Ultrastructure of Goat Testes: Intercellular Bridge between Germ Cells

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ABSTRACT. The fine structure of intercellular bridge (ICB) of goat germ cells was studied using testicular samples fixed by perfusion. In the seminiferous tubules, the ICBs were observed between sister cells of spermatogonia, spermatocytes and spermatids. As a result of incomplete division of germ cells, the ICB first appeared as a midbody containing a remnant of the bundle of microtubules (spindle fibers). These microtubules then disappeared and were replaced by a shutter apparatus which was composed of multiple lamellar cisternae (bridge-partitioning complex). The inner part of the ICB was reinforced with a layer of electron dense mass (bridge density) which persisted up to the residual cytoplasm of spermiation. After complete reconstruction of the sister cells, the cisternae of the bridge-partitioning complex disappeared and the channel of the ICB was opened. Evidently (see electron micrographs), almost all of the cytoplasmic organelles could pass through the channel of the ICB. In the longitudinal section, the appearance of the ICBs between sister spermatogonia and between sister spermatocytes was observed as a double linear or drum shape, and that between sister spermatids was noticed as a horseshoe-like or concave formation. With the process of spermatogenesis, the ICBs gradually became widened and shortened. The functional significance of the ICB in the goat was discussed.—**KEY WORDS:** goat germ cell, intercellular bridge (ICB), ultrastructure.

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Intercellular connection in the epithelial cells first discovered in the human a century ago [6]. This connection is furnished with a narrow thread-like short cytoplasmic apparatus to bind sister cells. In the seminiferous epithelium, the germ cells formed clones each of which was composed of several sister cells connected by such apparatuses between them. With a transmission electron microscope, about four decades ago, this narrow passageway was clarified in the cat spermatids [3], and it was named as an intercellular cytoplasmic bridge (ICB). The existence of the ICB between spermatogenic sister cells has been confirmed in various mammals [4, 5, 7, 8, 10, 16–18, 20, 23, 24]. Since then, as a connection device of sister cells after cell division, the ICB has been extensively noticed in germ cells (both sexes) not only in mammals [8, 9, 25] but also in the other animals such as birds [19] and insects [12, 14]. However, detailed information about the ultrastructural feature of the ICB in the goat testis appears to be lacking at present.

The present study was undertaken to obtain an additional information about the morphogenesis of the ICB with its relation to the cellular organelles, in the goat testis. Thus, this report is the first of its kind which deals with the ICB of goat germ cells.

MATERIALS AND METHODS

Twelve adult mongrel goats (*Capra hircus*) were used in this study. The processing of testicular tissue samples for electron microscopy was basically similar to that described previously [15]. Briefly, the animals were castrated under anesthesia. The testes were initially cleared of blood and vascularly perfused with glutaraldehyde fixative. Small pieces of tissues were post-fixed with osmium tetroxide. The tissues were stained *en bloc* with uranyl acetate solution. After dehydration, the samples were embedded in epoxy resin and sectioned. The sections mounted on uncoated grids were again stained with lead citrate, and examined with a JEOL 1200EX electron microscope.

RESULTS

Except Sertoli cells and few spermatogonia, the ICBs were noticed in all germ cells between their sister cells at the same stage in the goat seminiferous tubules. During the telophase stage of cell division in both mitosis and meiosis, the newly formed nuclei were separated away from the middle part of cytoplasm by a cleavage furrow (Fig. 1), and then the furrow left a slender duct or a narrow channel. As a result of incomplete cytokinesis, the narrow channel connecting two daughter cells included a

Fig. 1. Micrograph of a spermatogonium during mitosis at cytokinesis. Note the cleavage furrow (arrows) at the early anaphase, and the nuclei of daughter cells are in the state of reconstruction. B; basement membrane. $\times 9,000$.

