

Quantitative and Qualitative Characteristics of the Stages and Transitions in the Cycle of the Rat Seminiferous Epithelium: Light Microscopic Observations of Perfusion-Fixed and Plastic-Embedded Testes'

REX A. HESS2

Department of Veterinary Biosciences, University of Illinois, Urbana, Illinois 61801

ABSTRACT

The stages of the cycle in the rat seminiferous epithelium are illustrated for testes fixed by vascular perfusion and embedded in plastic resins. Improved cellular resolution in plastic sections permitted a clearer demarcation of the stages than in paraffin. Quantitative data are presented to support the recognition of stages, particularly those in transition. Stages IV, V, VII, XI, and XII had the highest frequencies of transitional characteristics. Stage IV was redefined to be more consistent with the occurrence of a high percentage of mitotic figures and to clarify transitions in this stage. Although the resolution of cellular detail was greatly improved with the use of plastics, the thinner sections contained fewer identifying features together within a single tubule cross section and sometimes major characteristics were absent. Therefore, additional characteristics were used for stage classification, such as nuclear diameter and the presence or absence of mitotic figures. A binary decision key is provided to improve consistency among laboratories in the identification of the stages in plastic-embedded testes.

INTRODUCTION

spermatogenesis in the rat is a process of gradual transformation of germ cells into spermatozoa over a period of 48-53 days [1]. As the germ cells advance within the seminiferous epithelium, older cells become associated with younger ones in a specific 12 to 13-day cycle that begins with mitotic division of spermatogonia and proceeds through meiosis and finally ends with the release of sperm. This cycle of the rat seminiferous epithelium was divided into 14 stages by Leblond and Clermont [2]. As illustrated in Figure 1a, each stage represents a different combination of germ cell status, including spermatogonia, spermatocytes, and spermatids. In the testis, the stages are repeated in consecutive order along the length of the seminiferous tubules [3]. Because the tubules are folded repetitively, a cross section of the testis will contain several hundred tubule sections, each of which can be identified as one of the 14 stages. Primary recognition of the stages was based upon morphologic changes in the spermatid [2,4]. This excellent classification of stages has served as the foundation for numerous studies of biochemistry and physiology of spermatogenesis [5] and has provided the standard to which testicular histopathology is compared.

Nevertheless, recognition of the stages in the cycle can be difficult for some individuals, depending upon their training and optical acuity, as well as the type of histologic fixatives and stains that are used [6]. Because of these difficulties, additional clarification of the stages was intro-

duced to facilitate their routine use in paraffin sections [4,7]. In particular, precise changes in the acrosomic system and nucleus of the spermatid were shown to be the most accurate characteristics by which stages could be divided [4,7]. However, cross sections of seminiferous tubules may contain a mixture of features from two adjacent **stages, indicative** of the continuous process of spermatogenesis [3]. Thus, consistent and precise recognition of the stages requires a knowledge not only of the general characteristics [2], but also perception of the subtle changes of the epithelium through transition.

Although these early descriptions of the stages have been adequate for most purposes, they were based upon observations of tissues fixed and processed by use of classical histologic methods. More advanced techniques of histology and ultrastructure have been developed to improve the preservation of cells and to increase the resolution of cellular detail [8, 9]. Improvements in resolution through the use of electron microscopy have contributed much to our understanding of the evolution of spermatids in the seminiferous cycle [10, 11, 12,13,14]. With these improvements it has become clear that there are differences between the appearances of stages in thick paraffin and ultrathin plastic sections. However, attempts to clarify the light microscopic characteristics in plastic remain limited and **insufficiently illustrated** [10, 15, 16].

Recognition of germ cells in plastic sections can be more difficult than in paraffin [17], probably due to the lack of clear illustrations of the stages and a more precise definition of the transitions. This difficulty led to the combination of stages in one study because of the "lack of reliable criteria to differentiate stages I-IV in the present material . . ." [18]. Therefore, the present study offers a comprehensive evaluation of the stages in the cycle as observed in testes fixed by vascular perfusion and embedded in plastic resins.

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2Correspondence: Department of Veterinary Biosciences, University of Illinois, 2001 S Lincoln, Urbana, IL 61801.

Special attention is given to the recognition of transitions between stages (Figs. 1-5, pp. 534-539) and a binary decision key is provided in the Appendix (pp. 540) to improve consistency among laboratories in stage identification.

MATERIALS AND METHODS

Glycol

Methacrylate

After administration of anesthesia (sodium pentobarbital) to 10 Sprague-Dawley rats, the testes were preserved by vascular perfusion through the descending aorta at 100 cm Hg pressure. The blood was rinsed from the vasculature with Ringer's bicarbonate solution containing 0.5% procaine HCl and 10 IU heparin/ml (2 min), and tissues were fixed with 3% glutaraldehyde in sodium cacodylate buffer (0.1 M, pH 7-3). The fixed testes were removed, cut, and stored as thin transverse samples in the fixative at 4°C.

Fixed cross sections of testis were dehydrated in a graded series of ethanol and embedded in a modified glycol methacrylate (JB-4; Polysciences, Inc., Warrington, PA) that was polymerized at 0-4°C. Thin sections, 1.5-2.0 µm, were stained with a modified periodic acid-Schiff's reaction (PAS) and counterstained with Alum hematoxylin [19]. For comparison, testes from other Sprague-Dawley rats were fixed by immersion in Bouin's fluid and embedded in paraffin.

Epoxy

Testes of 10 Sprague-Dawley rats were preserved by vascular perfusion, similar to the method of Forssmann et al. [20]. After ether anesthesia, the blood was rinsed from the vasculature with Ringer's bicarbonate solution containing 0.5% procaine HCl and 10 IU heparin/ml (2 min). Then the tissues were fixed first with phosphate-buffered (0.1 M, pH 7.2) 1.5% glutaraldehyde/ 1.5% paraformaldehyde solution containing 2.5% polyvinylpyrrolidone (40 000 MW) for 3 min at room temperature and under 100 mm Hg pressure. A second fixative of 3% glutaraldehyde/3% paraformaldehyde/0.05% picric acid in the phosphate buffer with 2.5% polyvinylpyrrolidone was then perfused for 15 min at 100 cm Hg. The fixed testes were removed, cut, and stored as thin transverse samples in the second fixative.

Fixed tissues were cut into thin (1 mm) but broad faced pieces (4 x 4 mm) to include several cross sections of seminiferous tubules. The samples were rinsed in phosphate buffer overnight at 4°C, post-fixed in 1.5% osmium tetroxide (phosphate-buffered) for 5 h at 4°C, rinsed 3 times in distilled water, washed in 10% ethanol for 30 min, and stained en bloc with 4% uranyl acetate in 10% ethanol for 1 h at 4°C. The samples were dehydrated in a graded series of ethanol and embedded in Polybed 812 epoxy (Polysciences, Inc.). Thin sections, 0.5-1.0 µm were stained at 70°C with 1% toluidine blue in 1% sodium borate.

Quantification

Observations for counts (frequency of stages in transition and frequency of mitosis) were made with high resolution light microscopy using either a 40X (0.95 N.A.) or 100X (1.4 N.A.) planapochromatic objective. To determine the mean distance of spermatid heads from the basement membrane, measurements were made of all heads with a visible acrosomal tip, which was assumed to be a random event. To determine the linear extension of the acrosome over the round spermatid nucleus, the nuclear diameter (D) and the portion of the diameter not covered by the acrosome (D - h) were measured. The height of the acrosomic system (h) was equal to D - (D - h). The ratio of h/D was proportional to the acrosomic surface area (Sa/

nuclear surface area (S_n); where $S_a = Dh$ and $S_n = \pi D^2 / 4$. A highly selected population of cell nuclei were used to obtain more approximate cross sections of the acrosomic system. Only the largest nuclei (>9 µm diameter) were used in sections that included the acrosomic granule. To determine the range in diameter of round spermatid nuclei, 100 nuclei (with the acrosomic granule and head cap visible) were measured in epoxy and methacrylate sections (10 nuclei/rat).

Measurements were made using a Zeiss photomicroscope with a 100 x objective and a light tube through which an LED cursor was viewed. The movable cursor dot (approximately 0.1 µm in diameter) was viewed with the specimen for outlining regions for measurements. Jandel Scientific Sigma Scan@ Image Analysis software (Jandel Scientific, Corte Madera, CA) and a Numonics; Graphics Digitizer (Numonics Corporation, Montgomeryville, PA) were used for processing the measurements. The repeatability of measurements varied depending upon the length. The coefficient of variation (CV) for 10 measurements at 10 µm was 0.74%, and a CV of 5.8% was obtained for measurements at 0.7 µm. The minimum resolvable distance was 0.073 µm. Differences between means were determined using the t-test with significance set at p < 0.01.

Photomicrography

Representative areas of the stages were photographed using a 100X planapochromatic objective (1.4 N.A.). Color prints were made from Kodak Type 40A positive slides and black and white prints were made from Kodak Technical Pan Film 2415 (Eastman Kodak Co., Rochester, NY).

RESULTS

General Comments

Recognition of stage characteristics in the cycle of the seminiferous epithelium was superior in plastic compared to paraffin sections. With improved resolution, tubules exhibiting transitional features were clearly defined. How

ever, as with any histologic method, certain artifacts were noted; in particular, distortion and swelling of the round spermatid nuclei was more common in the methacrylate sections than in epoxy. Important stage characteristics and differences between the two types of plastics are discussed below. A binary decision key for the recognition of stages is presented in the appendix.

