Three-dimensional Structure of the Sertoli Cell in the Shiba Goat*

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Summary. The three-dimensional structure of the Sertoli cell in the miniature “shiba” goat was examined using scanning electron microscopy. In the basal portion of the seminiferous epithelium, spermatogonia and/or spermatocytes were located in compartments enclosed by adjacent Sertoli cells. From the basal aspect, they were situated in successive recesses. In the middle portion, early round spermatids halfway embedded in the Sertoli cell were recognized. The exposed surfaces of these spermatids were wrapped with ramifying processes which were derived from the Sertoli cell. In the apical portion, only the heads of the maturing spermatids invaded the Sertoli cell. As the spermatid matured, the apical Sertoli process varied in range to finally release the spermatid head. It is probably that the maturing spermatids gradually leave the apical Sertoli process and ultimately segregate themselves from the seminiferous epithelium.

The Sertoli cells, which nurture maturing germ cells, play an important role in the process of spermatogenesis. Although a number of studies in many mammals have been made on their morphological characteristics using light and transmission electron microscopy (ELFTMAN, 1950, 1963; NISHIDA, 1954; FAWCETT and BURGOS, 1956; DYM, 1973; FAWCETT, 1975; KAYA and HARRISON, 1976; OSMAN and PLOEN, 1978, 1986; PLOEN and RITZEN, 1984; EKSTEDT et al., 1986), the three-dimensional configuration was never accurately described, probably because of its complicated morphological features as well as its close attachment to germ cells.

In recent years, however, some investigators have tried to demonstrate the three-dimensional profile of the Sertoli cell. First, GRAVIS (1978) observed the three-dimensional features of the Sertoli cell in the Syrian hamster using scanning electron microscopy, clearly displaying the spermatid stalk connecting the cytoplasmic lobe to the maturation-phase spermatid. In addition, the three-dimensional structure of the Sertoli cell in the rat and monkey was also reconstructed from electron micrographs of semiserial sections (WONG and RUSSELL, 1983; WEBER et al., 1983; RUSSELL et al., 1983, 1986). Moreover, HAMASAKI (1981, 1987) described in detail certain processes of the rat Sertoli cell by scanning electron microscopy. The present study also deals with the three-dimensional structure of the Sertoli cell in the miniature “shiba” goat, with

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the relationship between the Sertoli cell and the germ cells observed by scanning electron microscopy, especially in the seminiferous epithelium containing maturation-phase spermatids. In regard to designations for structures in the apical region of the seminiferous epithelium, we adopted those of Russell's (1984).

MATERIALS AND METHODS

Six miniature "shiba" goats bred in the closing colony of the stock farm of the University of Tokyo were used in this study. Because of its small size, obedient nature, robust health and non-seasonal breeding habits, the shiba goat has gradually come to be used in Japan as a new experimental ruminant over recent years (Kano et al., 1977; Sawaiaki et al., 1979).

The testes from sexually mature shiba goats were fixed in 2.5% glutaraldehyde with 0.1 M phosphate buffer, after perfusing with the same fixative through the testicular artery. They were cut into smaller pieces to be fixed in 2.5% glutaraldehyde overnight. The materials were then washed in 0.1 M phosphate buffer containing 8% sucrose and rinsed in 8 N hydrochloric acid at 60°C for 20-30 minutes. The specimens, washed repeatedly in Hank's solution, were also washed with an ultrasonic oscillator for about 10 minutes. They were postfixed in 1% osmium tetroxide, dehydrated with graded ethanol and dried with liquid CO₂ at the critical point. Finally, they were coated with gold by sputtering in a vacuum evaporator and observed under a Hitachi S-430 scanning electron microscope.

RESULTS

The Sertoli cells extend from the basement membrane of the seminiferous tubule to its lumen, presenting a columnar shape with many processes (Fig. 1). In the present study, the seminiferous epithelium was divided into apical, middle and basal portions for convenience in examining the relationship between the Sertoli cells and each germ cell in epithelium containing maturation-phase spermatids.

