Ultrastructure of Boar Testis: Spindle Shape Body of Spermatid

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(Received 8 May 1990/Accepted 15 June 1990)

ABSTRACT. The fine structure of the spindle shape body (SSB) of boar spermatid was studied using testes samples fixed by perfusion. This structure appeared on the middle piece which served as the upper border of the fibrous sheath of principal piece during the transition period between the late acrosome phase to the maturation phase of spermiogenesis. The formation of this thread-like spindle form coincided with the development of the postnuclear sheath with perinuclear ring just prior to the growth of the equatorial segment of the acrosome. Likewise, the ribs of the fibrous sheath on the principal piece were observed to have already formed but have not yet completed the mitochondrial sheath. The total size of the SSB and its consisting microtubule were measured. The functional meaning of this transitory construction may involve a threshold condition on the sperm middle piece.—KEY WORDS: boar spermatid, SSB, ultrastructure.

The process of spermiogenesis in several mammalian species is marked by a transitory tubular structure that appears on the proximal end of the principal piece called spindle shape body (SSB) [9, 11, 14]. However, the sequence of events of boar spermiogenesis concerned with this structure has not yet been clarified in detail.

The present study aims at resolving problems related to the structure on SSB of the spermatid of boar testis. This could be the first report showing the SSB of boar spermatid.

MATERIALS AND METHODS

Testicular samples taken from proven sires of Landrace (4), Yorkshire (2) and Hampshire (2) at the slaughter house were cut into small pieces with a razor blade before depletion and then immersed into 3% glutaraldehyde fixative buffered with sodium cacodylate solution (pH 7.3) [12]. The samples were fixed in the same fixative for 1–12 hr, washed in the buffer alone and subsequently immersed in a 1% osmium tetroxide in the buffer.

Embedding was done in Epon [8] and thin sections by means of Porter-Blum MT-1 ultramicrotome, were stained with uranyl acetate [15] and lead citrate [13] and examined using two electron microscopes (JEOL T-7 or 1200EX).

For descriptive purposes, the process of spermiogenesis is subdivided into four phases; Golgi, cap, acrosome and maturation phases [2, 5].

RESULTS

The SSB of boar spermatid appeared during the late acrosome to the mid-maturation phase of spermiogenesis. Such formation occurred between the period of the development of postnuclear sheath with the perinuclear ring, disappearance of the micro-tubular bundle of the manchette and just before the formation of the equatorial segments on the head (Figs. 1 and 2). Similarly, the ribs of the fibrous sheath on the principal piece were observed to have developed but have not yet completed the
Fig. 1. An electron micrograph of a late spermatid with condensed nucleoplasm. The head cap (acrosome) does not consist of an equatorial segment and the manchette disappeared. A perinuclear ring (arrow head) however, does exist and the precursor of the annulus is moving to its terminal location. Note the developing SSB (arrow) at the upper part of the fibrous sheath of the principal piece. The mitochondrial formation of the middle piece has not yet started. ×12,000.

Fig. 2. Longitudinal section of a late spermatid (maturation phase of spermiogenesis). The period of mitochondrial sheath formation is characterized by the condensation of the nucleoplasm with the redundant nuclear envelop (*), absence of an equatorial segment and the presence of SSB (arrow). ×9,000.
mitochondrial arrangement of the middle piece (Figs. 1–7).

At this point, the precursors of the annulus with the chromatoid body changed the position and located themselves in the border of the middle piece as the Jensen’s ring (Figs. 5–7). The average size of a well developed SSB was ca 0.53 μm in diameter and 0.93 μm in length (n=25).

The SSB consisted of a thread-like spindle ring of microtubular complex (Figs. 7 and 8). This microtubule had an average diameter of ca 30 nm (n=62) and arranged themselves spirally (2–5 layers) around the axial fibrillar bundle of the sperm tail. Additionally, incomplete C-shaped microtubules were present cross-sectionally (Figs. 4 and 7).

**DISCUSSION**

The fine structures of spermatogenesis in most mammalian species have been well considered [3]. However, only a few undertakings have been reported in boar testis [10].

A peculiar spindle-shaped swelling (SSB) was first observed in rabbit [9] and was confirmed in baboon, bull and cat spermato-tids [1, 5, 16]. A more detailed structural interpretation of this tubular complex was carried out in cotton marmoset by Rattner and Brinkley [11]. In human testis, this same structure was first called “unusual microtubular body” [4]. However, after further studies and considerations on the morphological formation of this structure, it was later called SSB by Holstein et al. [6, 14].
Spermiogenesis is characterized by the elongation of the axoneme of spermatid from the distal centriole at the basal part of the round nucleus (Golgi phase). Consequently, the transition period from the Golgi phase to the early cap phase is marked by the enlargement of the head cap (acrosome cap) containing acrosome substances covering the anterior half of the round nucleus. At this point, the centriolar adjunct appears on the proximal centriole in the implantation fossa of the sperm head, in the neck.