Two types of transition were found: 1) stages with spermatids mixed from adjacent stages, and 2) transitions between spermatid steps. Stages IV, V, VII, XI, and XII were found frequently (>20%) in either an early or late transition (Table 2). A stage exhibiting transition was not difficult to classify, necessarily. If the transition was due to the mixing of spermatids from adjacent stages, the stage represented by the majority of spermatids was selected. If the transition was between spermatid steps, then other criteria were used in making a precise identification of the stage.

Stage I. Recognition of Stages I-IV was dependent upon a clear view of the Golgi apparatus in early round spermatids. These stages were among the most difficult to classify because, frequently, the Golgi zone was out of the plane of section due to thinness of the plastic. However, when the Golgi apparatus was in view, the presence or absence of granules was clearly delineated. Early Stage I was recognized by the presence of round spermatids with very small heterochromatic nuclei (Fig. 1a) and the absence of a prominent Golgi apparatus. Early step 15 spermatids were recognized by a pronounced curvature of the nuclear apex and a bulge of the caudal region of the acrosome (caudal fin) along the dorsal edge of the nucleus.

in mid stage, the step 1 spermatid nucleus enlarged and a distinct Golgi apparatus stained PAS+ (Fig. 1b). Also, the nucleus of step 15 spermatids was less curled. Due to the thinness of sections, the caudal fin was rarely seen in epoxy (Fig. 2).

The Golgi apparatus of late step 1 spermatids contained very small PAS' granules that were readily apparent only with high resolution using an objective with a numerical aperture of 1.4. A lower power objective could not resolve these granules until Stage 11 (Figs. 1b and 2).

TABLE 1. Mean relative distances from the head of elongated spermatids to the basement membrane in Stages I-V.'

Stage	Spermatid step	Distance (µm)'	Minimum distance (µm)c
1	15	45.4 3. 1 d	9.2 ±
	1.99		
11	16	39.0 2.6 de	6.9 ±
	2.31		
111	16	36.2 2.11	7.7 ± 0.79
	17	26.7 0.8f	3.8 0.7h
IV	17	26.4 2.7f	3.0 0.5h
	17		

'Measurements from the tip of the acrosomic system to the beginning of the PAS' basement membrane in glycol methacrylate. 'Mean ± SEM. N = 3 animals; 1 tubule per stage per animal; between 33 and 95 measurements per tubule. 'Mean ± SEM. N = 3 per stage.

''Significant differences are indicated by different superscripts within a column (p < 0.01).

TABLE 2. Frequency of stages in transition within the cycle of the rat seminiferous epithelium.'

Stage	Percent transitional (%)		Total
	Early	Late	
1	0	8	8
11	16	10	26
111	16	10	26
IV	22	26	48
V	22	2	24
VI	18	14	32
VII	26	18	44
VIII	2	4	6
IX	8	10	18
X	6	12	18
X1	10	20	30
XII	18	40	58
XIII	16	10	26
XIV	0	0	0 ^{ob}

aN 50 cross-sectional tubules per stage

in glycol methacrylate.

'Type 11 spermatocytes were present with-

out meiotic figures in 8%.

Stage II Classification of this stage depended upon the recognition of 1-6 small proacrosomic granules, 0.3-0.6 µm in diameter, that usually were surrounded by vesicles (Figs. 1b and 2). The vesicles were resolved better in mid to late step 2 spermatids. Thus, for consistency, Stage 11 was classified only when the proacrosomic granules were visible at 400X magnification or when the granules were surrounded by vesicles at 600-1000X magnification. Also, the caudal fin of the acrosome, visible in step 15 spermatids, was flattened in step 16 of Stage 11.

Stage III. Transitions between Stages II and III, and III and IV were common (Table 2). A mixture of the corresponding spermatid steps 2 and 3, or 3 and 4, was often found in this stage. The average presence of step 2 spermatids was 12% and the maximum was 28%. The average for step 4 spermatids was 9.5%. Steps 2 and 4 were never observed together. To discriminate between Stages 11, 111, and IV, at least 10 spermatids per tubule cross section were examined to determine the predominate cell type used to classify the tubule.

In mid Stage 111, acrosomic granules in step 3 spermatids had diameters between 0.6 and 0.8 µm and were larger than proacrosomic granules in step 2 (Figs. 1b and 2). In epoxy, a clear zone surrounded these granules in the Golgi region, making their appearance more distinct. In methacrylate sections, acrosomic vesicles were less distinct and stained slightly PAS+, but were surrounded by a more intensely PAS+ Golgi apparatus.

The mean distance between the heads of elongated spermatids and the basement membrane showed a gradual decrease from Stage I to V, with maximum penetration during Stage V (Table 1). However, because there were large variations in the penetration of spermatid heads into the epi

TABLE 3. Surface area of the acrosomic system and nucleus in round spermatids of the rat seminiferous epithelium.^a

Stage	Spermatid step	Diameter ^b nucleus (μm)	Height acrosomic cap (μm) ^c	Ratio of surface area ^d acrosome/nucleus
Late V	5	9.5 ± 0.1 ^d	1.8 ± 0.07	0.184 ± 0.0071
Early VI	6	9.5 ± 0.1 ^d	2.2 ± 0.06	0.236 ± 0.006 ^e
Late VI	6	9.4 ± 0.1 ^d	2.7 ± 0.07	0.280 ± 0.0071
Early VII	7	9.2 ± 0.1 ^d	2.7 ± 0.05	0.294 ± 0.0061
Late VII	7	9.5 ± 0.1 ^d	3.2 ± 0.08	0.335 ± 0.007 ^e
VIII	8	9.4 ± 0.1 ^d	3.3 ± 0.07	0.349 ± 0.006 ^e

^aEstimated from the diameter of the nucleus and the height of the acrosomic head cap in glycol methacrylate sections. Surface area of the nucleus = D²; surface area of the acrosomic system = Dh.

^bVariation reflected in the SEMs represents variability among a population of cells selected with nuclei cross sections greater than 9 μm in diameter, which might differ substantially from the true variability in dimensions of whole nuclei.

^cMean ± SEM. N = 25 for each spermatid step.

^dSignificant differences are indicated by different superscripts within a column (p < 0.01). Differences between height of caps was not determined.

thelium, this feature was useful only for distinguishing between Stages III and IV.

Stage IV. Stage IV was recognized primarily by the presence of one or more configurations of the acrosomic system in step 4 spermatids. The acrosomic granule had a diameter between 0.8 and 1.1 μm. The literature does not provide clear criteria for distinguishing spermatids in transition between Stages III and IV. In the present study, step 4 was identified when the acrosomic vesicle formed a distinctly flat edge at the nuclear membrane (approximately 0.3-μm-thick) and the acrosomic granule contained within the vesicle rested on this straight margin. The acrosomic granule frequently was found to one side of the flattened vesicle (Fig. 1b). If mitotic figures were present in a tubule having both late Stage III and early Stage IV characteristics, the stage was classified as IV. Early Stage IV had a high per

centage of mitotic figures, whereas Stage III typically did not exhibit mitosis (Table 4).

The length of the forming acrosomic system was clearly enlarged between early and late step 4 spermatids (Table 6). In the early period, the acrosomic vesicle did not stain intensely PAS⁺, although there was a slight reaction at the flat margin. However, in late step 4 spermatids, the thin straight margin was strongly PAS⁺ as the acrosomic vesicle flattened onto the nuclear membrane. In epoxy, this region appeared as a thin dark line that stained intensely basophilic (Fig. 2). An additional characteristic for the identification of Stage IV was recognition of step 17 spermatids by the extension of the acrosomal head cap beyond the tip of the nucleus. This feature, however, was rarely observed in epoxy sections due to the thinness of sections.

In late Stage IV, the haloed appearance of the acrosomic vesicle in methacrylate sections disappeared as the acrosomic system spread to form the future head cap of spermatids. In epoxy, the vesicle remained as a clear, half-moon region covering the acrosomic granule and sloping toward the edges of the acrosomic margin (Fig. 2). In late step 4 spermatids, there were small clear spaces on the lateral sides of the acrosomic granule in both types of plastics. Whether

TABLE 4. Frequency of mitosis in the stages of the cycle of the seminiferous epithelium^a

Stage	Tubules counted	Tubules with mitotic figures	Percent (%)
1	50	7	14
11	50	4	8
111	50	0	0
Early IV	14	3	21
Mid IV	21	14	67
Late IV	15	8	53
V	50	0	0
Early VI	15	9	60
Mid-Late VI	35	8	23
VII	50	0	0
VIII	50	0	0
IX	50	9	18
X	50	0	0
	50	1	2
XL	50	8	16
XIII	50	1	2
XIV	50	13	26

^aNuclei of spermatogonia exhibiting either metaphase or anaphase mitosis.

TABLE 5. Diameter of the nucleus at the acrosomal-nuclear junction in step 10-step 13 spermatids in the rat.^a

Stage	Spermatid step	Diameter (μm) ^b
X	10	3.79 ± 0.071
X1	11	2.56 ± 0.04 ^c
XII	12	1.75 ± 0.041
XIII	13	1.22 ± 0.02 ^c

^aSagittal sections of the acrosomal-nuclear junction in glycol methacrylate (see Fig. 1). ^bMean ± SEM. N = 50 for each step. ^cSignificant differences are indicated by different superscripts (p < 0.001).

this clear space was part of the acrosomic vesicle could not be resolved.

