Descriptive Histology of the Seminiferous Epithelium of the Rat Testis : Pictorial Introduction to Reproductive Biology

Descriptive Histology of the Seminiferous Epithelium of the Rat Testis: — Pictorial Introduction to Reproductive Biology —

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Abstract

Histology of the seminiferous epithelium of the rat testis is described at the light microscope level. The morphologies of male germ cells (spermatogonia, spermatocytes, and spermatids) undergoing the spermatogenesis are depicted chronologically using PAS-stained materials, and correlated with 14 stages of the seminiferous epithelium, 11 phases of diploid germ cells, and 19 steps of transformation of haploid germ cells.

Developmental sequence of the spermatogenesis at any portions of the seminiferous tubules occurs continuously but at a certain moment the germ cell types observed *in situ* are distinctive in morphology and limited in number. Each facet of the epithelium displays several time-sliced windows of the continuous spermatogenetic sequence. The assembly patterns of male germ cell types characterize the stages of the epithelium (stage I to XIV), which are determined primarily by cellular and subcellular transformations of spermatids. Since the spermiogenesis of spermatids is comprised of 19 steps according to the acrosomal development, the remaining steps are overlaid in the cycle of stages. Thus, the two different, successive generations of spermatids are compiled in the same facets of the epithelium, in particular in stage I to VIII. The same applies to spermatocytes, different phases of which are stratified deep in the epithelium. The correlation between the chronological sequence of the spermatogenesis and the pictorial presentation of male germ cells can provide a clue for understanding the stratified cellular components in the epithelium, and will help identify and describe the male germ cell types properly for diverse arrays of analyses in advanced studies.

Key Words: seminiferous tubule, spermatogenesis, spermiogenesis, male germ cell, Sertoli cell

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Introduction

During several decades, the testis, or histogenesis and male fertility, of mammals has been garnering attention of basic and clinical scientists. Many reviews and monographs have been published on these topics (for a monograph[1]). A large number of ongoing researches continue to come out in current journals. The researches are highly diversified; reproductive biology and medicine, toxicology, oncology, pharmaceutical science (fertility and contraceptive), mining race of new molecules, and so on.

Although much information of the male reproductive system has been gathered, the spermatogenesis is still a complex process. For this process are requisite the increment of cells, perfect accuracy of genetic recombination, morphological differentiation and fertility. The process can produce male gametes, or spermatozoa, having a streamlined head and sleek whip-powered tail and a half amount of chromosomes with huge genetic diversities. Moreover, male gametes should be perfect physiologically and genetically; healthy and fertile. This complex spermatogenesis is, however, impaired easily by a variety of chemical, physical, genotoxic and endogenous genetic factors which contain potential to lead to male infertility (morphological and functional abnormalities). Compared with the resting cells, in particular, developing and differentiating cells are susceptible and vulnerable to chemical as well as physical alterations in their micro-environments.

Our contemporary societies have introduced a large number of chemical compounds into our life. Cells in the body are unconsciously irrigated with such compounds regardless of nutrients or toxicants. The testis containing vigorously differentiating cells attracts people's attention because of serious family problem infertility and furthermore extinction of species. That implies some chemicals we use make us an endangered species in the surely coming future. The testis is, therefore, used to evaluate a variety of factors, either chemical or physical, and to assess the spoiled environments, either large or small. In spite of the essential requisite for toxicological analyses, the histology and histogenesis of the seminiferous epithelium of the testis is still complex to understand, in particular for fledgling scientists or beginners.

The present study aims at providing a simplified scheme of spermatogenesis in the rat testis at the light microscope level, and helps understand the morphologies of the male germ cells in the seminiferous epithelium. The pictorial presentation with corresponding descriptions appears to be helpful for further advanced studies.

Materials and Methods

The testes from three young adult Sprague-Dawley rats (postnatal 10-11 weeks) were used in the present study. The rats were maintained in an artificial condition of 12-hour day/night cycle at 25 degrees Celsius and acclimatized for at least 1 week before sacrifice. The rats were anesthetized deeply with pentobarbital sodium (Nembtal, 1 mL/kg body weight, i.p., Dinabott Inc.), and perfused transcardiacly with 0.9% saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2). The testes were dissected out and postfixed in the same fresh fixative overnight in refrigerator, and then processed routinely for paraffin-embedded histological materials. Cross and longitudinal sections of the testes were cut at 5 μm on a microtome. One group of sections was processed according to a PAS (periodic acid Shiff)-reaction protocol for staining glycoprotein. The other group was stained immunohistochemically using a mouse monoclonal antibody against

the proliferating cell nuclear antigen (PCNA: NeoMarkers, CA, USA) according to the manufacture's instruction.

The animals used in the present study were treated in accordance with the guideline of the Animal Study Committee of the Kagawa Prefectural College of Health Sciences.

Results

This *Results* is descriptive to explain the chronological morphological changes of the spermatogenetic development of the male germ cells in the seminiferous epithelium, rather than typical *Results* presenting experimental data. This seems to be convenient for having readers convince the entire story of spermatogenesis in a straightforward way.

Histology of the Testis

The parenchyma of the rat testis, as in other mammals, consists of two major divisions, the interstitium and the seminiferous tubules. In the parenchyma are packed the seminiferous tubules, which are convoluted and looped in a complex fashion (Fig. 1A). Both far ends of the long looped seminiferous tubule are connected to the excurrent duct system (the rete testis) (Fig. 1A). The tubular wall or the seminiferous epithelium is comprised of two categories of cells; supporting cells or Sertoli cells and stratified male germ cells at different developmental phases of the spermatogenesis (Fig. 1B and 2A-F).

