Ultrastructure and Function of Sertoli Cell Processes in the Korean Native Goat

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Summary: The ultrastructure of Sertoli cell in the testes of Korean native goats was investigated by scanning and transmission electron microscopy to elucidate the relationship between germ cell and Sertoli cell processes in the spermiogenetic cycle. Type-A Sertoli cells at stage V of the spermiogenetic cycle and type-B Sertoli cells at stage VII of the spermiogenetic cycle were used for analysis. Morphologically, the Sertoli cell processes were classified into sheet-like and slender cord-like processes. The sheet-like process originated solely from the Sertoli cell column while the slender cord-like process projected either from the Sertoli cell column or the sheet-like process. Periodic acid (PA)-thiocarbohydrazide (TCH)-silver protein (SP)-physical development (PD)-positive granules were found diffusively both in the sheet-like and slender cord-like processes near the round spermatid, whereas they had accumulated near the head of the elongated spermatid. The morphological variation and glucoconjugate histochemical reaction of the Sertoli cell processes reflect nourishment, movement and transformation of the spermatogenic cells in accordance with spermiogenesis.

Korean native goat has been used as an experimental animal because of its small body size and short puberty period. However, there have been few studies on the structures of and relationship between germ cells and Sertoli cells in the spermiogenetic cycle.

Sertoli cells, columnar-shaped, non-spermatogenic cells of the testis, extend centripetally from the basal lamina towards the lumen of the seminiferous tubule. The lateral contour of the cell bears numerous cellular processes and multiform recesses that trap germ cells either partially or completely inside (Fawcett, 1975; Russell, 1977; Russell *et al.*, 1983; Vogl *et al.*, 1985; Weber *et al.*, 1983). This complex structure derived from an irregular appearance of the cell surface. During spermatogenesis, Sertoli cells play a strategic role in transfer of spermatocytes to the adluminal compartment of the seminiferous tubule via cell processes (Morales and Clermont, 1982; Russell, 1977; Vogl *et al.*, 1985). The processes penetrate the

spermatid cytoplasm to form a canalicular complex, which contributes to the removal of residual body (Sakai *et al.*, 1988, 1989; Takahashi-Iwanaga and Fujita, 1986). Morphology of the cell processes has been described as either ramified or conical wedge-like, cup-like, sheet-like, flat club-like or slender cord-like shapes (Hamasaki, 1987; Hamasaki and Murakami, 1986; Kurohmaru and Nishida, 1987; Park *et al.*, 1993; Wong and Russell, 1983). To determine the function of Sertoli cells and the relationship between Sertoli cells and spermiogenetic cells, investigations of the testis by means of lectin histochemistry have been carried out in rats (Raychoudhury and Millette, 1997; Martinez-Menarguez *et al.*, 1992), chicks (Nagano *et al.*, 1995), musk shrews (Kurohmaru *et al.*, 1995), Shiba goats (Kurohmaru *et al.*, 1991).

In this study, we therefore investigated the structure and physiological mechanism of Sertoli cell processes in testes of Korean native goats.

Materials and Methods

Four healthy and sexually mature Korean native goats in breeding season were used in this study. The goats were anesthetized with xylazine HCL (0.2 mg/kg) and castrated bilaterally. The testes were diced and prepared for transmission and scanning electron microscopic observation.

For analysis by transmission electron microscopy, the left testes from four goats were diced (approximately 1 mm³). The specimens were prefixed at 4°C for 2 hrs in 3% glutaraldehyde. The tissues were post-fixed in 1% osmium tetroxide at 4°C for 1 hr, dehydrated, embedded in Quetol 812, and sectioned with a diamond knife. The sections were stained with uranyl acetate and then counterstained with lead citrate. For cytochemical analysis, the tissue was cut into small pieces and placed in 0.1 M phosphate buffer containing 4% paraformaldehyde (pH 7.4) at 4°C for approximately 2 hrs. After washing with the same buffer and dehydrating with a graded ethanol series, the samples were embedded in LR-White (The London Resin Co., UK; Newman *et al.*, 1983). The tissues were sectioned and reacted with periodic acid (PA)-thiocarbohydrazide (TCH)-silver protein (SP)-physical development (PD) according to the method of Kitamura (1990).