In the basal portion of the seminiferous epithelium, spermatogonia and/or early spermatocytes were located in compartments enclosed by adjacent Sertoli cells. No slender processes existed in this area, whereas a number of Sertoli processes grew in a cluster in the upper area (Fig. 2). Figure 3 shows the seminiferous tubule viewed from the basal aspect. In this micrograph, some spermatogonia and successive recesses are

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**Fig. 1.** Whole configuration of the Sertoli cell. The Sertoli cell extends from the basement membrane into the lumen. Some short processes (arrowheads) project from the lateral side of the Sertoli cell. The large space between individual Sertoli cells results from the exfoliation of spermatocytes and spermatids. In the apical portion, several elongated spermatids (asterisks) invade the Sertoli cell with their heads. ×2,600

**Fig. 2.** Seminiferous epithelium from the basal portion to the middle portion. In the basal portion, spermatogonia and/or spermatocytes are located in compartments enclosed by adjacent Sertoli cells. In the middle portion, slender Sertoli processes project in large numbers. ×1,300

**Fig. 3.** Basal portion of the seminiferous epithelium from the basal aspect. Some spermatogonia (asterisks) are situated in successive recesses. These recesses consist of continuous Sertoli cells. BM basement membrane, SET Sertoli trunk. ×1,800
Three-dimensional Structure of Sertoli Cell

Fig. 1-3. Legends on the opposite page.
clearly recognized, since the basement membrane has been exfoliated by the artificial shock.

In the middle portion, early round spermatids were observed successively, being halfway embedded in the Sertoli cell. The exposed surfaces of these spermatids were wrapped with ramifying processes derived from the Sertoli cell (Fig. 4). The Sertoli cell was provided with many slender processes in this portion, unlike that in the basal portion. These processes ramified into secondary and tertiary branches, occasionally showing a digitate shape (Fig. 4). Ring-like tips were sometimes seen (Fig. 5, Inset) in the Sertoli processes in addition to the usual pointed tips. Shallow crater-like dents were frequently found on the exposed surfaces of the early round spermatids (Fig. 4), probably representing artifacts caused by the hydrochloric acid treatment. The spermatocytes and spermatids in this portion were more easily exfoliated from the Sertoli trunk during specimen preparation.

In the apical portion, many spermatids often attached to the Sertoli cells (Fig. 1). In this area, the spermatids at the maturation phase invaded the Sertoli cell only with their heads. The spermatid head of the shiba goat presented a flat and elliptical shape (Fig. 5, 6). These spermatids, surrounded by apical Sertoli processes, were isolated from the trunk of the Sertoli cell, since the apical Sertoli process connected to the Sertoli trunk by a narrow and short Sertoli stalk (Fig. 5). As the spermatid matured, the apical Sertoli process varied in range, finally releasing the spermatid head. Some

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Fig. 4. Artificially colored micrograph of the middle portion of the seminiferous epithelium. The yellow colored area shows the Sertoli cell, and the green area the spermatids. Some early round spermatids are embedded in the Sertoli cell. The exposed surfaces of these spermatids are wrapped with ramifying Sertoli processes. Crater-like dents are frequently seen on these surfaces. ×5,500
Three-dimensional Structure of Sertoli Cell

Apical Sertoli processes completely surrounded the head (Fig. 5), others only one-third of it (Fig. 6). The cytoplasmic lobe (asterisk) is connected to the maturing spermatid by the spermatid stalk (arrowheads). ×5,500. Inset. Ring-like tips (arrowheads) of the Sertoli cell are sometimes seen among the usual pointed tips. ×5,400

Apical Sertoli processes completely surrounded the head (Fig. 5), others only one-third of it (Fig. 6). The cytoplasmic lobe, which logically becomes a residual body after spermiation, was attached to the neck region of the maturing spermatid by a slender spermatid stalk (Fig. 5) of varying widths. Maturing spermatids with no spermatid stalk were frequently seen (Fig. 6), showing that their stalks detached from the spermatids before the apical Sertoli process was isolated from the spermatid head. The surface of the apical Sertoli process possessed numerous small pores. These pores also