In the narrow interstitial space, there are seen different types of cells in the loose connective tissue; lymphatic and blood capillary endothelia, arterioles, venules, myoid cells, and glandular cells known as the Leydig cells. The Leydig cells are the major source of androgen (e.g., testosterone) and a source for a variety of other steroids (Fig. 2B). The myoid cells are localized on the

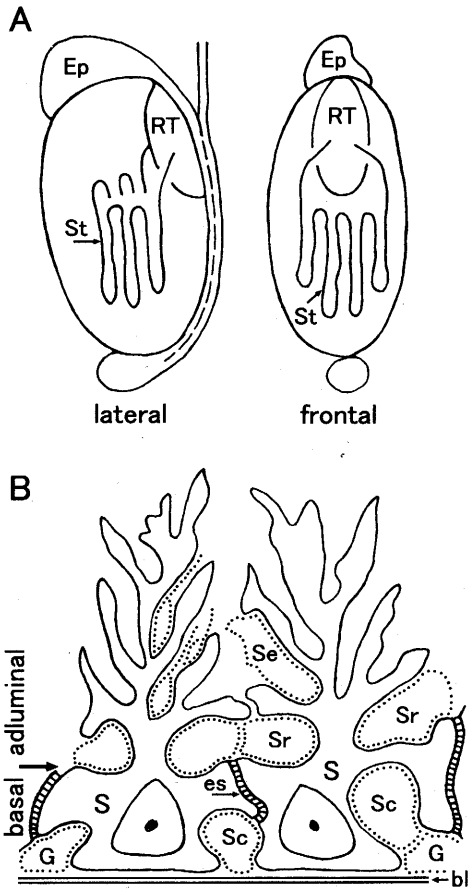


Fig. 1 A: Schematic drawing of the rat testis in lateral and frontal views to show a single representative seminiferous tubule. Ep: epididymis, RT: rete testis, St: seminiferous tubule. B: Schematic drawing of two adjacent Sertoli cells in the seminiferous epithelium. The baso-lateral cytoplasmic processes of the Sertoli cells make contact with each other via tight junctions, and establish the basal and adluminal compartments and simultaneously the blood-testis barrier. Dotted lines imply male germ cells in accordance with developmental phases of the spermatogenesis. bl: basal lamina, es: ectoplasmic specialization, G: spermatogonium, S: Sertoli cell, Sc: spermatocyte, Se: elongate spermatid, Sr: round spermatid

