An Ultrastructural Study of the Sertoli Cell in the Shiba Goat

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ABSTRACT. The ultrastructure of the Shiba goat Sertoli cell was observed using light and transmission electron microscopy. In the basal portion of the Sertoli cell, the nucleus was located near the basement membrane and was irregular in outline. A number of filaments with intermediate width were observed around the nucleus. A multivesicular nuclear body, composed of numerous vesicles of different sizes and ribosome-like structures, was detected within the nucleus. The majority of cell organelles was situated in this basal portion of the cytoplasm. Oval mitochondria were randomly distributed. A well-developed laminated smooth endoplasmic reticulum (sER) was frequently found within the cytoplasm of Sertoli cell. Lysosomes and large lipid droplets were also recognized. While, the outline of the basal lamina was almost straight. No nodular processes were observed in the lamina. The Golgi complex appeared only in the supranuclear region. In the middle and apical portions of the Sertoli cell, elongated mitochondria and microtubules were oriented parallel to the long axis of the cell. The laminated sER was also detected in these portions. The apical head of slightly-elongated spermatids was occasionally surrounded by the laminated sER. The head of maturing spermatids without laminated sER revealed a bleb-like shape. The microfilament bundles at the ectoplasmic specialization site were regularly arranged. Judging from the present observations, we have suggested that only the multivesicular nuclear body and the laminated sER may be the peculiar structures which are commonly found in the Sertoli cell of ruminants.—**KEY WORDS:** ectoplasmic specialization, laminated sER, multivesicular nuclear body, Sertoli cell, Shiba goat.

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The Sertoli cell has been subjected to the matter of numerous studies [3, 22, 24, 27], since it plays an important role during spermatogenesis. The Sertoli cell is mainly reliable to transport spermatogenic cells from the basal region toward the tubular lumen, and it is also involved in the phagocytosis of degenerated cells, as well as nutrition and protection of spermatogenic cells [10].

The morphology of the Sertoli cell has been described not only in experimental animals, but also in domestic animals whose damaged fertility causes a great economic loss.

It is generally accepted that the Sertoli cell of ruminants has some peculiar structures different from other species, for example, multivesicular nuclear body [2, 5, 6, 13, 20, 31, 32], aggregated ribosomes [13, 32], laminated sER [2, 5, 6, 13, 20, 31, 32], irregularly arranged of microfilament bundles at the ectoplasmic specialization site [2, 13] and nodular processes [5, 30]. Among these structures, the multivesicular nuclear body has been certainly described in the Sertoli cell of ruminants hitherto examined. However, it is controversial whether the above mentioned structures other than the multinuclear body are common in the ruminant Sertoli cells. For example, the aggregate of ribosomes was described in the bovine by Zibrin (1972), whereas other authors who studied the bovine Sertoli cell did not demonstrate its existence. In addition, most lines of information on the morphology of ruminant Sertoli cells are derived from the bovine. In order to confirm the peculiar structures commonly present in the ruminant

Sertoli cells, detailed ultrastructural study on other kinds of ruminants should be necessary.

The ultrastructure of the Shiba goat Sertoli cell has not yet been investigated in detail, although there are some publications on its structure using scanning electron microscopy [11, 12]. In the present study, the morphology of the Shiba goat Sertoli cell was studied by light and transmission electron microscopy to clarify the features commonly present in the ruminant Sertoli cells.

MATERIALS AND METHODS

Six sexually matured Shiba goats used in this study were obtained from the Stock Farm, the University of Tokyo. The testes were surgically excised from the anesthetized animals. After washing briefly with 0.9% saline, the testes for light microscopy were perfused through the testicular artery with Bouin's fixative. After fixation, the testes were cut into small pieces, dehydrated in a graded series of ethanol and embedded in paraffin. They were sectioned at 4 μ m, stained with PAS (periodic acid Schiff)-hematoxylin and observed by light microscopy.

For electron microscopy, the testes were perfused with 5% glutaraldehyde in 0.05 M cacodylate buffer after washing with saline. They were cut into small pieces (1 $\times$ 1 mm), postfixed in 1% osmium tetroxide/1.5% potassium ferrocyanide [21] and embedded in Araldite. Thick sections (about 1 μ m) were stained with toluidine blue and observed by light microscopy. Ultrathin sections were mounted on a copper grid, stained with uranyl acetate and lead citrate and examined in a JEM-1200 EX transmission electron microscope at 80 kV.

