded with age. The regressive tubules occupied only 2.2% at 18 months of age, but extended to 63.0% at 33 months.—**KEY WORDS:** age-related change, BDF₁ mouse, testis.

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It is well known that the reproductive capacity of male mammals gradually declines with ageing [2, 4]. The regressive histological changes in seminiferous tubules and the decrease in daily sperm production occur in advance with age. Such changes have been reported in men [10, 16, 17, 20], mice [5, 22] and stallions [7, 11]. Johnson *et al.* have reported that the age-related decrease in daily sperm production is due to a higher rate of germ cell degeneration during postprophase of meiosis [8]. This germ cell degeneration may have a strong relation to the increased serum FSH and LH levels and to the decreased testosterone level [6, 10]. While, Sasano has pointed out that the ischemic changes by gerontal circulatory damages may lead to the testicular dysfunction [19].

In degenerating tissues of old animals, it is difficult to distinguish gerontal changes from pathologic changes. In this point, (C57BL/6 × DBA/2) F₁ mice [BDF₁ mice] are of great advantage for studying gerontal changes, since they hardly cause testicular tumors and inflammations in heredity, and since they can live more than 31 months [23]. In the present study, the testes of BDF₁ mice from 3 to 33 months of age were used as materials. Therefore, both the initiation of regressive changes and the subsequent degeneration in testes could be observed in detail by light and transmission electron microscopy.

MATERIALS AND METHODS

Virgin male BDF₁ mice used in this study were obtained from Charles River Japan, Inc. and bred in Laboratory for Experimental Animals, Tokyo Metropolitan Institute of Gerontology. The mice were reared in a specific pathogen-free condition, and fed autoclaved CRF-1 pellets (Oriental Yeast Co., Ltd.) and sterilized water, both *ad libitum*. They were used for observation at 3, 6, 12, 18, 21, 24, 27, 30 and 33 months of age. Their average body

weights at respective months of age were 29.2 g, 37.6 g, 42.3 g, 46.1 g, 44.0 g, 36.6 g, 34.5 g, 33.1 g and 31.2 g.

Light microscopy: Four to 6 mice were used in each age group. Under sodium pentobarbital (5 mg/100 g b.w.) anesthesia, testes of both sides were perfused through the heart first with Ringer's solution and then with Bouin's fixative. The specimens were then dehydrated in a graded series of ethanol and embedded in paraffin. They were sectioned at 4 μm, stained with periodic acid Schiff (PAS)-hematoxylin and examined by light microscopy.

Transmission electron microscopy: The testes were perfused through the heart first with Ringer's solution and then with 5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. Testes of both sides were excised from two mice in each age group. They were then washed briefly with 0.1 M phosphate buffer, cut into smaller pieces (1 × 1 × 2 mm), and postfixed in 1% osmium tetroxide containing 1.25% potassium ferrocyanide [18] at 4°C. Tissue blocks were dehydrated in a graded series of ethanol, infiltrated in propylene oxide, and embedded in Araldite. Thin sections were cut with an ultramicrotome, stained with uranyl acetate and lead citrate, and examined with a JEM-1,200 EX transmission electron microscope at 80 kV.

More than 1,000 round seminiferous tubules were randomly selected in each mouse and were observed by light microscopy. Histologically, seminiferous tubules were roughly classified into 4 types according to age-related degenerative changes as follows: (1) Showing normal seminiferous epithelia. (2) Showing vacuolated seminiferous epithelia with some degenerated germ cells. (3) Showing thin seminiferous epithelia with very few spermatids. (4) Showing extremely thin seminiferous epithelia without spermatocytes and spermatids.

Figs. 1-6: Light micrographs. PAS-hematoxylin stain.

Fig. 1. (12 months) Spermatogenesis is active in the seminiferous epithelium. No atrophic changes are detected in the tubules. $\times 200$.

Fig. 2. (18 months) One large vacuole (arrow) is found in the seminiferous epithelium. $\times 200$.

Fig. 3. (21 months) PAS-positive round spermatocytes (arrow) seem to be degenerated. $\times 200$.

Fig. 4. (21 months) Vacuoles (arrows) are increased in number, and few elongated spermatids are recognized. $\times 200$.

Fig. 5. (24 months) PAS-positive cells (arrows) tend to increase in number in the interstitial region. $\times 200$.

Fig. 6. (27 months) Most of spermatids have disappeared in the thin seminiferous epithelium. $\times 200$.

Fig. 7-11: Light micrographs. PAS-hematoxylin stain.

Fig. 7. (27 months) A PAS-positive extracellular matrix (asterisk) appears in the interstitial region. $\times 200$.

Fig. 8. (33 months) Most of spermatocytes and spermatids disappear in the extremely thin seminiferous epithelium. $\times 200$.

Fig. 9. (33 months) Amorphous materials (arrow) are recognizable in the lumen. $\times 200$.

Fig. 10. (33 months) Aggregations of spermatozoa (arrows) are found in the lumen. $\times 200$.