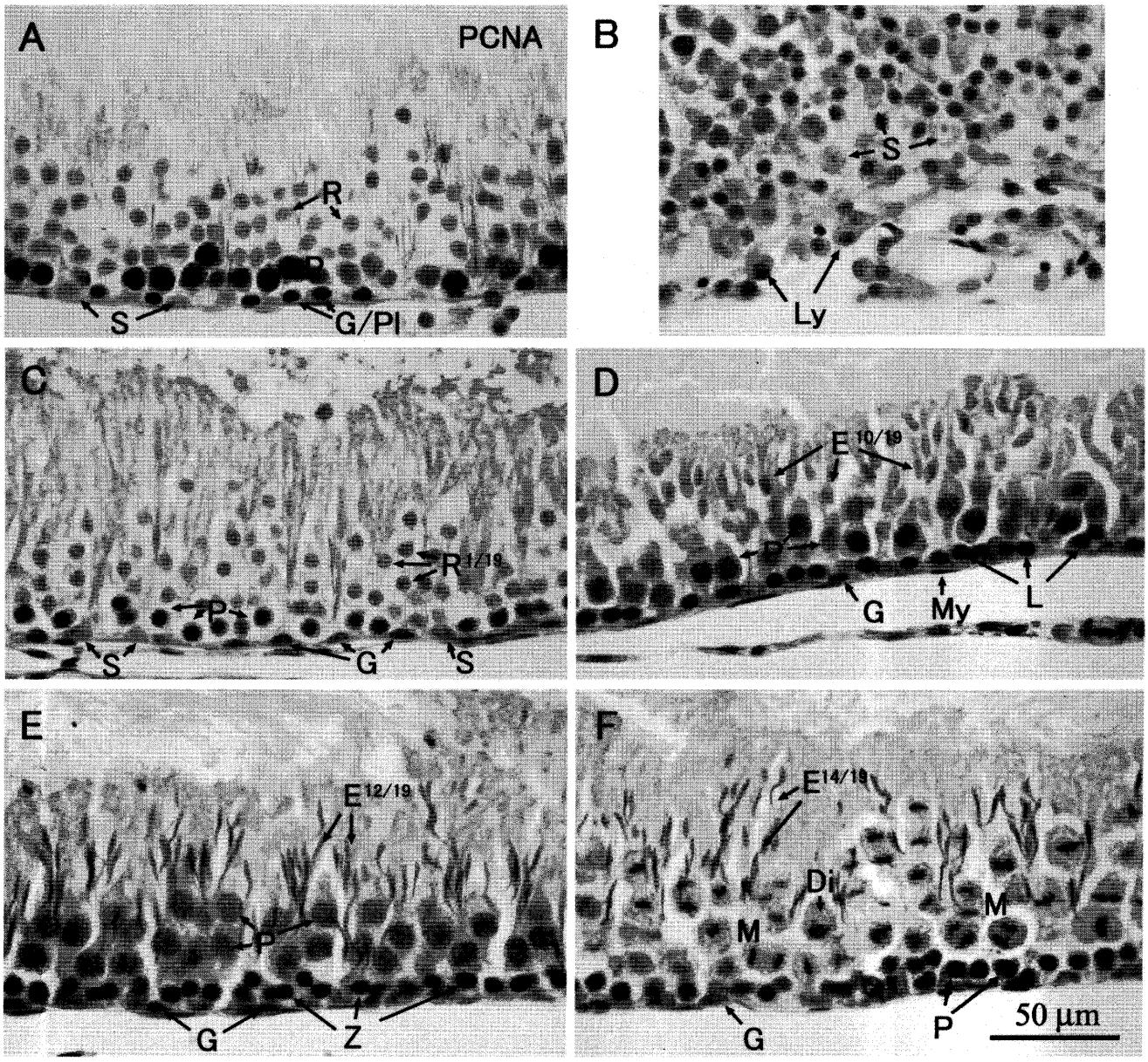


Fig.2 A: Seminiferous epithelium immunostained with anti-PCNA antibody. Positive signals (solid black) were observed on nuclei of either young preleptotene (labeled G/Pl) or pachytene (P) spermatocytes. Nuclei of Sertoli cells (S) are not stained, implying not-proliferating cells. PCNA: proliferating cell nuclear antigen. B: Leydig cells (Ly) in the interstitium, adjacent to the seminiferous tubule sectioned tangentially. C-F: Representative stages of the seminiferous epithelium containing distinctive assemblies of male germ cells. The germ cells in the selected stages display different morphologies in size, shape, position, stainability, and appearance. Di: diplotene spermatocyte, E: elongate spermatid, G: spermatogonia, L: leptotene spermatocyte, M: meiosis, My: myoid cell, P: pachytene spermatocyte, R: round spermatid, Z: zygotene spermatocyte

external surfaces of the seminiferous tubules, and thought to provide the propulsive force to sperm in the tubular lumens (Fig. 2D). The others are mostly the same to solitary, migratory, or constituting cells seen ubiquitously in many different tissues.

Sertoli Cells

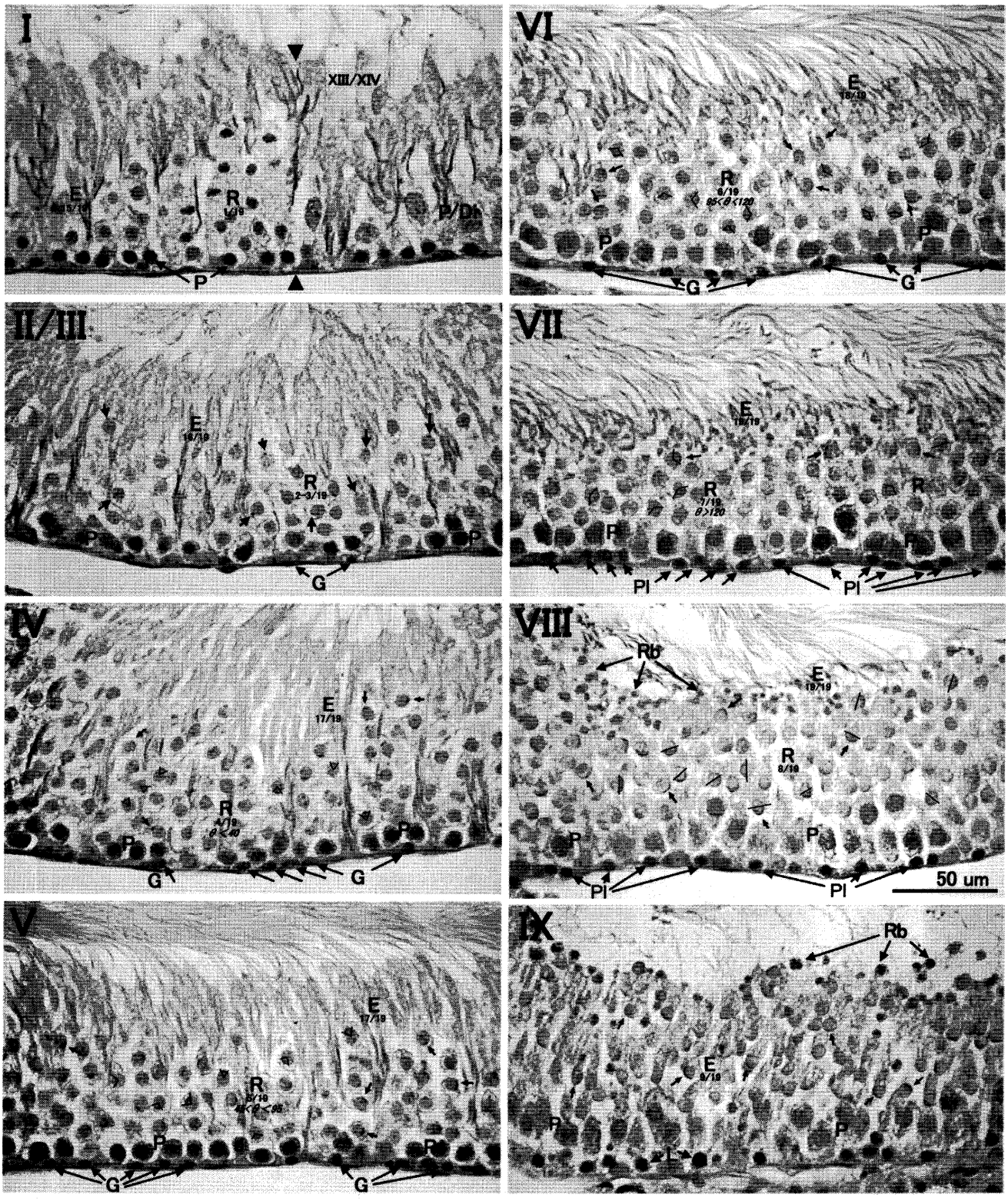
Sertoli cells are somatic cells residing among germ cells of the seminiferous epithelium and maintain the epithelial structures and the spermatogenetic development. They repeat mitoses until puberty to yield a large number of their progenies enough for their functions in the adult epithelium. During pubertal development, Sertoli cells cease to divide and extend from the basal lamina into the lumen of the seminiferous tubules. The Sertoli cells in adult rat testes were, thus, not immunostained with anti-PCNA (proliferating cell nuclear antigen) antibody among immunoreactive germ cells (Fig. 2A). Perikarya of the Sertoli cells are located along the basal lamina of the epithelium and contain a pale, triangular nucleus. Differentiated Sertoli cells are elaborately equipped to maintain the spermatogenesis structurally and chemically, so that they can serve as structural underpinning and humoral regulators in the epithelium. Although cytoplasmic arborizations of the Sertoli cells are equivocal by light microscopy, their baso-lateral surfaces around the cells make contact firmly with each other via tight junctions according to accumulated knowledge (Fig. 1B). This ectoplasmic specialization between the adjacent Sertoli cells constitutes the blood-testis barrier, or the Sertoli cell barrier, which also divides the seminiferous epithelium into two major compartments; basal and adluminal compartments, basally and apically, respectively. Owing to this barrier, newly born male germ cells, which are more or less genetically recombined, in the adluminal compartment are isolated from autoimmune onslaughts. Another intermediate compartment temporally appears as early phases of spermatocytes migrate upward through the cytoplasm of Sertoli cells. During spermatogenesis, Sertoli cells keep physically interacting with all different phases of male germ cells until sperm release (or spermiation) and also chemically controlling their development and differentiation by tuning adluminal humoral environment (Fig. 2A-C).

