Ultrastructure of Goat Testes: Tubulobulbar Complexes between Spermatids and Sertoli Cells

Yoshio KOJIMA

Laboratory of Reproductive Physiology, Faculty of Agriculture, Shizuoka University, 836 Ooya, Shizuoka 422, Japan

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ABSTRACT. The fine structure of tubulobulbar complex (TBC) of goat seminiferous epithelium was studied using testicular samples fixed by perfusion. During the maturation phase of the spermatid, the TBC appeared on the periphery of the head before spermiation, dorsal and ventral parts of the head, both sides of the acrosome and on the postnuclear sheath region. At an early stage of the development of the TBC, a partial loss of smooth endoplasmic reticulum in the ectoplasmic specialization of a neighboring Sertoli cell was observed; filament bundles disappeared and a small fragmented smooth endoplasmic reticulum was left. Subsequently, a spermatid vesicle (coated vesicle) expanded to be in contact with the Sertoli cytoplasm by a small channel. These tubular projections extended to form a bulbous balloon-like structure forming sometimes a bi-balloon appearance, until torn off from the spermatid into the Sertoli cytoplasm and probably digested by the Sertoli cell. The significance of this structural development was morphologically discussed.—KEY WORDS: goat spermatid, TBC, ultrastructure.

Russell and Clermont [16] reported an interesting invaginating structure located between Sertoli cells and germ cells of the rat seminiferous tubules, a structure being later named the tubulobulbar complex (TBC). These structures are observed not only in rodents but also in other mammals [17] including farm animals [3, 8, 9].

However, no information has been available on the morphological study of goat testis with respect to this structure, and whether the TBC exists also in the seminiferous tubules of goat has not been clearly understood.

This study was therefore undertaken to investigate morphologically the existence of TBC in goat with particular interest to its function.

MATERIALS AND METHODS

Eight adult mongrel goats (Capra hircus) were used in this study. Anesthesia was induced by intramuscular injection of 2% Lylazine HCl (0.8–1 ml, Rompan, Bayer). Collection of testes was performed by rough castration using the standard operating procedures. The testis was perfused through the testicular artery or its branch in an ice water bath at 0°C, and the pampiniform plexus was incised to permit egress of blood and perfusate [2]. About 130 ml of Dextran 40 (10% v/v Dextran; MW 40,000 in 0.9% saline), saline or Ringer solution followed by 80–140 ml of 5% glutaraldehyde buffered with 0.22 M s-collidine [1] or cacodilate solution were successively permitted to flow through the testis for 30 min. One- or 2-mm cubes of the tissue were then cut out with a razor blade, immersed in a glutaraldehyde fixative solution containing 1% tannic acid [7] for additional 90–120 min, and post-fixed in 2% osmium tetroxide using the same buffer solution.

After washed thoroughly with distilled water, the tissues were stained en bloc with
1% uranyl acetate solution at 5°C overnight [5]. After dehydration with a graded alcohol series and substitution with acetone, the samples were embedded in epoxy resin [6], and sectioned on a Sorval MT-1 ultramicrotome with glass knives. The sections were mounted on uncoated grids, stained with saturated lead citrate [10], and examined by JEOL T-7 and 1200EX electron microscopes.

RESULTS

During an early phase of the development of the TBC, particularly at the hemi-close junction, there was a partial loss of smooth endoplasmic reticulum in the ectoplasmic specialization (EcS) of the Sertoli cell adjacent to a spermatid head.

At first, the TBC appeared as a small invagination of the Sertoli cell membrane showing a form of bristle-coated vesicle (BCV) on the spermatid cell membrane, this phenomenon resembling micropinocytosis of the Sertoli cell (Figs. 1 and 2). From the viewpoint of the germ cell side, it appeared as an exocrine figure. The formation of the TBC was followed by the extension of the vesicle with a small narrow tube (Ø=ca 10 nm, up to 1-3 µm length) into the Sertoli cytoplasm. This special tubule or deep channel was in contact with the Sertoli cell membrane with a gap of about 0.5 nm. Moreover, the contact point was always

![Fig. 1](image1.png)

Fig. 1. Micrograph of a sagittal section of a goat spermatid. On the acrosome area, a typical bristle-coated pit (arrow) of the Sertoli cell has started to form the TBC with a short tube from the spermatid cell membrane. ×90,000.

![Fig. 2](image2.png)

Fig. 2. Narrow tubes are expanding into the Sertoli cell at the sites of equatorial zone (acrosome) and postnuclear sheath. Note the deficiency of ectoplasmic specialization (+) of the Sertoli cell around the TBC area. ×20,000.