For analysis by scanning electron microscopy, the right testes from four goats were cut into small cubes (2 × 2 × 5 mm). The samples were immersed in 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for 5 minutes followed by continuous prefixation with glutaraldehyde solution for 3 hrs. The samples were then washed 3 times in phosphate buffer and placed in 1 N NaOH at 60°C for 20–30 minutes (Takahashi-Iwanaga and Fujita, 1986). After washing in distilled water and treating with an ultrasonic oscillator for about 10 minutes, the cubes were processed by the modified tannin-osmium method (Murakami, 1974). The samples were then dehydrated and freeze-dried using the t-butyl alcohol substitution method (Inoue and Osatake, 1988), coated with gold particles, and finally examined under a field-emission scanning electron microscope (JAM-6000F, JEOL, Tokyo, Japan).

Criteria for classification of Sertoli cells were based on the different stages of the spermiogenetic cycle. Type-A and type-B Sertoli cells were classified as Sertoli cells of spermiogenetic stages V and VII, respectively (Park *et al.*, 1993).

Results

The type-A Sertoli cell, just before spermiation,

was identified by elongated mature spermatids on its apical portion (Fig. 1a). The type-B Sertoli cell, after spermiation, was recognized by the presence of round, immature spermatids on its luminal region (Fig. 2b).

Basically two different types of process, sheet-like and slender cord-like processes, were found in both type-A and type-B Sertoli cells. These two processes underwent metamorphosis according to spermiogenesis and transformed to either ramified leaf-like, club-like, or ring-like shapes.

The sheet-like processes having simple and smooth margins originated solely from the Sertoli cell column. They surrounded a half to one-third of the surfaces of the spermatocytes and spermatogonia at the base of the seminiferous tubule (Figs. 1a, 1b, 2a, 2b). The processes that were still attached to the spermatids gave off short club-like structures in the middle and apical portions of the seminiferous tubules (Figs. 1c, 2c).

The slender cord-like processes were found in different areas. Processes that existed in the middle and apical portion of the seminiferous tubules projected from the Sertoli cell column and/or the sheet-like processes (Figs. 1a, 1b, 2a, 2b), while processes that were still attached to the round spermatids had fine elliptical tips that finally transformed into leaf-like, club-like or ring-like structures (Figs. 1c, 2c).

In the type-A Sertoli cell before spermiation, positive reaction of the PA-TCH-SP-PD with materials accumulated in the cell column and sheet-like processes was observed near the heads of the elongated spermatids (Fig. 1d). In the type-B Sertoli cell after spermiation, the round spermatids were surrounded by sheet-like processes that contained numerous PA-TCH-SP-PD-positive granules (Fig. 2d).

Discussion

Our results were slightly different from results of ultrastructural studies on Sertoli cells in other mammalian species. Previous TEM and SEM observations indicated that configurations of the Sertoli cell processes were different in species such as the Syrian hamster (Gravis, 1978), rat (Hamasaki and Murakami, 1986; Wong and Russell, 1983), Shiba goat (Kurohmaru and Nishida, 1987), dog (Park *et al.*, 1993) and monkey (Russell *et al.*, 1986). The Sertoli cell processes observed in the present study were different from those observed in the above species.

Based on SEM findings, Hamasaki and Murakami (1986) reported four types of primary proc-

ess, bough-like, sheet-like or sleeve-like, retracted, and wrapper-like processes, extending from the rat Sertoli cell. They also reported that the apical processes were adorned with bleb-like appendices, arboreal expressions, or ramifying tentrils. In the Shiba goat, configurations of the Sertoli cell processes were classified into slender, ramifying and ring-like tip processes. The shapes of these processes differed in the portion of the seminiferous tubule (Kurohmaru and Nishida, 1987).

The Korean native goat is a close species to the Shiba goat. In this study, we observed only two types of process extending from Korean native goat Sertoli cells: sheet-like and slender cord-like processes. The slender cord-like process have many modified cell processes. We named these cell processes were shapes. The characteristics of these processes seem to be insignificant, distinction on contour of the border of the sheet-like processes overlying a large portion of each developing spermatid in the seminiferous tubule. A simple border was found in Sertoli cells from the Korean native goat and Shiba goat (Kurohmaru and Nishida, 1987), whereas a complicated border was evident in the Sertoli cells from the monkey (Russell *et al.*, 1986), Syrian hamster (Gravis, 1978) and rat (Hamasaki and Murakami, 1986). The reasons for these incompatibilities are not clear. The variations may be due to species specificity. Apical varieties of the modified slender cord-like processes assume various positions on the germ cell in correlation with its advancing maturation. The modified slender cord-like processes might play a role in disengagement of the residual body from the head of the spermatid just before spermiation (Hamasaki, 1987; Park *et al.*, 1993).