Germ Cells

Male germ cells in the seminiferous epithelium display distinctive morphologies according to their developmental phases of spermatogenesis; spermatogonia (proliferative cells), spermatocytes (meiotic cells), and spermatids (transforming cells) (Fig. 2A-F). These germ cells proliferate to increase in number and/or transform in either quality or morphology in every facet of the epithelium. Corresponding to three categories of the germ cells, the spermatogenesis is divided into three phases: 1) the proliferative phase in which spermatogonia undergo rapid, successive mitotic divisions and contain immunoreactive PCNA (Fig. 2A), 2) the meiotic phases (Meiosis I and II) in which genetic sequences of spermatocytes are recombined and then divided to yield a huge number of genetic diversities (Fig. 2D-F), and 3) the spermiogenesis in which round type of spermatids differentiate into sleek streamlined spermatozoa or haploid male gametes (Fig 2 C-E).

Proliferation of Spermatogonia

Spermatogonia are primitive and small, and are located basally facing the basal lamina of the seminiferous epithelium, or in the basal compartment (Fig. 2A, C-E). It is theoretically assumed that a single primitive spermatogonium repeats mitotic divisions to produce thousands of its descendants. For


I
XIII/XIV
E
R
P/Dh
P
G
VI
E
16/19
R
6/19
85<θ≤120
P
G
II/III
E
16/19
R
2~3/19
P
G
VII
E
16/19
R
7/19
θ>120
Pl
IV
E
17/19
R
4/19
θ≤40
P
G
VIII
Rb
E
18/19
R
8/19
P
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IX
Rb
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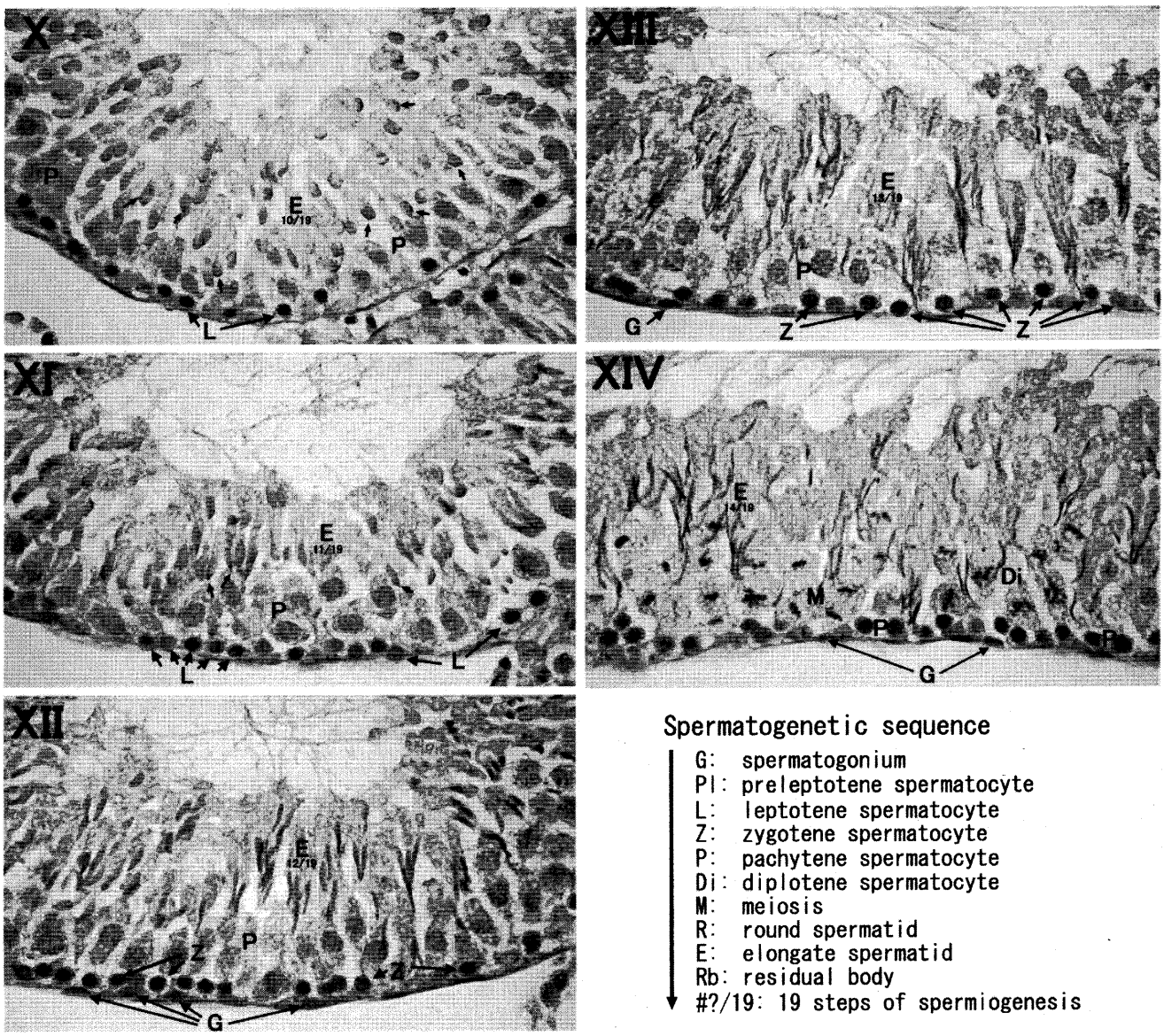