substantial bundle of longitudinal microtubules (residual spindle fibers). The channel changed gradually with the completion of the cell division with a lapse of time. The inner part of the cytoplasmic membrane of the narrow channel was reinforced with dense materials called the bridge density [24]. The ICB between the two sister cells then began to form. With the shortening of the spindle fibers, dense filamentous materials appeared and formed a plate-like arrangement which is called the midbody [1, 21]. After the completion of cytokinesis, the spindle microtubules disappeared and the midbody was soon substituted by a shutter apparatus composed of multiple smooth endoplasmic reticulum (sER) layers dubbed as the bridge-partitioning complex [24] (Figs. 2–4). The bridge-partitioning complex consisted of many membranous cisternae of sER arranged side by side at an approximately right angle to the bridge duct. The mean number of cisternae was approximately 11 (range, 8–17; $n=35$). In the next step, the sER layers became shortened and the shutter apparatus was opened (Figs. 7–10). The ER layers then disappeared, and the inner part of the ICB was reinforced with a dense layer of the bridge density in spermatogonia and spermatocytes (Figs. 5 and 7), or with a special

organization in spermatids (Figs. 8 and 9). Thus, in spermatids, following the disappearance of block organelles of the bridge-partitioning complex (multiple sER layers of the shutter apparatus), a dense splint plaque (bridge density) appeared and adhered inside the neck part of the ICB.

Sometimes, the ICB formed a barrel-figure or a bulging ring with the wrinkled surface (Figs. 5 and 10). This could be a kind of shrinkage or convergence of membrane system of the ICB. In the longitudinal section, the bridge density of the ICB showed a linear- or cramp-form between sister spermatogonia and between sister spermatocytes (Fig. 7), but it changed to show a convex outline, a horseshoe-like figure, between sister spermatids (Fig. 8). The diameter of the ICB was increased gradually as spermatogenesis progressed (Fig. 11).

When the bridge density and/or the special organization was established, the passage of cytoplasmic substances and organelles including sER, mitochondria, Golgi complexes, ribosomes, microtubules, microfilaments, vacuoles or vesicles, multivesicular bodies, nuages (dense bodies or chromatooid bodies), etc. (Figs. 7–9, 11) through the sister cells became possible.

Fig. 2. A higher magnification of developing ICB's shutter apparatus (bridge-partitioning complex) between sister spermatogonia. Three cisternae are transversing the bridge channel, but the others are expanding and developing from the bridge density. Dense matrix with each cistern is clearly visible. $\times 40,000$.

Fig. 3. A fully developed shutter apparatus (bridge-partitioning complex) composed of multiple parallel transverse cisternae between the divided spermatogonia. Cohering dense materials adhere to the cisternae closing the cytoplasmic channel of the ICB. Seventeen disc-shaped cisternae are seen in this section. $\times 24,000$.

DISCUSSION

Origin of ICB: At first, this structure appears as a narrow remnant of incomplete division of germ cells, and the narrow channel is composed of a bundle of microtubules called spindle fiber [1, 2, 22]. The assembled mass of spindle fibers changes to form an electron dense midbody [1, 21]. The midbody is a dense and flat structure forming a discoid which occupies the central part of the bridge to block the channel. Regardless of the dividing stage or the inner construction, this channel is called the ICB from the midbody stage, since most of such narrow channels persist throughout the metamorphogenic process of spermatogenesis. Moreover, the surrounding cytoplasmic membrane shows a marked convexity as the process progresses. It is assumed that the midbody of the ICB swells out to form multiple layers of aligned transverse cisternae of sER. These multiple layers of sER seem to be a kind of shutter apparatus and are called as the bridge-partitioning complex in the rat germ cells

[24]. In the present study, the mean number of the cisternae in the goat was approximately 11 and not more than 17. The cisternae then start to be resolved from the center and shorten toward the channel-wall, and the channel of the bridge is opened.

Persistence of this shutter apparatus (bridge-partitioning complex) may defend the cell against of any abnormal effect by its sister cell and also by its sister cells already connected by the other ICBs, during the cell division.

Based on the occurrence rate, it is apparent that the time required for the formation of the bridge-partitioning complex is rather short as compared with the collapsing process which takes place at a comparatively slow pace. Thus, this protects the related cells that have already formed. The ICB was previously thought to be a component of the Sertoli junctional complex which bundles the filaments interposed between the cytoplasmic membrane and the subsurface cisterna of the sER of the germ cell. However, the junctional complex is not formed in this part, and the thickness (bridge density) of the

Fig. 4. Collapsing cisternae of the bridge-partitioning complex between sister spermatids. Note the partition of cisternae at the central portion of the bridge channel. $\times 30,000$.

Fig. 5. A newly formed ICB in spermatocytes. Note the scattered sER in the cytoplasm. $\times 12,000$.

Fig. 6. Three spermatocytes connected by ICBs (arrows) at the prophase stage. In the nuclei of the cells, synaptonemal complexes of chromosomes are clearly visible. $\times 6,600$.

germ cell membrane is left only in the ICB.