Fig. 5. Seminiferous epithelium from the middle portion to the apical portion. Ramifying Sertoli processes are observed to a great degree. The apical Sertoli process surrounds the spermatid head. The cytoplasmic lobe (asterisk) is connected to the maturing spermatid by the spermatid stalk (arrowheads). ×5,500. Inset. Ring-like tips (arrowheads) of the Sertoli cell are sometimes seen among the usual pointed tips. ×5,400
seemed to be artifacts caused by the hydrochloric acid treatment, as were also the crater-like shallow dents on the exposed surfaces of the early round spermatids.

DISCUSSION

To comprehend the precise configuration of the Sertoli cell, the observation of its

Fig. 6. Maturing spermatid in the apical portion. Numerous Sertoli processes are also seen around the maturing spermatid. The maturity of these spermatids is further progressed than those shown in Fig. 5. In some maturing spermatids (asterisks), the spermatid stalk (arrowheads) vanishes and the apical Sertoli process encloses only one-third of the spermatid head. These spermatids appear at a point just before their segregation from the Sertoli cell. ×7,500
Three-dimensional Structure of Sertoli Cell

Three-dimensional structure by scanning electron microscopy is advantageous to that of its two-dimensional structure by transmission electron microscopy.

Although artifact-like structures such as small pores on the apical Sertoli process

Fig. 7. Diagram showing a Sertoli cell and germ cells. The yellow area shows the Sertoli cell and its ramifying processes, and the green area the germ cells. Abbreviations; ER·SPD early round spermatid, MP·SPD maturation-phase spermatid, SES Sertoli stalk SPC spermatocyte, SPG spermatogonium, SPS spermatid stalk.
and crater-like dents on the exposed surface of the early round spermatid were recognized and presumed to have been caused by the hydrochloric acid treatment, we can say that a three-dimensional view of the Sertoli cell in each portion was obtained through the present study. Figure 7 shows one Sertoli cell and its supporting germ cells at a certain stage of the seminiferous epithelium as a conclusion of this study.

It is generally accepted that the shape of the Sertoli cell continually changes in association with the progress of spermatogenesis (Elftman, 1950, 1963). Russell and coworkers (Wong and Russell, 1983; Russell et al., 1986) classified the configuration of the Sertoli cell into Type A and Type B. The cell observed in this study corresponds to their Type A.

In the basal portion of the seminiferous epithelium, the germ cells seem to be situated in compartments enclosed by adjacent Sertoli cells. Furthermore, they were located in successive recesses as viewed from the basal aspect. These recesses were formed by continuous Sertoli cells, presumably being equivalent to the so-called basal compartment (Fawcett, 1975).

In the middle portion, the Sertoli cell has many slender processes, and contact between the Sertoli cell and germ cells seems looser than that in the basal portion. In fact, during the preparation of specimens, germ cells in this portion tended to exfoliate from the Sertoli trunk. In this way, the middle portion differs in appearance from the basal portion. Therefore, the middle portion, provided with many slender processes, probably belongs to the adluminal compartment (Fawcett, 1975).

Russell (1984) described how the cytoplasmic lobe, which was attached to the maturing spermatid by the spermatid stalk, would eventually be segregated as a residual body from the spermatid at spermiation. Gravis (1978) also indicated that the spermatid stalk acted to moor the spermatids to the seminiferous epithelium after the apical Sertoli processes lost their grip on the spermatids. In contrast, the results obtained from this study showed that the apical Sertoli processes acted to anchor the spermatids after the spermatid stalk lost its grip, since the spermatid stalk separated from the maturing spermatid earlier than the apical Sertoli process.

These observations suggest that, as the spermatid matures, the spermatid stalk becomes gradually thinner, to finally vanish. The spermatid head also may gradually leave the apical Sertoli process, resulting in the spermatid detaching itself from the seminiferous epithelium.

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