Fig. 3 I-XIV: Stages of the seminiferous epithelium of the rat testis. Fourteen stages of the epithelium are primarily distinguished on the basis of the acrosomal development in spermatids and concurrently characterized by their distinctive assemblies of the other differentiating male germ cells (described in detail in text). In the panel of stage I, the two stages (stage I vs XIII/IX) are discernible with distinct demarcation. In stage I to XI, the developing acrosomes are indicated by the corresponding angles and/or small arrows. Tissue sections were processed according to a PAS-reaction protocol to show acrosomes in spermatids and counterstained with hematoxylin.

Table 1. Stages (I-XIV) of the Seminiferous Epithelium of the Rat Testis

Stage	*L*	*cell*	*Histology of Spermatogenesis and Spermiogenesis by Light Microscopy*
I	Ad	Se	Step 15 E-spermatids intervene in flocks between step 1 R-spermatids.
		Sr	No acrosome is seen in cytoplasm of step 1 R-spermatids.
		Sc	Pachytene spermatocytes are aligned in the bottom of the adluminal compartment.
	B	G	A few spermatogonia are seen along the basal lamina of the seminiferous epithelium.
II/III	Ad	Se	Step 16 E-spermatids intervene in flocks between step 2-3 R-spermatids.
		Sr	Acrosomal spots become recognized in cytoplasm of step 2-3 R-spermatids.
		Sc	Pachytene spermatocytes and their nuclei grow in size.
	B	G	A few spermatogonia are seen along the basal lamina of the seminiferous epithelium.
IV	Ad	Se	Step 17 E-spermatids intervene in flocks between step 4 R-spermatids.
		Sr	The angle subtended by acrosome is less than 40° in step 4 R-spermatids.
		Sc	Pachytene spermatocytes and their nuclei continue to grow in size.
	B	G	Spermatogonia increase in number and display heterochromatin in their nuclei.
V	Ad	Se	Step 17 E-spermatids intervene in flocks between step 5 R-spermatids.
		Sr	The angle subtended by acrosome extends from 40° to 95° in step 5 R-spermatids.
		Sc	Pachytene spermatocytes and their nuclei continue to grow in size.
	B	G	Increased spermatogonia display distinct heterochromatin in their nuclei.
VI	Ad	Se	Step 18 E-spermatids move to the luminal surface of the seminiferous epithelium.
		Sr	The angle subtended by acrosome extends from 95° to 120° in step 6 R-spermatids.
		Sc	Pachytene spermatocytes and their nuclei continue to grow in size.
	B	G	Increased spermatogonia display heterochromatin in their nuclei.
VII	Ad	Se	Step 19 E-spermatids move to the luminal surface and concomitantly stripping down of cytoplasm occurs and residual bodies appear. Sperm are released into the tubular lumen.
		Sr	The angle subtended by acrosome becomes greater than 120° in step 7 R-spermatids.
		Sc	Pachytene spermatocytes and their nuclei continue to grow in size.
	B		Preleptotene spermatocytes become recognized along the basal lamina of the epithelium.
		G	Spermatogonia become indistinguishable between their descendant preleptotene cells.
VIII	Ad	Se	Sperm release or spermiation continue to disengage step 19 E-spermatids (sperm or spermatozoa). Residual bodies increase in amount.
		Sr	Nuclei of step 8 R-spermatids are slightly deformed in shape (oval) and concomitantly their nuclei make contact with a portion of the cell membrane.
		Sc	Pachytene spermatocytes and their nuclei continue to grow in size.
	B		Preleptotene spermatocytes contain large and round nuclei, distinct from spermatogonia.
		G	Spermatogonia are barely seen.
IX	Ad	Se	Sperm release or spermiation has been completed. Step 9 E-spermatids (next generation of germ cells) occupy the luminal half of the seminiferous epithelium.
		Sr	R-spermatids have been transformed into step 9 E-spermatids.
		Sc	Pachytene spermatocytes and their nuclei continue to grow in size.
	B		Leptotene spermatocytes grow in size and commence to migrate toward the lumen.
		G	Spermatogonia are barely seen.