In this study, observation of the elliptical tips of the slender-like processes that had attached to the spermatids and transformed into leaf-like structure suggests that the Sertoli cell processes do not only anchor to the germ cells but also actively support them. However, it was suggested in previous reports that the Sertoli cell fixes germ cells with its processes and moves the cells to the lumen of the seminiferous tubule (Murakami, 1974; Russell, 1977). The Sertoli cell might therefore be involved in the exchange and elimination of excessive sperm cellular substances (Park *et al.*, 1993; Russell *et al.*, 1983, Sakai *et al.*, 1988, Sakai and Yamashina, 1989).

The distribution pattern and properties of the carbohydrate residues in the germ cell and Sertoli cell investigated by use of the PA-TCH-SP-PD reaction are relation to the functions of and relationship between the Sertoli cell process and spermiogenetic cells. The positive reaction indicated the

presence of glucoconjugates such as glycogen, D-galactose and multiple fucosyltransferases (Kurohmaru *et al.*, 1995; Raychoudhury and Millette, 1997; Raychoudhury *et al.*, 1997.). Numerous positive materials are found in the sheet-like and slender cord-like processes in different areas. Reacted materials accumulate in the sheet-like processes of the pre-spermiating type-A Sertoli cell near the head of the elongated spermatid, whereas they are scattered in the sheet-like process of the post-spermiating type-B Sertoli cell near the round spermatid. The glycoconjugates containing D-galactose change stage-dependently in Sertoli cells of the musk shrew (Kurohmaru *et al.*, 1995). Glycoconjugates localized on the Sertoli cell surface may play an important role in testicular cord formation (Nagano *et al.*, 1995). The multiple fucosyltransferases and their lectin ligands are involved in mediation of germ cell-Sertoli cell adhesion to form a cohesive epithelium and in assistance of the germ cell in translocating to the adluminal compartment of the seminiferous tubule (Raychoudhury and Millette, 1997; Samir *et al.*, 1997). These findings suggest that Sertoli cell processes are involved in nourishment, movement and transformation of germ cells in accordance with the spermiogenetic cycle toward the tubular lumen.

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Explanation of Figures

Plate I

Fig. 1a–d. 1a: SEM images of Type-A Sertoli cell. An elongated spermatid (E) has extended from the basal lamina toward the lumen of the seminiferous tubule. The sheet-like process (SH) and slender cord-like process (SL) originated from the middle portion of the Sertoli cell column. SP, spermatocyte; Bar is 10 μ m. 1b: TEM images of middle and apical portions of a type-A Sertoli cell. A sheet-like process (SH) has surrounded the head of an elongated spermatid (E). A slender cord-like process (SL) originated from the Sertoli cell column (SC). R, round spermatid. Bar is 5 μ m. 1c: SEM images apical portion of a type-A Sertoli cell. A sheet-like process (*) have extended onto the elongated spermatid (E). Bar is 10 μ m. 1d: Apical portion of a type-A Sertoli cell. PA-TCH-SP-PD-positive granules (arrowhead) are clustered in the vicinity of the elongated spermatid (E). Bar is 2 μ m.

Plate II

Fig. 2a–d. 2a: SEM images of Type-B Sertoli cell. A round spermatid (R) has located in the apical portion of the Sertoli cell. A sheet-like process (SH) is wrapped around the surface of a spermatocyte (SP). SL, A sheet-like. Bar is 10 μm . 2b: TEM images of middle and apical portions of a type-B Sertoli cell. A sheet-like process (SH) has surrounded the round a spermatid (R). Club-like shapes (CL) and a slender cord-like process (SL) originated from the sheet-like process. R, round spermatid. Bar is 5 μm . 2c: SEM images apical portion of a type-B Sertoli cell. Slender-cord like process (SL) and a slender cord-like process (SL) have extebded onto the elongated spermatid. R, A round spermatid. Bar is 10 μm . 2d: PA-TCH-SP-PD-positive granules of the sheet-like process in a type-B Sertoli cell. Numerous granules are scattered in the sheet-like process (SH) and slender cord-like process (SL). R, round spermatid. Bar is 2 μm .