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Fig. 1a. In the basal portion of Sertoli cell cytoplasm, some lysosomes (ly), mitochondria (arrowheads) and other organelles are distributed at random. Multivesicular nuclear body (square) is clearly seen within the Sertoli cell nucleus; BM: basement membrane. $\times 3,500$.

Fig. 1b. Higher magnification of square in Fig 1a. The multivesicular nuclear body consists of different sized vesicles (v) and aggregate of ribosome-like structures (arrowheads). $\times 25,000$.

Fig. 1c. Higher magnification of the multivesicular nuclear body. Some nuclear vesicles (v) are situated close to the nuclear membrane. Intermediate filaments (arrowhead) are surrounding the Sertoli cell nucleus. M: mitochondria. $\times 20,000$.

RESULTS

Light microscopy: Active spermatogenesis was recognized in the seminiferous tubules of all goats used. The interstitial region did not occupy a large area of the testis.

Similar to other mammalian species, the Sertoli cell of the Shiba goat was columnar in shape, lying on the basement membrane of seminiferous tubules. It extended toward the tubular lumen. The nucleus of Sertoli cell was complicated in contour and was located in the basal region of the cell.

The dense area around the apical region of the young spermatid head was visualized. No dense area was detected around the maturing spermatid. Large lipid droplets were frequently observed close to the basement membrane in some seminiferous tubules.

Transmission electron microscopy: As observed by light microscopy, the nucleus of Sertoli cell was irregular in outline, showing numerous infoldings in its membrane. Some organelles, such as mitochondria, free ribosomes and cisternae of smooth endoplasmic reticulum (sER), were observed in the indentation of the nucleus.

A characteristic structure, called "multivesicular nuclear body" [32], was detected within the nucleus of Sertoli cell (Fig. 1a). It was composed of numerous vesicles of different sizes, tubules and ribosome-like structures (Fig. 1b). Generally, the position of the multivesicular nuclear body was central, although some nuclear vesicles were occasionally located close to the inner layer of the nuclear membrane (Fig. 1c). Tubules were rarely found within this

structure. Some vesicles contained electron dense materials, whereas others apparently lacked contents. They were obviously limited by a unit membrane.

Numerous mitochondria were observed in the cytoplasm of Sertoli cell. In the basal portion of the cell, mitochondria were distributed at random, while in the middle and apical portions they were oriented parallel to the long axis of the cell. The mitochondria in the trunk of Sertoli cell (middle portion) were elongated in shape, compared with the oval-shaped mitochondria in the basal region. Only a few mitochondria were visualized between the nucleus and the basement membrane.

The sER was abundant throughout the cytoplasm of Sertoli cell. A laminated sER, surrounding the apical head of the young spermatid nucleus, was encountered in the middle and apical portions of the Sertoli cell (Fig. 2a). Probably, the laminated sER associated with the acrosome of young spermatids was corresponding to the dense area observed by light microscopy. While, a concentric whorl of sER in no contact with spermatids was sometimes recognized in the basal region (Fig. 2b). The head of maturing spermatids invading the Sertoli cell revealed a bleb-like shape. They were not surrounded by laminated sER.

The Golgi complex appeared in the supranuclear region of Sertoli cell. Lysosomes and numerous lipid droplets were mainly found in the basal portion. The lipid droplets tended to coalesce and increase in size, and to be surrounded by sER. Intermediate filaments, approximately 10 nm in diameter, were densely distributed around the

Fig. 2a. Well-developed laminated sER is found in the basal portion of the Sertoli cell. It contains a few amount of ribosomes.

BM: basement membrane; lp: lipid droplet. my: myoid cell. Asterisk: laminated sER. $\times 8,000$.

Fig. 2b. Laminated sER is observed in close association with the apical head of a young spermatid (Spd). AC: acrosome. $\times 12,000$.

nucleus (Fig. 1c). Microtubules were found in the trunk of Sertoli cell and were oriented parallel to the cellular axis. They were also observed in the processes of Sertoli cell. Ectoplasmic specialization between adjoining Sertoli cells (Fig. 3a), or between Sertoli cell and spermatid (Fig. 3b), consisted of bundles of microfilaments and subsurface cisterned sER close to the inner layer of the cellular membrane. The microfilament bundles at the ectoplasmic specialization site were regular in arrangement. The basal lamina was slightly infolded, but almost straight. Small projections into the cytoplasm of Sertoli cell was rarely found. No nodular processes were detected.

DISCUSSION

The present study has demonstrated that the Sertoli cell of the Shiba goat has some notable characteristics.