Table 1. Stages (I-XIV) of the Seminiferous Epithelium of the Rat Testis (continued)

Stage	*L*	*cell*	*Histology of Spermatogenesis and Spermiogenesis by Light Microscopy*
X	Ad	Se	Step 10 E-spermatids contain a elongated nucleus and short, thick cytoplasmic tail.
		Sr	none
		Sc	Pachytene spermatocytes and their nuclei continue to grow in size.
			Leptotene spermatocytes grow in size and loose contacts with basal lamina of the epithelium.
	B	G	Spermatogonia are barely seen.
XI	Ad	Se	Step 11 E-spermatids become anchored deep in crypts of the Sertoli cells.
		Sr	none
		Sc	Pachytene spermatocytes and their nuclei continue to grow in size.
			Leptotene spermatocytes grow in size and migrate toward the lumen.
	B	G	Spermatogonia are barely seen.
XII	Ad	Se	Nuclei of step 12 E-spermatids are carved into streamlined heads of mature spermatozoa.
		Sr	none
		Sc	Pachytene spermatocytes and their nuclei grow in size.
			Zygotene spermatocytes succeed to lineage of leptotene spermatocytes and grow in size.
	B	G	A few spermatogonia become recognized again along the basal lamina of the epithelium.
XIII	Ad	Se	Step 13 E-spermatids contain a sleek , streamlined nucleus and intervene in flocks between fully grown pachytene spermatocytes.
		Sr	none
		Sc	Pachytene spermatocytes reach the fully grown situation just before diplotene phase.
			Zygotene spermatocytes continue to grow in size.
	B	G	A few spermatogonia are recognized along the basal lamina of the epithelium.
XIV	Ad	Se	Step 14 E-spermatids intervene in flocks between spermatocytes undergoing Meiosis I.
		Sr	none
		Sc	Diplotene spermatocytes undergo two successive meiotic divisions.
			Pachytene phase of spermatocytes start following zygotene phase.
	B	G	A few spermatogonia are recognized along the basal lamina of the epithelium.

Since all stages of the seminiferous epithelium is concentrically layered, compartments (*L*) and germ cells (*Cell*) are arranged in a upright position from top (luminal surface) to bottom (basal lamina of the epithelium).

Abbreviations: I-XIV, fourteen stages in the seminiferous epithelium; L, layered compartment; Ad, adluminal compartment; B, basal compartment; Se, elongate spermatid; Sr, round spermatid; Sc, spermatocyte; G, spermatogonium; E-spermatid, elongate spermatid; R-spermatid, round spermatid

yielding a large number of cells, it is natural that spermatogonia should undergo rapid successive mitoses at a higher rate, but there are barely encountered typical mitotic divisions within the basal compartment in light microscopic preparations. By scrutinizing using high-power magnification, spermatogonia might be divided into three types (successively, stem-cells, proliferative cells and differentiating cells) with further subdivisions on the basis of the amount of nuclear chromatin, as in a monograph[1] (e.g., stem-cell spermatogonia: type A isolated [A_{is}]; proliferative spermatogonia: type A paired and aligned [A_{pr} and A_{al}]; differentiating spermatogonia: type $A_{1, 2, 3, 4}$, Intermediate type [In], and type B [B]). Slight differences between the spermatogonia can be discerned on the basis of the nuclear appearance and the cell size. However, labeling spermatogonium types strictly is a risky undertaking for lack of clear-cut, discriminating criteria by light microscopy. Apart from the subtypes, it is noted that the population of spermatogonia appears to fluctuate in number in the basal compartment where they reside (Fig. 3; compare stage IV-VI with the others).

Meiosis of Spermatocytes

Following the final mitosis of spermatogonia (type B), small young primary spermatocytes termed preleptotene are born (Fig. 2A). Thereafter, all postmitotic spermatocytes undergo Meiosis I and II of the spermatogenesis. During the prophase (5 subphases: preleptotene, leptotene, zygotene, pachytene and diplotene phases) of Meiosis I, genetic codes on the paternal and maternal chromosomes are paired strictly and recombined in millions of ways by unknown mechanisms. In the next Meiosis II, genetic materials are reduced to half in haploid daughter cells (ploidy: n). Through the two successive meiotic divisions, individual spermatocytes are quadrupled in number and result in the production of haploid male gametes, or spermatozoa, with genetic diversities (Fig. 2C-F).

At first, preleptotene (Pl) spermatocytes, which are the last cells to pass through the DNA-synthetic phase of the cell cycle in the entire course of the spermatogenesis, duplicate genetic materials (ploidy: $4n$) (Fig. 3 VII, VIII). Thereafter, preleptotene cells commence Meiosis I. These preleptotene cells are located in the basal compartment, or the outside of the blood-testis barrier.

The next phase of the first meiotic division is the leptotene (L) phase which actually implies the initiation of Meiosis I. Leptotene spermatocytes start to move away gradually from the basal lamina and then are engulfed temporally into the cytoplasm (intermediate compartment) of Sertoli cells as the spermatocytes migrate upward (Fig. 2D). The prophase of Meiosis I lasts for about three weeks, so that different phases of the spermatocytes (5 subphases) occur in every stage (Stage I to XIV; described below) of the seminiferous epithelium. During the long-lasting prophase of Meiosis I, various cellular and subcellular transformations proceed in both nucleus and cytoplasm. Spermatocytes transit from one phase to another without clearly discernible landmarks (Fig. 3I-XIV). As a whole, the spermatocytes and their nuclei increase gradually in size as the prophase advances (Figs. 2D and 3IX-XI). Altered appearances of spermatocyte nuclei are helpful to subdivide the evolving meiotic prophase.

In zygotene (Z) phase, the spermatocytes transit from the basal to adluminal compartments (Figs. 2E and 3XII, XIII). Zygotene spermatocytes contain a larger round nucleus than the predecessor leptotene cells (Fig. 2E). In this phase, the homologous chromosomes become paired for recombining genetic sequences derived from the paternal and maternal chromatids.

Pachytene (P) spermatocytes and their nuclei continue to increase more and more in size in the bottom of the adluminal compartment for about two weeks (Fig. 2C-E and 3I-XIV). The pachytene phase, therefore, consumes two-thirds of the prophase of Meiosis I. Throughout this phase, the fully paired chromosomes are maintained and the most outstanding genetic recombination known as crossing over occurs within the nucleus. The strictly accurate recombination of genetic sequences allows subsequent germ cell progenies to possess a unique combination of genes.