![Fig. 3](image3.png)

Fig. 3. Two TBCs situated on the ventral and dorsal sides of the acrosome area. Tubular evagination is extending into the Sertoli cell. Note a bristle-coated pit at the depth of the Sertoli recess. ×20,000.
reinforced by a sparse filament bundles in the Sertoli cytoplasm (Figs. 2-4). At a later phase of the development of the TBC, a round bulk ($\phi$=ca 0.5 $\mu$m) was formed at the tip or side of the tube (Figs. 4-7). The bulk was usually surrounded completely by a very thin smooth endoplasmic reticulum of the Sertoli cell. In some occasions, the bulk showed a double barrel- or bi-balloon-like structure (Figs. 6 and 7). The bulk was then torn off in the Sertoli cytoplasm (Figs. 8 and 9) and might be digested by the cell. During the spermiating process, the TBCs were completely separated from the spermatid.

DISCUSSION

Ultrastructural studies on the testis of most mammalian species have been carried out. From these studies, it has been well known that special structures, called EcS, appear in the Sertoli cytoplasm close to the Sertoli-Sertoli and the Sertoli-germ cell apposition [4]. Beside these, another type of structure, called TBC, is present. The TBC is a deep invagination of the Sertoli cell membrane, which contains a membrane protrusion from the late spermatid.

Nicander [8] first reported this tubular invagination in the bull and named it a "deep pocket" of the Sertoli cell membrane. Similar findings in the same species were also obtained by Ekstedt et al. [3]. In the rat [16], the membrane protrusion of the spermatid was plurally observed on the ventral side of the sickle-shaped head of the step 19 spermatid, and it was thought to be an anchoring device for spontaneous spermiation; thus it was called TBC. Russell [12, 13] examined critically the three dimensional
form of this apparatus in the rat testes. His results included the clarification of the Sertoli-germ cell relationship and the mechanism of spermiation [14, 15]. This process in the boar testes was called a tubular device [9]. The TBC has been also found in several other mammalian species [17]. Russell [14, 15] discussed the functional meaning of this structure with the mechanism of spermiation.

Proper handling and processing of testicular samples would yield a better view of the TBC around the head surface of the maturating spermatid in the testis of various mammals. It has been reported that the TBC bulk in the rat contains acid phosphatase activity [17].

In the goat seminiferous epithelium, bristle-coated pit of the Sertoli cell engulfing a vesicle of the plasma membrane of the late spermatid, first appeared on the ventral and dorsal sides of the head, the acrosome and the postnuclear sheath area. The vesicle formed a bulk with a narrow stalk expanding into the surrounding Sertoli cytoplasm as an evaginating structure from the spermatid. This bulk reached its full development in the later phase of spermatid maturation (Figs. 1–5). The late spermatid with TBC was situated close to the lumen of seminiferous tubule in preparation for spermiation, and the TBC lost the covering components of the subsurface endoplasmic reticulum of the Sertoli's EcS where only the filament bundles were left. By this time, the formation of the middle piece (mitochondrial arrangement with annulus) and of an equatorial segment of the acrosome had.
already been completed.

These morphological changes (see Figures) may indicate that the TBC is a kind of structure for the removal of excessive germ cell membrane and fluid of the spermatid, suggesting the start of the spermiation process.

It is suggested that the TBC acts not only as an anchoring device for the late spermatid but also as an absorption mechanism of the residual membrane system and the excessive cytoplasmic substance of the cell, prior to spermiation [17].

REFERENCES


要 約

ヤギ精巣の微細構造：セルトロ細胞と精子細胞間の tubulobulbar complex (TBC)：小島義夫（静岡大学農学部家畜繁殖学教室）—ヤギ精巣を観流固定し、TBC の形成並びにその形態について詳細な観察を行った。ヤギの TBC は精子形成の最終段階で精子細胞の背腹側に発生し、頭部部分のみならず後部核部も含め出現した。精子細胞の完熟期に、セルトロ細胞の表層膜が精子細胞膜を伴って微小食喰作用 (microinocytosis) 状の被覆小胞 (bristle coated vesicle) を形成することから TBC 形成が始まる (精子細胞側からは外分泌の被覆小胞の形となる)。この小胞は直径約10 nm の 1～3 μm にわたる細長い管状構造を呈してセルトロ細胞内へ伸びる。管状構造は両膜が数 nm の接触を保ち、セルトロ細胞側は微小線維によって補強されている。最も発達した時期には管の末端や中間で直径0.5 μm 大の円球状膨化をおこし、精子細胞膜が膨れる。膨化は二連球状となり中間で膨化する場合もあるが、何れもセルトロ細胞の胞面小胞体によって密に閉まれている。最終的には子切れてセルトロ細胞に貯蓄、吸収される。これらの構造物は文献で deep pocket (ウシ), anchoring device (ネズミ類), tubular device (ブタ) と呼ばれたもので、他の哺乳類同様、ヤギにも存在することを証明した。その機能としては anchoring device のみならず、精子細胞の核濃縮に伴う余剰膜や精子細胞内の余剰成分の処理に関与して、重要な意義を持つことが示唆された。