Stage V Although the linear extension of the acrosomic system was significantly different between late step 4 and early step 5 spermatids (Table 6), this slight morphometric difference was not visually apparent. Therefore, only when the acrosomic system of the round spermatids became slightly curved and extended on both sides of the acrosomic granule (one side may be longer in early step 5) was the tubule classified as Stage V. With this classification, 53% of the tubules in late Stage IV were found to contain mitotic figures (Table 4), which produced the type B spermatogonia predominant in Stage V. If the tubule contained equal numbers of step 4 and step 5 spermatids, then other features were considered, e.g. nuclei of the elongated spermatids continued to move deeper into the epithelium in Stage V and mitotic figures were present in Stage IV but not in Stage V.

Lateral curvature of the acrosomic projections was delineated on both sides of the acrosomic granule in step 5 spermatids. However, complete cross-sectional views of the acrosomic system were often difficult to find. Complete views were less frequent in the 1- μ m-thick epoxy sections (in only 12.7% of the spermatids) than in the 2- μ m methacrylate sections (in 34.3% of the spermatids). In epoxy sections, the acrosomic vesicle appeared as an unstained space over the acrosomic granule and thin lateral extensions over the nucleus (Fig. 2, IV). In methacrylate, the acrosomic system stained intensely PAS+. In the late step 5 spermatid, the clear space disappeared (Fig. 3, V) and lateral extensions of the acrosomic system increased in length and thickness to form a headcap that extended linearly over nearly 18% of the nucleus (Table 3). Stage V continued until the nuclei of step 17 spermatids began to move toward the lumen or until mitotic figures appeared (Figs. 1b and 3).

Linear extension of acrosomic head caps could only be relied upon for routine identification of spermatids in early step 5 and late steps 7 and 8. This difficulty was due to both nuclear distortion and to a large number of tubules in transition between stages (Table 2). Visual differences between

transitional spermatid steps were not discernable. However, morphometric differences were found between steps 5 and 6, and early and late steps 6 and 7 (Table 3).

Stage VI To distinguish between Stage V and early Stage VI (in transition 18%), features other than the acrosomic system had to be used. First, the length of the acrosomic system in very early step 6 and late step 5 spermatids did not differ (Table 6). Second, late step 5 spermatids had the same ratio of acrosomic to nuclear areas as step 6 spermatids in very early Stage VI tubules containing mitotic figures (0.194 ± 0.006 , $n = 2$). Consequently, the presence of mitotic figures was used to discriminate between stages at this transition. Displacement of step 18 spermatids away from the Sertoli cell nucleus also was used to recognize the 40% of early Stage VI tubules not containing mitotic figures (Table 4).

In mid Stage VI, the acrosomic system increased in size until it extended linearly across 25% of the step 6 spermatid nucleus (Figs. 1 and 3; Table 3). At this interval, step 18 spermatid nuclei were located either in the middle of the epithelium or along the luminal border in bundles. As step 18 spermatids moved toward the lumen in late Stage VI, distinguishing the transition between Stages VI and VII was difficult because the linear extension of the acrosomic head caps in late step 6 and early step 7 spermatids did not differ (Table 3). If the step 18 spermatid nuclei were aligned along the luminal border and bundles were not apparent, then other features were considered. For example, the basophilic granule in the distal cytoplasm of step 18 spermatids was small, compared to step 19; mitotic figures were present in Stage VI but not in VII; and type B spermatogonia were mixed with preleptotene spermatocytes in Stage VI.

Stage VII. Early Stage VII was distinguished from late Stage VI by the enlargement of the basophilic granule in the cytoplasm of the step 19 spermatid. As this stage progressed, the granule moved from its location distal to, but near, the nucleus until it rested within the ventral curvature of the nucleus in the step 19 spermatid (Figs. 1b and 3). Another important trait was the alignment of elongated spermatids along the luminal border.

The linear acrosomic extension could not be used to distinguish spermatids in early step 7 from late step 6, or late step 7 from early step 8 spermatids (Table 3). In methacrylate, the acrosomic system extended over nearly 35% of the linear diameter of the nucleus in both steps 7 and 8. In epoxy, these linear extensions were 42%. The difference between the resins may have been due to fixation or to shrinkage. The greatest difference between the two plastics was found in the diameter of the round spermatid nucleus. The mean diameter was $9.8 \pm 0.05 \mu\text{m}$ in methacrylate and $8.8 \pm 0.05 \mu\text{m}$ in epoxy.

Orientation of the acrosomic system in round spermatids toward the basement membrane was only a consistent feature for Stage VII. When an angle of 180° was ascribed

TABLE 6. Cross-sectional length of the acrosomic system in round spermatids.

Stage	Spermatid step	Acrosomic system length (μm) ^a
Early IV	4	1.56 ± 0.07^c
Late IV	4	3.01 ± 0.07^d
Early V	5	4.19 ± 0.09^e
Late V	5	6.32 ± 0.17^f
Early VI ^b	6	6.61 ± 0.09^g

^aMean \pm SEM. N = 10. Glycol methacrylate sections. ^bVery early Stage VI; heads of step 18 spermatids were penetrated deep within the epithelium, but mitotic figures were present. ^cSignificant differences are indicated by different superscripts ($p < 0.01$).

along a line between the basement membrane and the tubule lumen, 46.6% of the acrosomic head caps were positioned at less than 90° in Stage VII whereas 94.8% were seen with this position in Stage VIII.

Stage VIII. In the transition from Stage VII to Stage VIII, the basophilic granules moved to a location beneath the head of the step 19 spermatid. These granules then coalesced to form large cytoplasmic lobes characteristic of Stage VIII. Some Stage VII-VIII transitions had step 7 spermatids whose nuclei were located centrally within the cytoplasm even while the cytoplasmic lobes were seen beneath the nuclear curvature of step 19 spermatids. Only when the majority of round spermatid nuclei were displaced to one cytoplasmic pole was the tubule classified as Stage VIII. Nuclei were polarized in $90.8 \pm 17.3\%$ of the round spermatids in Stage VIII ($n = 10$ tubules per animal). Migration of the step 8 nucleus to one cytoplasmic pole (usually toward the basement membrane) was distinguished more readily in epoxy than in methacrylate (Figs. 1b and 3). The cytoplasmic membrane was more clearly outlined by the toluidine blue stain in thin epoxy sections. This movement of the nucleus corresponded with the release of spermatozoa, a characteristic of this stage.

Because there was no difference in the ratio of acrosomic and nuclear surface areas in spermatid steps 7 and 8 (Table 3), these cells could not be distinguished by use of this ratio. However, peripheral knobs formed by the acrosomic system in step 8 spermatids were a distinguishing feature, particularly in epoxy sections (Fig. 3). Also, a section of the nuclear membrane adjacent to the acrosomic knobs appeared thinner in step 8 spermatids than in step 7.

Stage DC. Step 19 spermatids were released in Stage VIII; however, a few could be found in early Stage IX. Two important features for the recognition of this stage were the asymmetry and slight compression of step 9 spermatid nuclei and the presence of residual bodies within the epithelium (Figs. 1b and 4). Also, mitotic figures were seen in Stage IX but not in adjacent stages (Table 4).

Stage X. Stage X was easily distinguished from Stage IX by an elongation of the acrosomic head cap to the caudal end of the step 10 spermatid nucleus. The caudal region of the nucleus extended as a wide band above the head cap. In sagittal section, the acrosomic system appeared as a V-shaped region covering the narrow lateral width of the spermatid head (Figs. 1b and 4).

The transition between Stage X and Stage XI contained a large number of mixed spermatids, so several spermatids were examined to make a decision. Between Stages X and XIII, the chromatin condensed and the diameter of the nucleus decreased (Table 5). Between spermatid steps 10 and 11, the nuclear diameter decreased by 32.5% at the acrosomic head cap position in sagittal section. In thin epoxy sections, tips of the forming spermatid heads often were

out of the plane of section. In such cases the diameter of the nucleus was used for identification.

Stage XI. The acrosome and apex of the nucleus of step 11 spermatids protruded as a tube at a sharp angle to the caudal portion of the nucleus. Between spermatid steps 11 and 12, the nuclear diameter decreased by 31.7% at the acrosomal-nuclear junction (Table 5). The chromatin was less condensed than in step 12 spermatids, as evidenced by the lighter-stained nucleus.

Stage XII. Stages XI and XII were difficult to distinguish because a large proportion of the tubules were in transition. Stage XII had the highest transition frequency (Table 2). In the spermatid transition between step 11 and step 12, the apex of the nucleus condensed and the acrosomic system covered the nucleus to its angle at the caudal edge. Step 12 spermatids were readily identified when their blunt acrosomic tips were present in the plane of section (Fig. 4). However, in methacrylate sections, 52% of the Stage XII cross sections (26/50) did not contain at least one acrosomic tip. Thus, characteristics including decreased nuclear diameter (Table 5), straightening and lengthening of the nucleus, and condensation of the apical chromatin, were used in staging. Mitotic figures were found in Stage XII but were seen rarely in adjacent stages (Table 4). In the late step 12 spermatid, the acrosomal head cap was less blunt and formed a slight bulge on the dorsal side of the nuclear tip.

Stage XIII. Subtle differences were found between late step 12 and early step 13 spermatids. In the early step 13 spermatid, the acrosomal head cap protruded primarily on the ventral side of the nucleus and the nuclear apex stained intensely due to chromatin condensation.