Fig. 11. (33 months) Epithelia in the vicinity of the tunica albuginea show a tendency to be more degenerative than those in other areas. $\times 30$.

RESULTS

Light microscopy: At 3–12 months of age, active spermatogenesis was observed. No atrophic changes were detected in seminiferous tubules (Fig. 1).

At and after 18 months of age, degenerative changes in the testis were recognizable. In one of four mice at 18 months, large vacuoles appeared in the seminiferous epithelia (Fig. 2). In most areas of the tubules, however, spermatogenesis was in an active state.

At 21 months, in two of four mice, such vacuoles were increased in number within the epithelium. PAS-positive spermatocytes were frequently seen in the epithelia (Fig. 3). There were some tubules bearing a few elongated spermatids (Fig. 4).

At 24 months, although degenerative seminiferous epithelia were observed in all mice testes, tubules having regressive changes were only 11.3% of the whole in section. Active spermatogenesis still occurred in the normal epithelia. In the interstitial region, PAS-positive cells appeared (Fig. 5).

At 27 months, degenerative seminiferous epithelia were observed in all mice, similar to the mice at 24 months. Tubules having regressive changes were 13.7%. In one of four mice, some seminiferous epithelia became thinner, probably due to exfoliation of elongated spermatids (Fig. 6). In the interstitial region, in addition to PAS-positive cells, PAS-positive extracellular matrix newly appeared region (Fig. 7).

At 30 months, thin epithelia were observed in two of four mice. Tubules having regressive changes were further increased up to 39.7%. Both the number of PAS-positive cells and the amount of extracellular matrix in the interstitium were gradually increased.

At 33 months, in four of six mice, the epithelia were extremely thin in appearance. Most of spermatids and spermatocytes disappeared in these thin epithelia (Fig. 8). Amorphous materials (Fig. 9) and aggregated spermatozoa (Fig. 10) were recognizable in the lumina of some tubules. The regressed area occupied 63.0% of the whole. The epithelia in the vicinity of the tunica albuginea showed a tendency to be more degenerative than those in other areas (Fig. 11). The lumina of seminiferous tubules were not closed. The PAS-positive cells and the extracellular matrix in the interstitium were qualitatively and quantitatively similar to those of mice at 30 months.

Damaged seminiferous tubules from 12 to 33 months of age were counted. Then, they were classified into the foregoing 4 types (Fig. 12). The numbers of tubules with degenerated seminiferous epithelia was increased with age. Regressive tubules occupied 63.0% of the whole at 33 months.

Transmission electron microscopy: After 18 months, germ cells were often phagocytized by Sertoli cells. In thin seminiferous epithelia of old mice, Sertoli cells with many lysosomes and lipid droplets lost their polarity to be flattened, and their cytoplasmic processes fell one upon another on the luminal surface of the epithelium (Fig. 13).

Fig. 12. Occurrence of four types of degenerated seminiferous epithelia in various aged mouse testes: Type 1; showing normal seminiferous epithelia, Type 2; showing vacuolated seminiferous epithelia with some degenerated germ cells, Type 3; showing thin seminiferous epithelia with very few spermatocytes, Type 4; showing extremely thin seminiferous epithelia without spermatocytes and spermatids. Tubules having degenerated seminiferous epithelia are increased in number with age. Regressive tubules occupy 63.0% of the whole at 33 months.

The seminiferous epithelia were net-like in appearance, probably due to sloughing of germ cells (Fig. 14). In a rare case, two-nucleate Sertoli cells were observed in these thin epithelia (Fig. 15), as confirmed by serial ultrathin sections. The basal lamina, including numerous bundles of collagen fibrils, was widely spread and distorted. Constricted myoid cells were frequently observed (Fig. 16). Even in such damaged tubules, the ectoplasmic specialization, consisting of microfilament bundles and subsurface-cisterned ER adjacent to the junction between adjoining Sertoli cells was normal in appearance (Fig. 17).

Macrophages rarely appeared in the lumen of these damaged seminiferous tubules, and phagocytized elongated spermatids (Fig. 18).

In aged mice, a whorl of sER or lipid droplets were frequently found in the cytoplasm of Leydig cells (Fig. 19). This peculiar structure was gradually increased in number in accordance with age. The PAS-positive cells in the testicular interstitium observed by light microscopy were obviously equivalent to mononuclear phagocytes observed by electron microscopy. While, the PAS-positive extracellular matrix contained low electron dense granules (Fig. 20).

DISCUSSION

No testicular tumors were recognized in all mice used in this study. Since no pathologic changes were detected in the testes of aged mice, it is suggested that the testicular disruption found in this study is due to the senescence. In the seminiferous epithelia, the appearance of vacuoles was

Figs. 13–16: Transmission electron micrographs.

Fig. 13. (33 months) Sertoli cells in the extremely thin epithelium lose their polarity to be flattened. The cytoplasmic processes of these Sertoli cells fall one upon another on the luminal surface of the epithelium (arrowheads). $\times 2,500$.