The multivesicular nuclear body, consisting of numerous vesicles, tubules and ribosome-like structures, was present. Such a body has been also found in other ruminants like the bovine [2, 5, 6, 31] and water buffalo [13, 20]. It is generally accepted that this structure is distinctive only in ruminants [6, 13]. As has been pointed out [6], the multivesicular nuclear body resembles the nucleolar channel system that appears in the human endometrial cell during the secretory phase of the menstrual cycle. A number of hypotheses on its possible function have been proposed, for example, transport of mRNA from the nucleus to the cytoplasm [15], metabolism

of RNA-containing materials and influence on the activity of cellular enzyme systems [26]. Although the position of the multivesicular nuclear body is usually central, some nuclear vesicles are occasionally located close to the inner layer of the nuclear membrane, suggesting that this structure may communicate with the perinuclear space or the cytoplasm of Sertoli cell [17].

Intermediate filaments were abundantly found around the nucleus of the Shiba goat Sertoli cell. They are thought to be mainly skeletal in function [8] and to be involved in maintaining the cell shape, anchoring organelles within the cytoplasm and positioning the nucleus [25].

Microtubules were not different in distribution from those observed in other mammalian species [1, 7, 28]. They play an important role in intracellular transport of particles and organelles [29]. The distribution of mitochondria parallel to the cellular axis must be due to the arrangement of microtubules in the trunk of Sertoli cell.

Large lipid droplets were present in the basal portion of the Sertoli cell. The presence of these large lipid droplets has been confirmed in the bull, ram, rat [14] and camel [17]. Thus, they are not characteristic only in ruminants, but seem to be common in the ruminant Sertoli cells.

A well-developed laminated sER was found in the Shiba goat Sertoli cell. Some amounts of such sER surrounded young spermatid heads. In large animals, such as bull [17], boar [16] and ram [17], numerous profiles of agranular endoplasmic reticulum accumulate in close

Fig. 3a. Ectoplasmic specialization (ES) between adjacent Sertoli cells. ES is composed of bundles of microfilaments (arrowheads) and subsurface cisterned sER (arrows). Microfilament bundles are regular in arrangement. Sc: Sertoli cell. $\times 80,000$.

Fig. 3b. Ectoplasmic specialization (ES) between Sertoli cell and spermatid (Spd). Microfilament bundles of ES are also regularly arranged. The acrosome of the matured spermatid is bleb-like in shape (Asterisk). $\times 20,000$.

association with the round or slightly oval nucleus of spermatids, just before the onset of nuclear condensation. Later, during the maturation phase, the sER is decreased in amount and begins to disintegrate, although the flattened subsurface cisternae of ectoplasmic specialization still persist. This finding is different from that in rats, because the stacks of sER surrounding the spermatid heads in rats persist throughout the acrosome and maturation phases [4], and also during the period just before spermiation [6]. On the other hand, a laminated sER that had no relation with spermatid heads was also observed in the cytoplasm of the Shiba goat Sertoli cell. There may be two possibilities to explain the function of the laminated sER. One is that the laminated sER may be associated with the production of steroid hormones. The existence of large lipid droplets seems to support this hypothesis. The other is that it may be the remnant of rough ER without ribosomes and without function. Lamination of ER has frequently been observed in secreting cells in an inactive state [7]. In addition, the laminated sER in the Shiba goat Sertoli cell contained a few amount of ribosomes. These facts may support the latter hypothesis. More detailed studies will be necessary to clarify its functional significance.

The filament bundles at the ectoplasmic specialization site in the Shiba goat Sertoli cell were regularly arranged as in most mammals examined to date, contrary to those irregularly arranged in the bovine [2] and water buffalo [13].

Nodular processes or knob-like protrusions to be formed for the basal lamina of the seminiferous tubules were lacking in the Shiba goat, although they have been observed in the bovine [30] and water buffalo [13]. In the Shiba goat, the basal lamina of the seminiferous tubules ran almost straightly and only a few small projections into the cytoplasm of Sertoli cell were seen.

From our observations, some peculiar structures, for example, aggregated ribosomes, irregularly arranged microfilament bundles at the ectoplasmic specialization site and basal nodular processes, were not detected in the Sertoli cell cytoplasm of the goat. On the other hand, other kinds of peculiar structures, such as the multivesicular nuclear body and the laminated sER were recognized in the Shiba goat Sertoli cell, suggesting that these two structures are common in the Sertoli cells of ruminants.

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