Step 13 spermatids of mid Stage XIII had a highly condensed, pointed nucleus that was beginning to curve. The acrosomal head cap was distinctly ventral and protruded beyond the tip of the nucleus (Figs. 1b and 5). When the apex of step 13 spermatid nuclei were out of the plane of section, the diameter and condensation of the spermatid nucleus (Table 5) and the presence of diakinetically spermatocytes were identifying characteristics. The nucleus of late diplotene and diakinetically spermatocytes were larger than those of other pachytene spermatocytes and contained more prominent nucleoli and chromatin clumps. Nuclei of diakinetically spermatocytes had thin, discontinuous membranes and large nucleoli with central cores.

Stage XIV Step 14 spermatids could not be used to classify Stage XIV as they were often seen in Stage XIII and Stage I. Stage XIV was only classified when meiotic figures (meiosis 1 or 2; Figs. 1b and 5) or secondary spermatocytes were present (Figs. 1b and 5). Because of this precise recognition, tubules in this stage were not listed as transitional (Table 2). Secondary spermatocytes were present without meiotic figures in 8% of the tubules classified as Stage XIV. Mitotic figures were found in 26% of Stage XIV tubules (Ta

ble 4) and usually were seen with secondary spermatocytes. In late step 14 spermatids, the nucleus was thin and curved and the acrosomic head cap extended from the nuclear apex to form a prominent ventral bulge.

DISCUSSION

This study provides descriptions and light microscopic illustrations of the 14 stages of the cycle in the rat seminiferous epithelium from testes fixed by perfusion and embedded in two types of plastic resins. Recognition of the stages in plastic sections by light microscopy is important as a prelude to electron microscopy and as a superior technique in histopathologic evaluation of the testis [8, 9, 15, 18, 21, 22]. Also, precise descriptions of the stages in plastic sections are needed if stage frequencies are to be determined as a means of assessing changes in spermatogenesis [23]. Many of the stage characteristics reported for paraffin sections [2, 4] were confirmed in plastic. However, this report is the first to clarify basic classifications of the stages by emphasizing quantitative differences in the transitions between stages.

Thin plastic sections are equated with superior delineation of stages whenever stage-specific features are visible. However, in thin plastic sections, many identifying features are missing or visible only at oblique angles in tubular cross sections. Thus, because some tubules are difficult to assign to stage by use of classical criteria, other characteristics must be used.

The early acrosomic system, which consists of a dense granule surrounded by a vesicle [24], originally was grouped as the idiosome and acrosomic granule [2,4,7]. In light microscopy, an inner and outer zone of this granule could be seen with different types of fixation [6], but the general description of the "acrosomic granule" remained [3]. Stage III was classified when the proacrosomic granules formed a single larger spherical structure [2]. A later clarification pointed out that the granule may touch or flatten on the nucleus [7]. Actually it is the acrosomic vesicle that first touches the nuclear membrane [13] and then flattens [24]. These descriptions of the forming acrosomic system have been confusing. Originally, the "granule" was described as flattened or hemispherical [2, 3, 4, 7], and forming the head cap of the acrosomic system [7, 10]. However, in plastic, the granule was spherical and electron microscopy has confirmed that the head cap is formed by the flattening and expansion of the acrosomic "vesicle" that grows by the addition of glycoproteins transferred from the Golgi apparatus [24]. Thus, light microscopic structures in paraffin and ultrastructural correlates were not equivalent. In plastic, the acrosomic granule and vesicle were resolved clearly. Therefore, classifying the beginning of Stage IV whenever the acrosomic granule touches the flattened margin of the vesicle is more precise and also corresponds with the presence of mitotic figures (Table 4).

Differences in the interpretation of the transition between Stages IV and V could influence the frequency of these stages in cross sections of the testis [23]. Perey et al. [31] reported that the granules in early step 5 spermatids were often seen only on one side of the head cap. In contrast, the present study found the granules to one side of the flattened acrosomic vesicle only in late transitional step 4 spermatids. Tubules containing these transitional spermatids should not be classified with Stage V because mitotic figures, which were found in a large percentage of these cross sections, have been shown to peak in Stage AI and not in Stage V [2, 25]. Originally, when the span of the acrosomic system was twice the diameter of the granule alone, the beginning of Stage V was recognized [7]. From observations of the characteristics seen in plastic, the acrosomic system was greater than twice the width of the acrosomic granule in late step 4 spermatids (Table 6). By requiring that the acrosomic system in plastic-embedded tissue exhibit slight curvature and extend on both sides of the granule, the beginning of Stage V was clearly resolved and corresponded to the lack of mitotic figures (Table 4).

Mitotic figures were present more frequently in Stages 1, IV, VI, IX, XII, and XIV, as previously reported in the Sherman rat [2,25]. However, there were considerable differences in their relative frequencies. Although the frequency of mitosis by stage of the cycle was not reported for the Sherman rat, the average number of spermatogonia, percent mitosis per tubule cross section, and the number of tubules examined were listed [25]. Thus, assuming one mitotic figure per cross section, an overestimate of frequency can be obtained from that data. It is surprising that in the Sprague-Dawley rat 50% of Stage IV, 34% of Stage VI, and 26% of Stage XIV tubules contained mitotic figures compared to 11.6%, 8.2%, and 10.5%, respectively, in the Sherman rat [25]. It is unlikely that this magnitude of difference could be explained by changes in stage classification, although minor differences may be found in early Stage IV. Because of the high frequency of mitotic figures in Stages IV and VI, this feature is an important discriminator in the recognition of these stages in transition.

Another major difference between the classification of stages in plastic and paraffin sections was the criteria for recognizing Stages V-VII. In paraffin, the acrosomic system covered one-fourth to one-third of the spermatid nucleus in Stage VI and one-third to one-half in Stage VII [7]. In plastic, particularly in the methacrylate, the acrosomic system could not be relied upon because there was less difference between stages. The round spermatid nucleus appeared swollen and slightly distorted in the methacrylate, which contributed to a reduction in the ratios of acrosomal-to-nuclear surface areas. In the methacrylate sections, the acrosomic system covered only one-third of the nucleus in Stages VII-VIII. Although methods used with epoxy embedment appeared to cause shrinkage, the acrosomic

rarely covered 50% of the nucleus. The type of fixative or resin did not appear to alter the structure of the other cell types.

Because the traditional classification of stages emphasized the morphology of the spermatids [2, 4], there could be considerable differences between laboratories in the identification of Stage XIV. In the transition between Stage XIII and XIV, a large number of step 14 spermatids were found associated with late diplotene and diakinetid spermatocytes of Stage 13. Therefore, it is recommended that only the dividing spermatocytes be considered in recognition of Stage XIV, as was emphasized in later clarifications of the stages [3, 7].

Studies requiring the classification of seminiferous tubules have routinely referred to the 14 stages first outlined by Leblond and Clermont [2,4]. However, recognition of the stages in paraffin or plastic should not be based solely upon the original descriptions, as substantial clarifications have demonstrated subsegments of the stages [3, 7]. Homogeneous "unit-segments" within a stage are thought to be clonal groups of cells derived from a pair of type A spermatogonia [3]. The "unit-segments" within a stage do not appear to change simultaneously and therefore most likely lead to the appearance of transitional stages. Much of the variation in reported frequencies of the stages [23] may be due to inadequate consideration of these stage transitions, as well as inconsistent recognition due to artifacts produced by the histologic methods [9]. The present study, by providing additional clarification and more accurate descriptions of the transitions between stages, will improve the consistency among laboratories in the recognition of stages in the cycle of the seminiferous epithelium.

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FIG. 1a. The 14 stages in the cycle of the rat seminiferous epithelium are illustrated (Leblond and Clermont [21 and after Dym and Clermont [26]). To demonstrate the transitional characteristics, the cell nuclei are depicted. Stages are identified by Roman numerals. Germ cell nuclei are labeled by letters or numbers. Mitosis is indicated by m and meiosis by Me, or Me2. Spermatogonia (A, 1, B); Spermatocytes (PI, preleptotene; L, leptotene; Z, zygotene; P, pachytene; D, diplotene; Di, diakinetik; SS, secondary); Round Spermatids (1-8); Elongating Spermatids (9-19).

FIG. 1b. Representative photographs of the 14 stages in the cycle of the rat seminiferous epithelium (I-XIV) as viewed in glycol methacrylate sections. S (Sertoli cell nucleus); A (type-A spermatogonia); I (intermediate spermatogonia); B (type-B spermatogonia); PI (preleptotene spermatocyte); P (pachytene spermatocyte); L (leptotene spermatocyte); Z (zygotene spermatocyte); D (diplotene spermatocyte); SS (secondary spermatocyte). Stained by the PAS reaction and hematoxylin. x550. Insets, x1 400.

Stage I. Step 1 spermatids have a light-pink-staining Golgi apparatus (G) that does not contain dense granules. A dorsal acrosomic fin (F) is visible on the step 15 spermatid.

Stage II. The Golgi apparatus of the step 2 spermatid contains two proacrosomic granules (Pg). The dorsal acrosomic fin (F) is no longer prominent as the acrosome has shifted toward the apex of the nucleus. C, chromatoid body.

Stage III PAS⁺, single acrosomic granules (Ag) are located within acrosomic vesicles of step 3 spermatids. The vesicle may be flattened somewhat on the nuclear membrane. The Golgi apparatus may be found to one side of the vesicle. C, chromatoid body.