Diplotene (D) spermatocytes are the largest among primary spermatocytes in the seminiferous epithelium (Fig. 3I, XIV). The diplotene phase of Meiosis I is so brief that typical diplotene cells are barely recognized in histological preparations (Fig. 3I, XIV). Their nuclei grow in size and are pale in appearance. In diplotene cells, chromosomal pairs are separated except at chiasmata. The diplotene phase is the last pass of the long-lasting prophase and the remainders of Meiosis I (metaphase, anaphase and telophase) are completed rapidly.

The cells formed after Meiosis I are secondary spermatocytes (2°) with short longevity. They undergo the second meiotic division (Meiosis II) rapidly to produce round spermatids (Figs. 2C and 3I). Each meiotic phase of Meiosis II is brief in duration. Dividing Meiosis II cells can be distinguished from larger Meiosis I cells. Meiosis II gives rise to haploid spermatids with recombined genetic diversities.

Spermiogenesis of Spermatids

Newly generated spermatids after completion of Meiosis II undergo intriguing processes of morphological alterations to produce mature spermatozoa. This process occurs without cell division. The major cytological events are acrosome cap formation, nuclear shaping and condensation, development of a flagellum, and elimination of cytoplasm. This series of processing is called spermiogenesis and consumes over three weeks for spermatids to differentiate to sleek, whip-powered spermatozoa (Fig. 3). The spermiogenesis is divided into 19 steps according to acrosomal development in round and elongate spermatids, as in the previous study[1].

Acrosome: Although newly formed spermatids lack acrosome (Table 1; Fig. 3I; step 1), the cells soon start to form small proacrosomal vesicles containing proacrosomal granules. Small proacrosomal granules coalesce to form a single acrosomal vesicle observable under the light microscope (Table 1; Fig. 3II/III; step 2-3). The acrosomal vesicle adheres to the spermatid nucleus and becomes flattened at the acrosomal pole of the nucleus (Table 1; Fig. 3IV; step 4), and then extends over a half of the nucleus (Table 1; Fig. 3V-VIII; step 5-8). Translocation of the spermatid nucleus to the cell surface comes to the next and the acrosomal cap as a result is positioned sandwiched between the cell and nuclear membranes (Table 1; Fig. 3VIII-X; step 8-10). Concurrently, nuclei and cytoplasm of spermatids start to elongate along the axis of the cell (Table 1; Fig. 3XI, XII; step 11, 12). Shapes of spermatid nucleus heads and overlying acrosome transform gradually during the late two-thirds (i.e., approx. 2 weeks) of spermiogenesis before sperm release or spermiation (Table 1; Fig. 3X-XIV to I-VIII; step 10-19).

Nucleus: On the basis of the nuclear shape, spermatids are divided into two; round (R) spermatids containing a spherical nucleus in early phases of the spermiogenesis (Figs. 2C and 3I-VII) and elongate (E) spermatids with an elongated or streamlined nucleus in later phases (Figs. 2D, E and 3X-XIV to I-IV). Round and elongated nuclei of spermatids are able to be distinguished easily but these seemingly

different cells are the same ones at discrete steps in single arrays of individual haploid germ cells. As elongate spermatids mature, their heads are carved elaborately into streamlined, or a sickle-shaped form in the rat (Figs. 2E, F and 3I-IV).

Flagellum: Sheaves of spermatid flagella become conspicuous in the luminal parts of the seminiferous epithelium at later phases of the spermiogenesis (step 17 or later; Fig. 3IV-VII). The formation of flagellum is, however, a continuous process which starts in young round spermatids and lasts until sperm release occurs (step 1 to 19). Detailed subcellular changes of the flagellum development in early phases are hardly discernible because of limited resolution of the light microscope. The flagellum is positioned at the flagellar pole of the spermatid nucleus, or at a position opposite to the acrosomal pole.

Cytoplasmic elimination: Stripping down of cytoplasm appears to be laborious for spermatids and loses about 75% of the original volume. The spermatid cytoplasm is pinched off and fragmented into a bunch of small cytoplasmic packages (Fig. 3VI). The debris named residual body left behind in the lumen of the seminiferous tubules is phagocytized by Sertoli cells (Fig. 3VIII, IX). Spermatozoa are equipped with a streamlined sickle-shaped head and a sleek whip-tail before sperm release or spermiation (Fig. 3VI, VII).

Stages of Spermatogenesis

Male germ cells are concentrically stratified in the seminiferous epithelium. Sertoli cells are arranged perpendicularly to the basal lamina of the seminiferous tubules and extend their cytoplasm from the basal lamina to the luminal surface. Male germ cells evolve from the bottom to the apex along the longitudinal axis of the Sertoli cell. Every facet of the seminiferous epithelium in microscopic preparations displays quite distinctive assemblies of different phases of male germ cells (Figs. 2 and 3). Representative facets of the epithelium are partitioned by morphological criteria of differentiating acrosome in spermatids and divided into 14 stages (Fig. 3; stage I to XIV). Since the spermiogenesis consists of 19 steps, the remaining steps are overlaid in turn in the cycle of the stages. In several stages, two different and successive generations of spermatids occurs on the same facets of the epithelium, sharing the common space either in tandem or in parallel (Fig. 3I-VIII). The same cellular arrangements as the spermatids are encountered in the long-lasting differentiation process of spermatocytes; different phases of spermatocytes are stratified in rows in the lower half of the epithelium (Fig. 3VII-XIV; combinations of [Pl & P], [L & P], [Z & P], and [P & Di]). These different phases of spermatocytes in discrete rows imply two different but successive generations of germ cells supplied continually from spermatogonia committed to spermatogenetic development. Therefore, in addition to chronological sequence of spermatogenesis facets of the epithelium should be considered properly as specific stages containing standard combination of different phases of male germ cells.