Accordingly, during the formation and elongation of the manchette, a microtubular sheath develops as a deep invagination of the cytoplasm surrounding the expanding axoneme. It forms both the cytoplasmic canal and the cytoplasmic sleeve. The anterior margin of this deep invagination is confined with a ring shape of dense body later called annulus or Jensen’s ring.

Throughout, the axoneme is elongating and dilating with the addition of the outer coarse fibers and the axial fibrillar complex (axoneme and 9 coarse fibers) distinctively composed of $2+9n+9$ pattern including the microtubules and its fibrillar components. Subsequently, the nucleus of the spermatid changes its orientation of the pole to the base of the seminiferous tubules. The elongated axial fibrillar complex was then covered with the fibrous sheath (tail sheath) at the distal part forming the principal sheath. The kinetics of the fibrous sheath formation was extensively studied in the rat spermatid [7].

Before the start of mitochondrial arrangement in the middle piece, a distinct growth of spiralled microtubular complex could be
found in the anterior border of the principal piece. This microtubular coil is the SSB, which develops and piles up assuming a thread-spindle like structure or the skeins of yarn.

Although the size of a fully developed SSB was not so apparent because of its transient formation, we managed to determine the average size of this structure in boar through random measurement of its longitudinal and cross sectioned samples. The size of the microtubules of boar SSB was comparatively similar with that of marmoset’s SSB [11].

Studies on the SSB of human spermatid indicated the existence of direct conversion of microtubules to the dense element of the fibrous sheath of the tail [14]. The relationship between the juxtaposition of the microtubules and cranial ribs of the fibrous sheath with the sheath of the principal piece strongly supports their role as a damming up of material in passage. In addition, the annulus forms the fibrous sheath via the microtubular system of the SSB [6].

On the other hand, Rattner and Brinkley [11] hypothesized that this transitory structure served as the opening of the middle piece. The present study conceded with the above consideration because the ribs of the fibrous sheath started its development just before the advent of the microtubular mass (SSB) in boar spermatid. And then, Irons and Clermont [7] claimed that the formation of ribs occurred in the earlier stage rather than the later stage in the rat spermatid.

The precursor of the annulus appeared during the acrosome phase in the deepest pocket area of the fossa of cytoplasmic canal before degenerating. It is also possible that
growth of the SSB is involved in the middle piece formation and its location determines the position of the annulus during the maturation phase of spermiogenesis. Furthermore, this study resolved the time sequence of the equatorial segment formation (thinner zone of the head/acrosome cap). In boar spermatid, the equatorial segment of the acrosomal cap was always formed after the disappearance of the SSB.

REFERENCES


Andrologia 8: 157-165.

要約

精巢細胞の微細構造: 精子細胞の Spindle shape body (SSB)：小島義夫（静岡大学農学部家畜繁殖学教室）——繁殖壁の明らかに8頭の精巢を放血前に取材し、固定、包埋後、超薄切片にウランと鉛の染色をして電顕で観察した。膜では SSB は頭部期から成熟期の間 (頭部で後部核根ができ、外套微管束が消失し始め、頭部の赤道带が未完成の時期) に、中皮部底端部に形成された。この SSB 全体の大きさは平均0.93×0.53 μm (n=25) となり、平均0.30nm (n=62) の微細管が2～4層に重層して尾部の軸線維束を囲繞した紡ぎ糸状構造を示した。SSB の出現時期は終輪の前筋体が急速に下降し、尾部の伸展によって形成された溝をとり巻いて下垂していた細胞質の軸をたくし上げることによって消失していく過程に当たり、既に尾部の線維鞘が形成されている。SSB の短期間の出現と消失は終輪の定義 (次いでミトコンドリア鞘の形成) と頭部の赤道帯形成の次期にとし引続きされ、SSB の機能については、尾部線維鞘の形成よりもむしろ中皮部の形成に勤っているものと考えられる。即ち、形態学的所見から見て、中皮部と尾部主部を区切る終輪の定義と成熟期に起こる中皮部形成に関与している可能性がある。SSB は分合、哺乳動物の精子形成過程に共通のものであろう。