Stage IV. In late step 4 spermatids, the acrosomic vesicle has flattened against the nuclear membrane, forming a PAS⁺ straight margin (M). The acrosomic granule (Ag) is applied against the straight margin and is sometimes found to one side. The Golgi apparatus (G) is found on one side of the forming acrosomic system. C, chromatoid body.

Stage V. The lateral extensions of the acrosomic system (Ac) form a slightly curved head cap in step 5 spermatids. The head of a step 17 spermatid (17) is buried adjacent to the Sertoli cell nucleus. C, chromatoid body.

Stage VI. The latest period of this stage is represented. The acrosomic system (Ac) covers approximately one-quarter of the step 6 nucleus. Step 18 spermatids have migrated toward the lumen and small basophilic granules (Bg) are present in their distal cytoplasm. The preleptotene spermatocyte nucleus (PI) is smaller than that of the type B spermatogonium.

Stage VII. The earliest period of this stage is represented. The acrosomic system covers nearly one-third of the step 7 spermatid nucleus (Ac). Large basophilic granules (Bg) are located near the nuclei of step 19 spermatids that line the lumen. Note the curvature of the late spermatid nucleus (19).

Stage VIII. The acrosomic system (Ac) and nucleus of a step 8 spermatid are positioned toward the basal pole of the cytoplasm. Future residual bodies (Rb) from the cytoplasmic lobe are layered between the step 19 spermatid nuclei and round spermatids. Typical for this stage, the Sertoli cell nucleus is positioned away from the basement membrane.

Stage IX. The acrosomic systems of early step 9 spermatids (Ac) are elongated. Step 19 spermatids no longer line the lumen, and residual bodies (Rb) are seen at various levels within the *epithelium*.

Stage X. The acrosomic system (Ac) of a late step 10 spermatid extends from the apex to the caudal region of the nucleus. A sagittal section of the spermatid nucleus exhibits a V-shaped structure (*) whose diameter at the acrosomal-nuclear junction has a characteristic width (see Table 3).

Stage XI. The step 11 spermatid features a prominent protrusion of the acrosomic system (Ac) and nucleus at a sharp angle to the caudal region of the nucleus. The diameter of the nucleus in sagittal section at the acrosomal-nuclear angle (*) is a distinguishing feature (see Table 3).

Stage XII. The nucleus of the late step 12 spermatid is more condensed and straight and thus displays a narrow width at the acrosomal-nuclear junction (*); see Table 3. The acrosomic system (Ac) extends slightly beyond the nuclear apex and forms a blunt tip that is rarely observed in plastic sections.

Stage XIII. The acrosomic system (Ac) of the late step 13 spermatid extends beyond the nuclear apex and forms a bulge on the ventral side of the nucleus. The diameter of the acrosomal-nuclear junction (*) is very narrow due to the further contraction of the nucleus (see Table 3). Large diplotene (D) spermatocytes with prominent nucleoli (Nu) are typical in this stage.

Stage XIVa. The first meiotic division is recognized by the presence of large meiotic figures (M) with clumps of chromatin forming scattered patterns in the center of the spermatocyte cytoplasm. The nucleus of a step 14 spermatid is highly condensed (14), and longitudinal views are rare in plastic sections.

Stage XIVb. Secondary spermatocytes (SS) are typically larger than round spermatids. Meiosis 11 is detected by the presence of smaller, centrally located meiotic figures that form dense clusters of chromatin (M).

FIG. 2. Stages I-IV in the cycle of the rat seminiferous epithelium from epoxy sections. S (Sertoli cell nucleus); A (type-A spermatogonia); B (type-B spermatogonia); I (intermediate spermatogonia); P (pachytene spermatocyte). Toluidine blue O stain. x1000.

Stage 1. The Golgi apparatus (G) is devoid of granules in step 1 spermatids. The chromatoid body (Cl) stains intensely near the nucleus and mitochondria outline the cytoplasm of each round spermatid. Most acrosomic details in step 15 spermatids were out of the plane of section.

Stage 11. Step 2 spermatids are characterized by small proacrosomic granules within acrosomic vesicles (arrows). Some nuclei of step 16 spermatids are embedded deeply in the Sertoli cell cytoplasm. Lipid-like bodies (L) and aligned mitochondria (M) are visible in the distal cytoplasm of late spermatids. An intercellular bridge is seen between two spermatids (arrowheads).

Stage III. Note the single, large acrosomic granule (Ag) surrounded by a clear acrosomic vesicle (Av) in the step 3 spermatid. The Golgi apparatus (G) forms an intensely-stained arch over the forming acrosomic system. Intercellular bridges (arrowheads).

Stage IV. A single acrosomic granule covered by a clear acrosomic vesicle touches the flatten margin (M) between the vesicle and nuclear membrane in a step 4 spermatid. The Golgi apparatus (G) forms an intensely-stained arch.

FIG. 3. Stages V-VIII in the cycle of the rat seminiferous epithelium from epoxy sections. S (Sertoli cell nucleus); A (type-A spermatogonia); B (type-B spermatogonia); PI (preleptotene spermatocyte); P (pachytene spermatocyte). Toluidine blue O stain. x1000.

Stage V. In the late step 5 spermatid, the acrosomic granule has flattened and the acrosomic system has formed thin lateral extensions (arrows) that curve over the nucleus. Nuclei of step 17 spermatids are embedded deeply within the Sertoli cell cytoplasm.

Stage VI. In this late period of Stage VI, the lateral extensions of the acrosomic system cover between one-fourth and one-third of the step 6 spermatid nucleus (arrows). Step 18 spermatids have migrated toward the lumen, but remain in sparse bundles.

Stage VII. Extensions of the acrosomic system cover between one-third and one-half of the early step 7 spermatid nucleus (arrows). The step 19 spermatids are distributed primarily along the luminal border with the basophilic granule (Bg) located near the nucleus.

Stage VIII. The nuclei of step 8 spermatids are located to the basal side of the cytoplasm, with the acrosomic system adjacent to the cell membrane (arrows). Small knobs are seen at the ends of the acrosomic extensions. Vacuolated residual bodies (Rb) are coalesced along the lumen beneath step 19 spermatid nuclei.

FIG. 4. Stages IX-XIII in the cycle of the rat seminiferous epithelium from epoxy sections. S (Sertoli cell nucleus); A (type-A spermatogonia); L (leptotene spermatocyte); Z (zygotene spermatocyte); P (pachytene spermatocyte). Toluidine blue O stain. x1000.

Stage IX. The nuclei of step 9 spermatids are slightly elongated. Residual bodies (Rb) are distributed throughout the seminiferous epithelium.

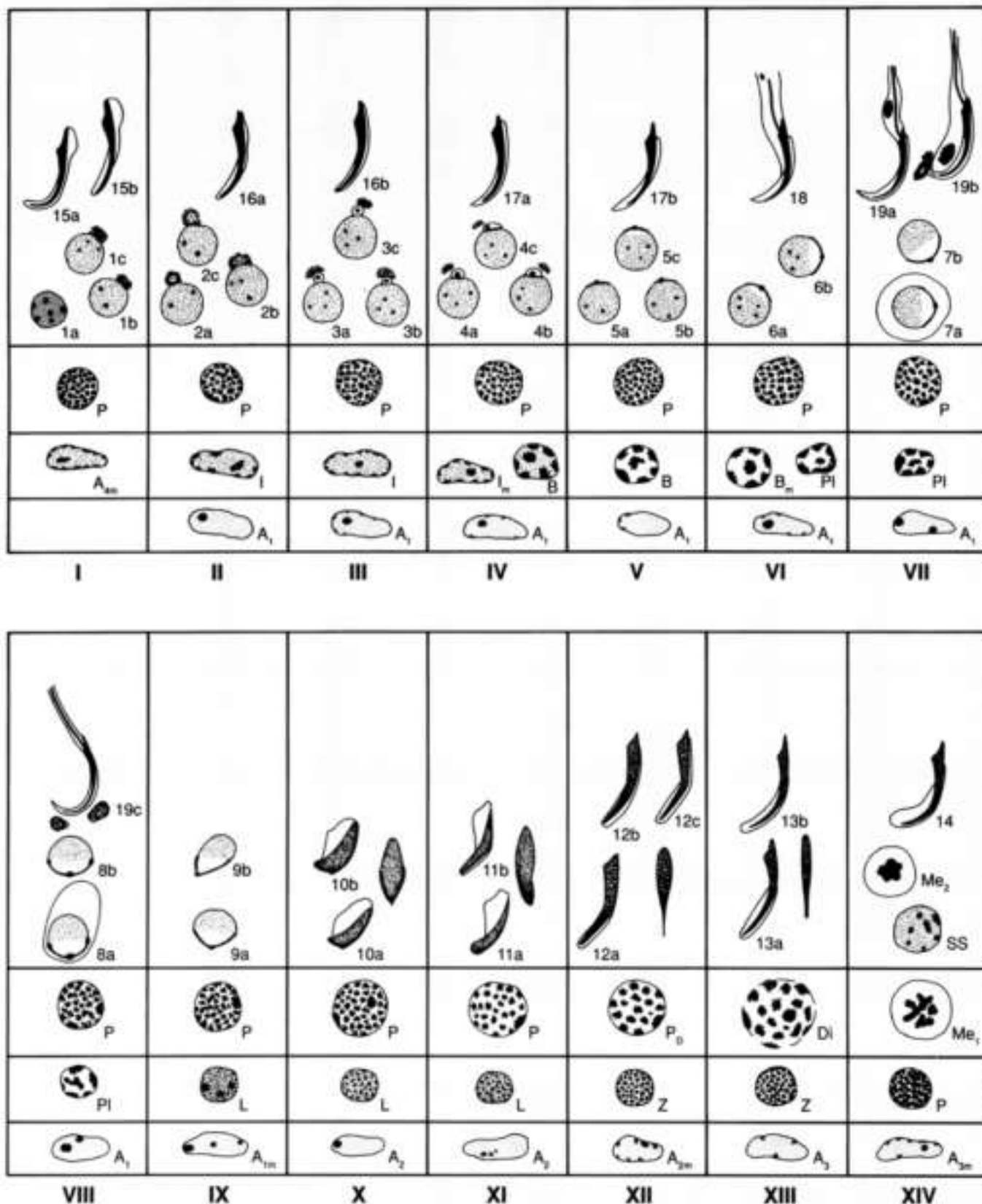
Stage X. Nuclei of step 10 spermatids are oblong and the acrosomic system forms the boundary of a ventral curvature that extends along one side of the nucleus (arrows). In sagittal sections, the apex of the acrosomic system is in a V-shape. Remnants of residual bodies (Rb) are found along the basal aspects of the tubule.