Discussion

Spermatogenesis is a dynamic proliferative and differentiating process yielding male gametes in the seminiferous epithelium of the testis. The process is one of the most outstanding histogenesis in the body. There are recognized three kinds of serious cytological events. First, spermatogonia make daughter cells to produce thousands of diploid progenies by repeating mitoses (proliferation) and at the same time reserve another daughter stem cells not committed to spermatogenesis for

future resources of spermatozoa (reservation of stem cell). Secondly, the two successive meiotic divisions recombine genetic sequences of the paternal and maternal genes to yield male gametes with a huge number of genetic diversities (genetic recombination) and increase postmitotic germ cells 4-folds in number. Thirdly, the morphological differentiation of haploid spermatids accounts for the competent spermatozoa having its partner ovum fertilize (spermiogenesis and fertility).

Spermatogenesis is a long-lasting, complex process, even though a simplified scheme can be depicted as in the present study or in more detail by the others[1]. In addition to histological complexity described in Results, the natures of male germ cells vary in resistance, susceptibility and vulnerability. Different phases of germ cells are differently exposed to their epithelial environments (chemical, molecular and physical factors). It is known, in general, that active cells in the cell cycle are subject to influences from their environments. The seminiferous epithelium is packed with actively proliferating and differentiating male germ cells. A possible assault to the epithelium may, thus, leave critical damages to specific phases of germ cells, or spermatogenesis and fertility. For example, exogenous substances such as toxins, food additives, and pharmaceuticals may adversely affect given phases of male germ cells as humoral constituents in the epithelium. Some medications used for treatments of certain disease can modulate the spermatogenesis via the pharmacological effectiveness in the target tissue or system. It is well known that the same applies to endocrine disruptors. A growing number of compounds are suspected to mimic the actions of native substances either to make male reproductive organs malfunction or to destroy them.

Sertoli cells may control and modulate germ cell growth through physical contacts and irrigated chemicals; e.g., synchronization of differentiating germ cells. Therefore, malfunctions of endogenous molecules (structures, enzymes and substances) cause severe damages to the spermatogenesis, such as arrested differentiation, teratogenesis, and reduction in number or motility. Accordingly, vulnerability and susceptibility of male germ cells to environmental constituents attract a great deal of attention from various research fields (toxicology, pharmacology, nutrition, reproductive biology, and others).

Sertoli cells

Sertoli cells play important rolls as structural underpinning and humoral controller of developing male germ cells. The whole cell images of Sertoli cells can be reconstructed on the basis of ultrastructural evidence by electron microscopy[2-5]. One of the major functions of Sertoli cells is to maintain the structural integrity of the seminiferous epithelium. The structural framework provides male germ cells with scaffolding for spermatogenesis. The close apposition between the germ cells and Sertoli cells may make it effective to influence physically and chemically each other.

The blood-testis barrier may be the most important structure by Sertoli cells. The barrier prevents free delivery of blood-derived extracellular substances into the adluminal compartment of the epithelium, and vice versa (back-flow)[5]. Furthermore, isolation of spermatocytes and spermatids, which are more or less genetically altered from their progenitor cells, in the adluminal compartment prevents them from autoimmune onslaughts[6]. The blood-testis barrier functions as an immunologic barrier.

Spermatogonia

Stem cell spermatogonia perform two important tasks. Following the initial

mitosis, one population is committed to spermatogenesis to produce thousands of somewhat differentiated spermatogonia (proliferative and differentiated). This contributes to continuous supply of spermatogonia for production of mature spermatozoa. The other is reserved as primitive spermatogonia (stem cells) for future resources of spermatozoa. Proliferating and differentiating spermatogonia are subject to influences of environments, while the resting stem cell spermatogonia are resistant to environmental stresses.

According to ultrastructural evidence[1], spermatogonia committed to spermatogenesis are classified into several populations. Spermatogonia which are considered as stem cells are type $A_{isolated}$ (A_{is}) spermatogonia, and the others are proliferative (A_{paired} [A_{pr}] and Aaligned [A_{al}]) and differentiated (A_1, A_2, A_3, A_4, Intermediate [In], and type B [B]) spermatogonia. The paired (A_{pr}) and aligned (A_{al}) spermatogonia are designated owing to their intercellular bridges, which connect the same type of spermatogonia via cytoplasmic continuity. Spermatogonia interconnected are probably the progenies derived from a single stem cell. Differentiating spermatogonia (A_1, A_2, A_3, and A_4) are discerned by the amount of chromatin along the inner aspect of the nuclear envelope. A transitional form (In) and type B spermatogonia may be distinguishable based on the amount of nuclear chromatin, moderate and large, respectively. Mature type B spermatogonia divide to form the young primary spermatocytes or preleptotene cells (Pl). To know resistance and vulnerability of these spermatogonia may be important to evaluate potentiality and fertility in reproductive medicine.

Spermatocytes

The most important task of spermatocytes is recombination of genetic sequences derived from paternal and maternal genes. For achieving the task, spermatocytes consume a long period, evolve morphologically[1,7] and shift their residence to the outside of the immune system of the body[8]. Spermatocytes also use the special pairing apparatus, or synaptonemal complex, to perform accurate recombination of genetic sequences[9]. Furthermore, through two successive meiotic divisions genetic materials of spermatocytes are reduced to half in the spermatids. It is probable that all behaviors of these germ cells are necessary to produce the new haploid male gametes genetically and immunologically independent from a host life.

Spermatids

Spermiogenesis occurs without cell division and is one of the drastic cell transformations in the body. Although cytological events in spermiogenesis are acrosome cap formation[10,11], nuclear shaping [12,13] and condensation[14,15], development of a flagellum[1], and elimination of cytoplasm[15], the purpose of this process is to yield sleek streamlined spermatozoa. Cytoplasmic elimination and a whip-powered flagellum are also important for spermatozoa to swim faster in the female reproductive tract.

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