Morphology of ICB: The narrow part of a dumb-bell shape in a cell division is the starting feature of the ICB development. Once the bridge is opened, the passageway continues throughout the whole process of spermatogenesis. After the completion of bridge formation (spermatogonia and spermatocytes), the passageway shows a cylindrical

form with two thick parallel lines (bridge density) in section. The germ cells are almost enclosed by a thin rim of the cytoplasm of the supporting Sertoli cell. However, the oppositely faced membrane of the Sertoli cell sometimes remains at a distance on the ICB part.

Throughout the spermatid maturation, the length of the ICB duct shortens gradually and shows a

Fig. 7. A longitudinal section of an ICB containing a vacuole and two multivesicular bodies between sister spermatocytes. Note the linear formation of the channel. Several cross-sectioned nuclear pores (arrow heads) are visible. $\times 18,600$.

Fig. 8. An ICB possessing multivesicular bodies between sister spermatids. Note the concave bridge density adhered by sER. $\times 18,600$.

Fig. 9. A channel of an ICB containing sER between sister spermatids. $\times 30,000$.

Fig. 10. An atypical form of an ICB between sister spermatocytes. This kind of wave and/or protrusion of bridge density may be due to the pressure of both daughter cells. The nucleus of the right cell is seen. $\times 18,600$.

Fig. 11. Broad ICBs (arrows) connecting three spermatids at the mature phase near spermiation. Note that the local thickening (bridge density) of the ICB is lessened. $\times 8,000$.

small arc in section. Following maturation, the membranous contact of Sertoli cell to the germ cell is loosened, and that of daughter cells is tightened.

In this study, the number of sister cells connected by ICBs was 3 in spermatogonia, 5 in spermatocytes and 10 in spermatids in sections. This group of sister cells has been referred to as a clone. In contrast, Dym and Fawcett [5] showed a larger number than we did. In the rodent spermatogonia, the number of cloned cells is approximately 16, though it is believed that there are thousands of spermatids theoretically [13].

In monkey spermatogonia, the ICBs are classified into 2 types, in cytoplasmic connection broad and narrow [10]. By scanning electron microscopy, in the rat germ cells, the ICBs are further subdivided into 3 types, long and narrow, intermediate (spermatogonia), and short and broad (spermatocytes and spermatids) [11]. Nevertheless, in the present study, these different types of the ICBs were not observed in the goat testes, though a long and slender connection existed especially in the spermatogonia. Such a flattened or long slender connection is thought to be a part of an extended cytoplasmic limb or sleeve.

Linares [16] measured the size of the ICBs of male germ cells of different mammalian species, and

differentiated the ICBs between the basal and adluminal compartments. The present author did not measure the size of the ICB because of their irregularly curved profiles and undesigned cut angles. The form of goat ICBs tends to differ morphologically between spermatogonia (in the basal compartment) and spermatocytes to spermatids (in the adluminal compartment). From the view point of sectioning, the ICB of the basal compartment shows a comparatively angular shape, but that of the adluminal compartment reveals a more round and smooth shape. Furthermore, Linares [16] reported a species difference in the size of ICBs among mammals. Togawa [23] demonstrated various forms of the ICBs in the human spermatogonia, and Weber and Russell carried out intensive studies on the ICBs of the rat testes [24]. These authors showed with text figures the structural changes of the ICBs in relation to the process of spermatogenesis.

Many reports on the structure and function of the ICBs in various mammalian testes [5, 7, 8, 11, 16, 18, 19, 24] have dealt with events related to synchronous maturation and differentiation of germ cells. Various lines of observation have been so far made on the cytoplasmic matrix including various cellular organelles and products in the ICBs with

special emphasis on the function and importance of bridge in the goat testes and other animals, as follows; 1) Synchronization of metamorphosis and maturation of the sister cells [5, 7, 13, 23–25]; 2) Maintenance of the position and restriction of germ cells in the seminiferous tubules (regulation of spermatogenic wave) [8, 24]; 3) Exchange of cellular materials and substances [13, 25]; and 4) Control of mitosis and meiosis (nature of seminiferous epithelial cycle) [8, 24]. As an essential part of the ICB, the bridge density may work to reinforce stiffen the bridge [6, 7, 23]. Meanwhile, the bridge-partitioning complex prevents further advancement of degeneration [5] and its strength may be able to maintain it during the active cell division [24]. Finally it probably serves as a temporary barrier between sister cells.

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