Stage XI. The nucleus and acrosomic system (Ac) of the step 11 spermatid protruded at an angle to the caudal region of the nucleus (Nu).

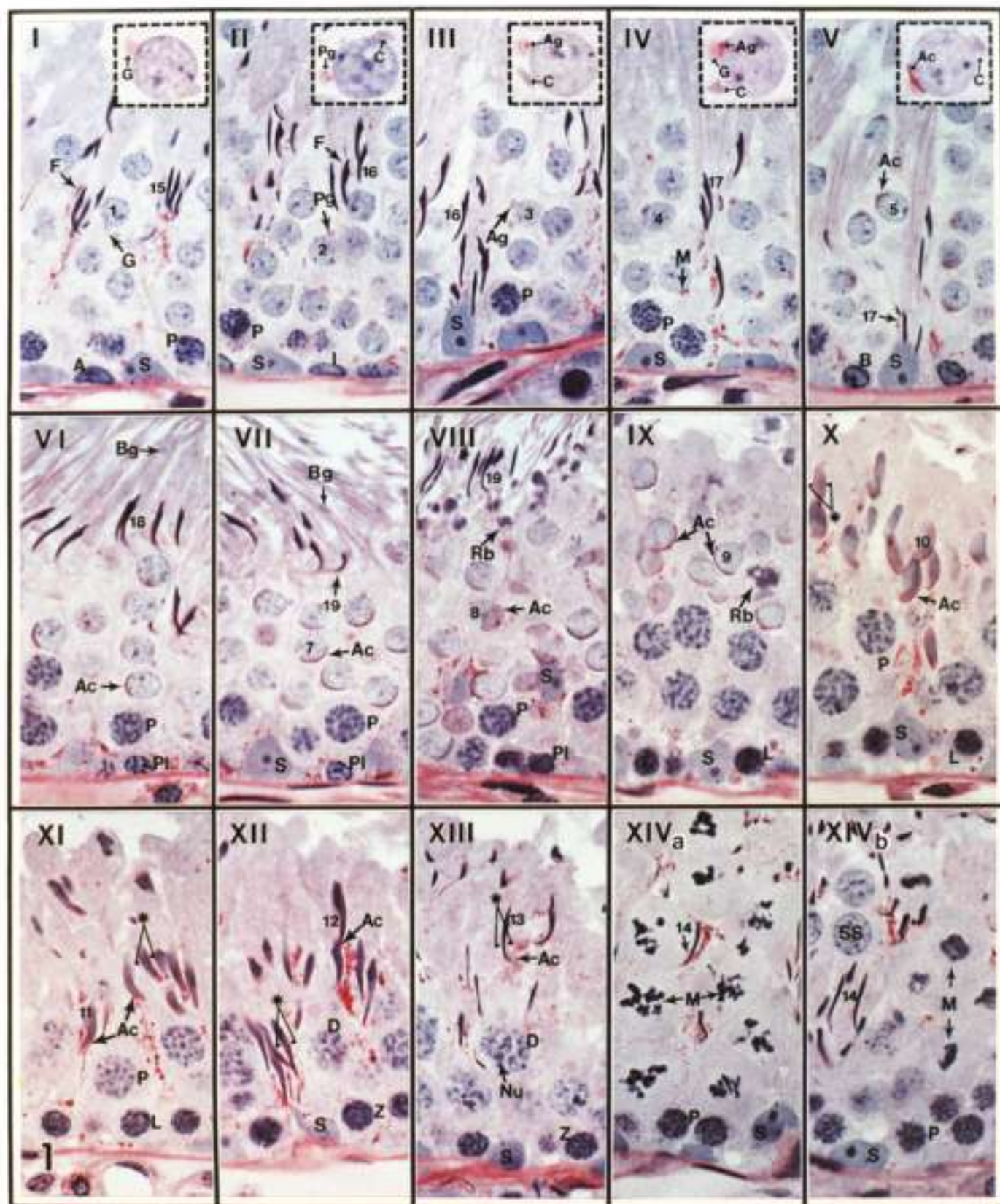
Stage XII. The long and narrow heads of step 12 spermatids are condensed and often have blunt tips (arrows).

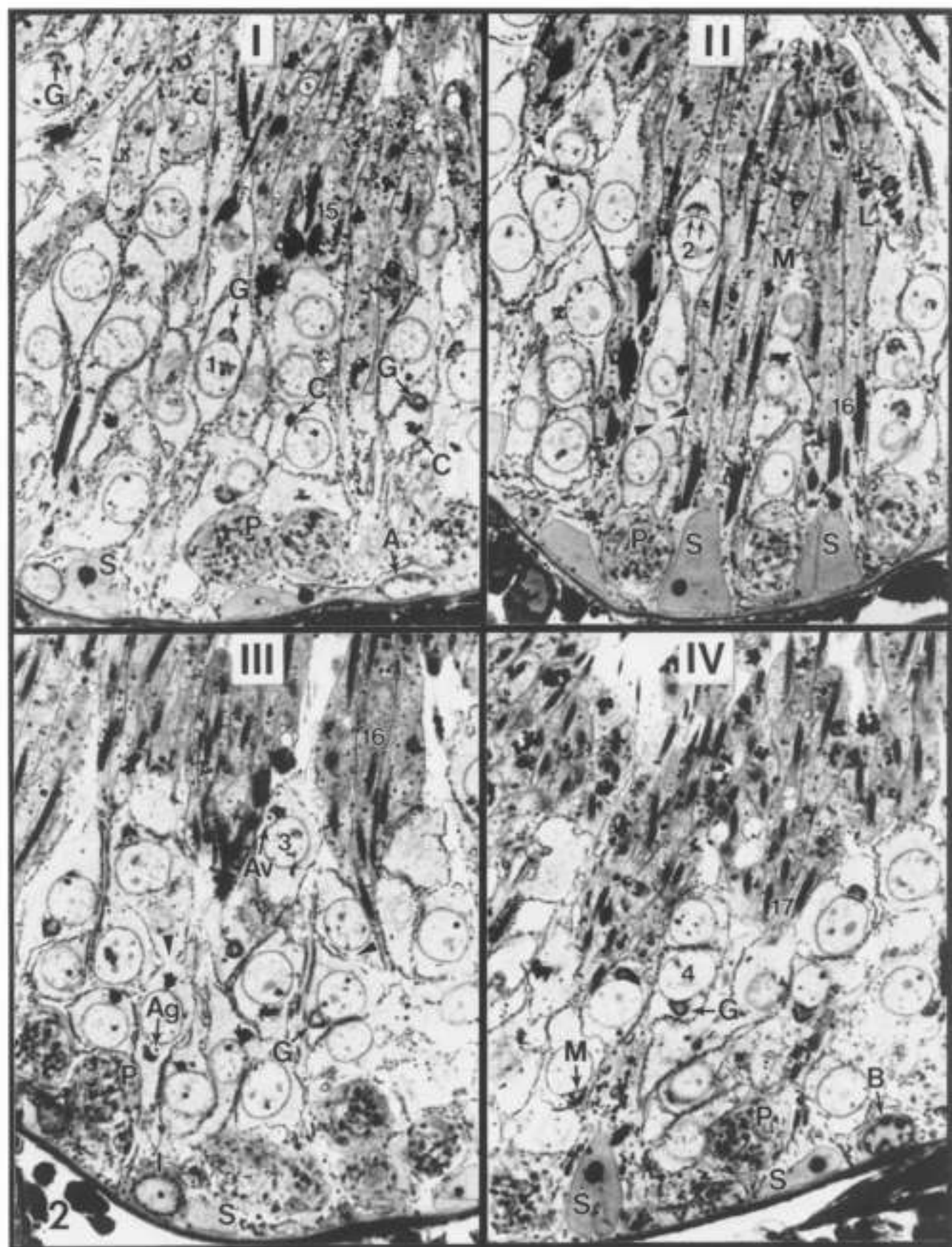
FIG. 5. Stages XIII-XIV in the cycle of the rat seminiferous epithelium from epoxy sections. S (Sertoli cell nucleus); A (type-A spermatogonia); P (pachytene spermatocyte). Toluidine blue O stain. x1000.