Fig. 14. (33 months) The seminiferous epithelium is net-like in appearance, probably due to the sloughing of germ cells. The cytoplasmic processes of Sertoli cells join each other. $\times 3,500$.

Fig. 15. (33 months) Two-nucleate Sertoli cells in thin epithelium. $\times 4,100$.

Fig. 16. (33 months) The basal lamina, including numerous bundles of collagen fibrils (arrowheads), is widely spread and distorted. A myoid cell is constricted (arrow). $\times 4,100$.

the first visible change at a light microscopic level. Germ cells were often phagocytized by Sertoli cells, as seen at an electron microscopic level. This finding indicates that the vacuoles found by light microscopy may be due to the sloughing of germ cells. Most of these vacuoles were located in regions from basal to middle of the epithelia. While, some degenerating germ cells were recognizable in the same regions. Therefore, the degeneration of germ cells seems to occur during postprophase of meiosis as

Johnson *et al.* have mentioned [8]. These vacuoles are thought to appear at an early stage of gerontal changes. Then, vacuoles spread over the whole area of the testis. Suzuki and Withers [21] have reported that the stem cells in the mouse testis are exponentially decreased in number with ageing, and that the survival rate of stem cells after X-ray irradiation declines with ageing. In the present study, however, there were no degenerated spermatogonia in the seminiferous epithelia of aged BDF₁ mice.

Fig. 17–20: Transmission electron micrographs.

Fig. 17. (33 months) The ectoplasmic specialization (arrowheads) at the junction between adjoining Sertoli cells is normal in appearance. $\times 16,500$.

Fig. 18. (33 months) A macrophage in the lumen of a damaged seminiferous tubule phagocytizes elongated spermatids (arrows). $\times 3,300$.

Fig. 19. (33 months) Whorls of sER (arrows) are frequently found in the cytoplasm of Leydig cells. $\times 6,100$.

Fig. 20. (33 months) A mononuclear phagocyte (arrow) is seen in the interstitial region. The extracellular matrix contains low electron dense granules (asterisk). $\times 10,500$.

Consequently, the spermatogonia seem to be in a normal state even in the aged BDF₁ mice.

Some phagocytized germ cells, many lysosomes and many lipid droplets were found in Sertoli cell of aged mice, suggesting that Sertoli cells at an advanced age still maintain the ability of phagocytosis. While, Sertoli cells lose their polarity to be extremely flattened at aged mice. These changes may be accompanied with the sloughing of

spermatids. On the other hand, Sertoli cells of the mutant mouse (*W/W^v*) with no germ cells or of the mutant rat (*as/as*) with no elongated spermatids retain their polarity [1, 13]. In the present study, the disappearance of polarity of Sertoli cells in aged mice may be due to the degeneration of Sertoli cells themselves.

Sertoli cells have a higher resistant property against ischemic damages than any types of germ cells [24]. In this

study, degenerated Sertoli cells were observed frequently in spite of the presence of spermatogonia. Therefore, the age-related dysfunction of the testis of the BDF₁ mice may not be mainly due to ischemic damages caused by gerontal circulatory changes. Although age-related changes in the basal lamina have been reported [5, 22], the relation between these changes and gerontal disruption of spermatogenesis is still not clear. Similar to two-nucleate Sertoli cells found in old BDF₁ mice testes, multinucleate Sertoli cells were also reported in the old human testis [16, 20]. Since the division of Sertoli cells ceases within three weeks in rats [15], these two-nucleate Sertoli cells may be derived from the cell fusion.

A lower serum testosterone level has been reported in aged men, mice, rats, and stallions [5, 6, 11, 14]. In this study, lipid droplets and a characteristic whorl of sER were frequently detected in the cytoplasm of Leydig cells of aged mice. These phenomena may indicate a decline of endocrine function in Leydig cells though the decline should be confirmed by measurement of serum testosterone levels.

The interstitial extracellular matrix has been reported in testes of diabetic men [3]. Its PAS-positive reaction demonstrates the presence of some kinds of glycoconjugates in it. Such a matrix may be one of waste materials, though its origin and properties are still obscure.

The degree of damage in seminiferous tubules varied obviously among regions of the testis. Extremely thin epithelia were observed more frequently in the vicinity of the tunica albuginea than in the central region of the testis. The reason for this phenomenon is not yet clear.

In the present study, it was confirmed that the first gerontal degeneration of the testis was the appearance of vacuoles in the seminiferous epithelia, probably due to the sloughing of germ cells. Sertoli cells in old mice may lose their ability to raise germ cells. But, it is still not clear why germ cells slough prominently in aged mice. The relation between age-related changes in hormonal level (FSH, LH, and testosterone) and these in testicular morphology also remains unsolved. There is a possibility that the differentiation of germ cells may be severely interrupted by hormonal changes in aged mice. On these points, such a possibility is needed to study in detail in the future.

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