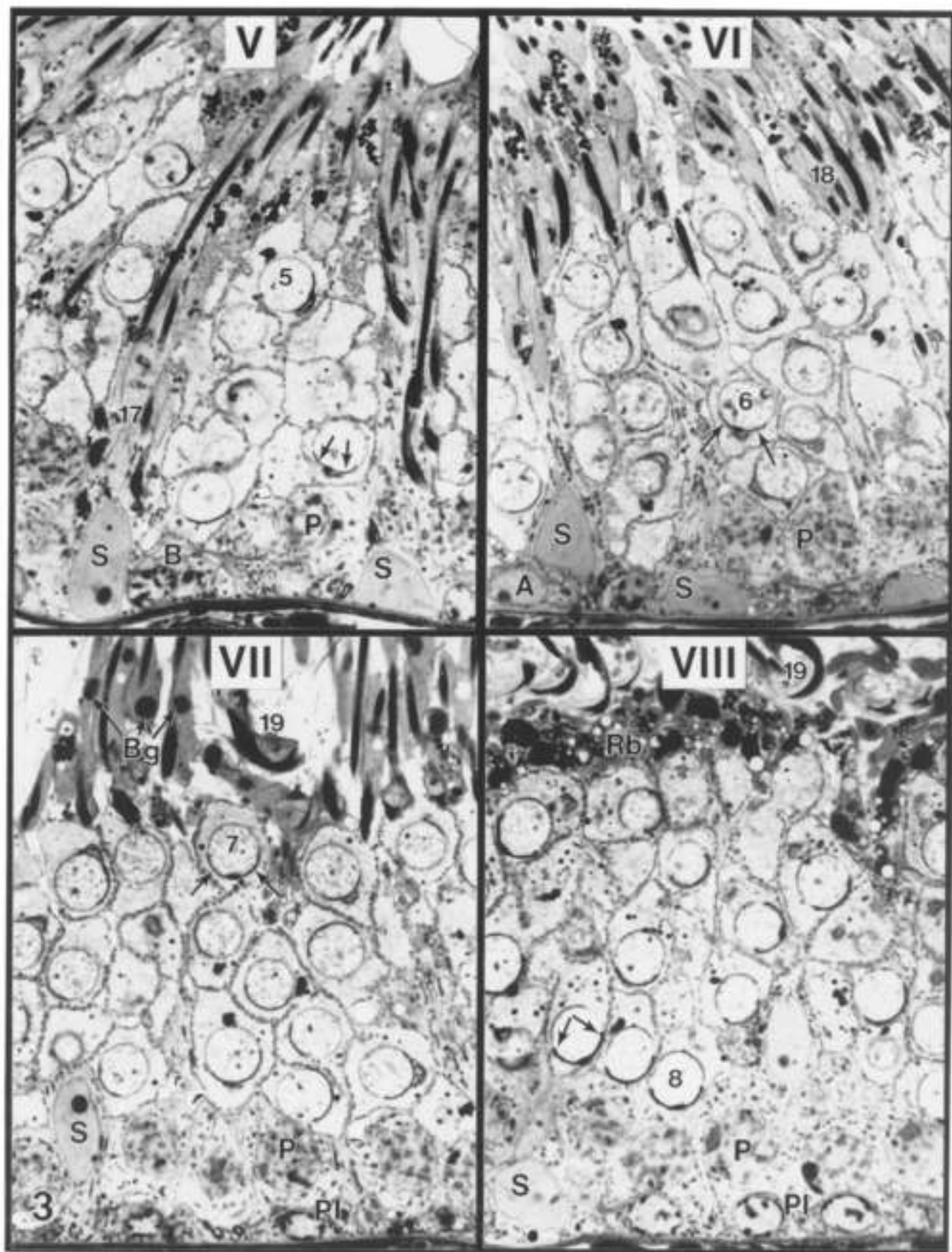
Stages and Transitions in the Cycle of the Seminiferous Epithelium

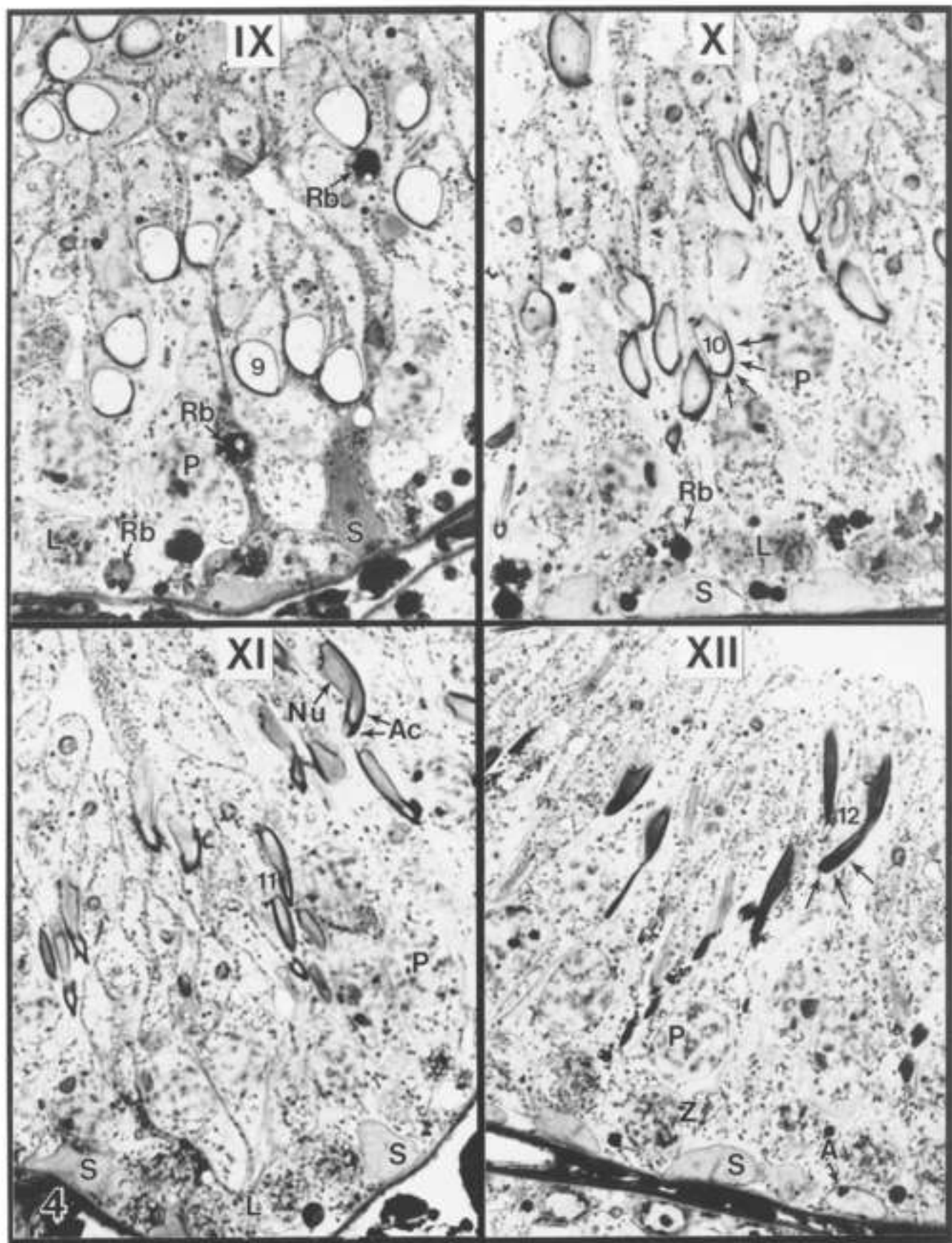


G. G. G. G.









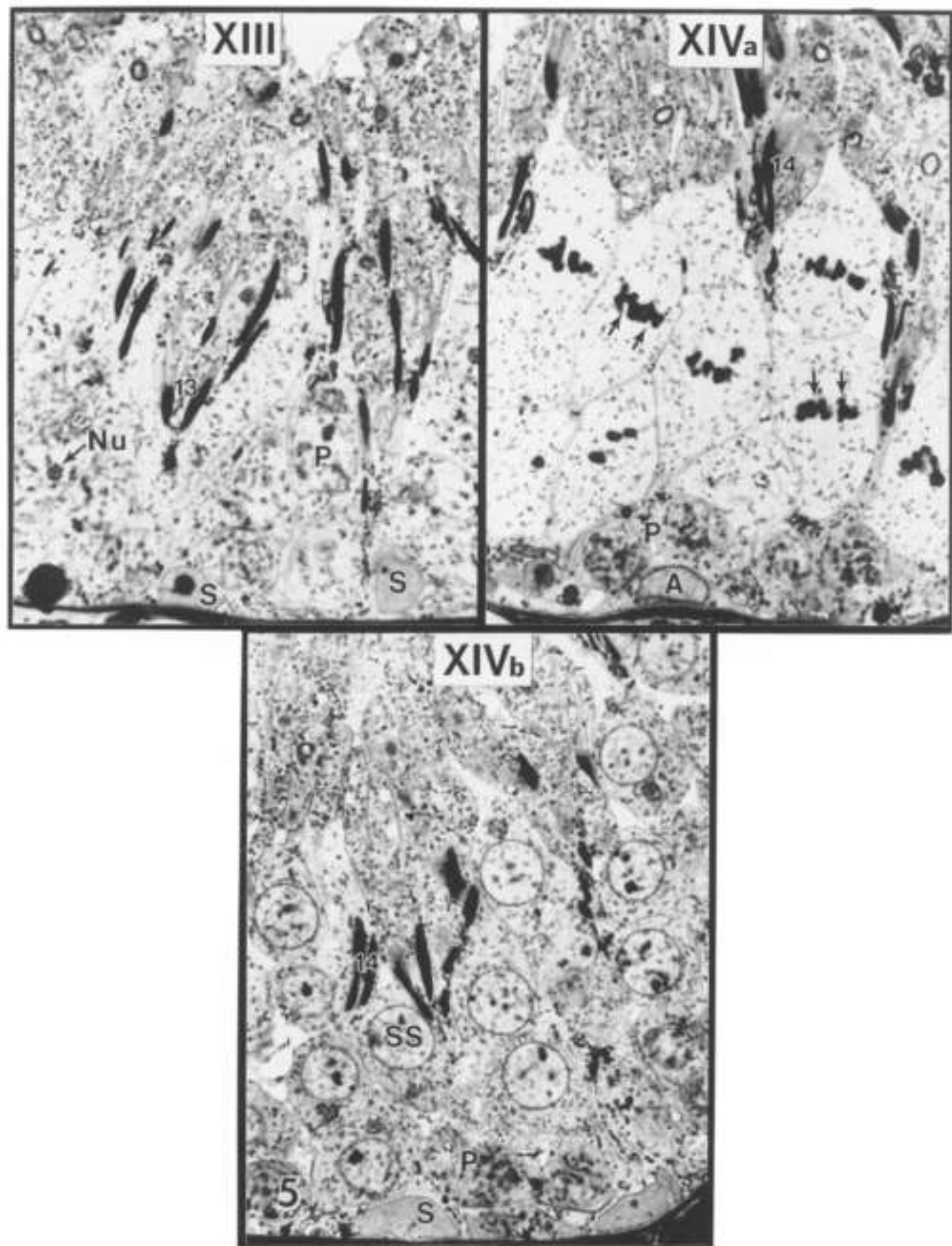


FIG. 5. (Cont'd.)

14 sper-

Stage XIII. The nuclei of step 13 spermatids are narrow and straight and in bundles, separated by large nuclei of pachytene spermatocytes (P). recognized by its nu-

first division in the early phase of this stage. The nuclei of step

matids are highly condensed and rarely exhibit their curvature. Stage XIVb. The secondary spermatocyte (SS) is

APPENDIX A BINARY DECISION KEY FOR THE IDENTIFICATION OF STAGES IN THE CYCLE

Note: Not all of the characteristics listed for a stage will be present in any one tubule. Each stage should be confirmed by more than one correct response because a feature may be represented infrequently due to the thinness of plastic sections. Each question applies to a particular stage or its adjacent stages. Major stage characteristics are in *italics*.

First Questions

1. Are 2 steps of spermatids present (round and elongated)? NO (Stages IX-XIII) YES (Stages I-VIII)
2. If YES in # 1, are elongated spermatids located near the lumen? NO (Late I-V) YES (Late VI-VIII)

Stage I

1. *Is there a faint PAS' Golgi apparatus adjacent to the nucleus of the round spermatid? NO (Newly formed step I spermatids) YES (mid to late step I spermatids)*
2. *Are nuclei of the round spermatids small and heterochromatic? NO (Probably late Stage I) YES (Early Stage I)*
3. Are 1-6 small proacrosomic granules seen in the Golgi apparatus? NO (Probably Stage I, unless out of plane of section) YES (Stage II, if present in only a few spermatid, their other characteristics must be considered)
4. *Do the elongated spermatids have a prominent bulge of the dorsal-caudal aspect of the acrosome (caudal fin)? (difficult to see in epoxy) NO (Stage II, although some overlap) YES (Stage I)*
5. Does the nucleus of the elongated spermatid have a pronounced hook? (rarely seen in epoxy) NO (Late Stage I) YES (Early Stage I)
6. Are the type A spermatogonia in mitosis? NO (Sometimes overlap Stage II) YES (Usually found in Stage I; also present in other stages)
7. Are step 15 spermatid clusters located more toward the lumen? NO (Not definitive) YES (Stage I)

Stage II

1. *Are 1-6 small proacrosomic granules (usually 1-2) present within the Golgi apparatus? (A surrounding vesicle may be visible only with the highest power) NO (Stage I) YES (Stage II)*
2. If there is a single granule in the Golgi zone, is the granule large in size and surrounded by a large, distinct vesicle? NO (Probably Stage I); very small pinpoint granules YES (Stage II); the vesicle is more distinct in epoxy)
3. *Do the elongated spermatids have a prominent bulge of the dorsal-caudal aspect of the acrosome (caudal fin)? NO (Stage I) YES (Sometimes overlap Stage II; reconsider Stage I)*
4. Are mitochondria distinctly aligned along the mid-piece? NO (Reconsider Stage I; begins in late Stage I) YES (Stage II more distinct in epoxy than in methacrylate)
5. Are mitotic figures present? NO (Not definitive) YES (Sometimes overlap Stage II)

Stage III

1. *Is there a single acrosomic granule surrounded by a vesicle (clear or slightly PAS' in the Golgi zone located adjacent to the round spermatid nucleus? (Vlore distinct in epoxy) NO (Stage I or II or out of the plane of section) YES (Stages II, III, or IV)*
2. *Is the acrosomic granule large in size? NO (Reconsider Stage II) YES (Stage III or IV)*
3. Does the acrosomic granule touch the nuclear membrane? NO (Stage III) YES (Consider Stage IV)
4. *Is the acrosomic vesicle flattened against the nuclear membrane? (More distinct in epoxy) NO (Reconsider Stage III) YES (Probably Stage IV)*
5. Does the apical acrosomal head cap extend beyond the tip of the late spermatid nucleus? (More distinct in methacrylate) NO (Probably Stage III) YES (Consider Stage IV)
6. Are mitotic figures present? NO (Probably Stage III, if other features indicated) YES (Consider Stage IV)

Stage IV

1. Does the acrosomic vesicle form a halo around the acrosomic granule? NO (Consider Stage IV) YES (Reconsider Stage III)
2. *Does the acrosomic granule touch the nuclear membrane? (More distinct in epoxy) NO (Stage III) YES (Consider Stage IV)*
3. *Does the acrosomic vesicle form a PAS' straight margin at the nucleus upon which the granule rests? NO (Consider early Stage IV or late Stage III) YES (Stage IV)*
4. *Are mitotic figures present? NO (Not definitive) YES (Stage IV)*
5. *Does the apical acrosomal head cap extend beyond the tip of the nucleus in the elongated spermatid? (Rarely seen in epoxy) NO (Probably Stage III) YES (Stage IV)*
6. Does the PAS' margin of the forming acrosomic head cap form lateral projections, that curve slightly beneath the acrosomic granule? NO (Stage IV) YES (Consider Stage V)
7. Is the acrosomic granule located to one side of the forming acrosomic system? NO (Not definitive) YES (Probably late Stage IV)
8. Is the caudal portion of the acrosome retracted from the dorsal tip of the elongated spermatid nucleus? (Rarely seen in epoxy) NO (Reconsider Stage III) YES (Stages IV-V)
9. Are spermatogonia of type B (large rounded nucleus containing thick clumps of chromatin) present?

- NO (Stage IV)
YES (Consider Stage V)
10. Are clusters of elongate spermatids located adjacent to the Sertoli cell nuclei?
NO (Not definitive)
YES (More common in Stages IV-V)
- Stage V
1. Are lateral projections present on both sides of the acrosomic granule? NO (Reconsider IV) YES (Consider Stage V)
2. Do the lateral projections of the acrosomic system form a curvature (from a slight curve to less than one-fourth of the nucleus)? NO (Reconsider Stage IV) YES (Stage V)
3. Are the clusters of late spermatid nuclei buried deep within the epithelium, adjacent to the Sertoli cell nucleus? NO (Consider Stages IV or VI) YES (Probably Stage V) Are most spermatogonia type B, rather than type A or intermediate? NO (Reconsider Stage IV) YES (Stage V)
5. Are mitotic figures present?
NO (Not definitive)
YES (Consider Stages A1 or 1.11)
6. Is the Golgi apparatus still present in the elongated spermatid cytoplasm? (Primarily seen in epoxy) NO (Consider Stage VI) YES (Stage V)
- Stage VI
1. Are the clusters of late spermatid nuclei buried deep within the epithelium, often adjacent to the Sertoli cell nucleus?
NO (Probably Stage VI)
YES (Reconsider Stage V, if the acrosomic system of round spermatids is small or if mitotic figures are absent)
2. Are the elongated spermatid nuclei between the basement membrane and the luminal border? NO (Consider Stage VII, if the nuclei line the luminal border) YES (Probably Stage VI)
3. Do some late spermatid nuclei occur in clusters?
NO (Consider Stage VII) YES (Probably Stage VI)
- YES (Stage VI)
5. Are small basophilic granules located in the distal cytoplasm of elongated spermatids?
NO (Rarely seen in Stage V; large granules near the nucleus in Stage VII) YES (Probably Stage VI)
6. Are type B spermatogonia predominant?
NO (Consider Stages VI or VII) YES (Reconsider Stage V)
7. Does the acrosomic head cap cover between one-fourth and one-third of the round spermatid nucleus?
NO (Reconsider Stage V)
YES (Stage VI or early VII)
- Stage VII
1. Do the elongated spermatid nuclei line the luminal border?
NO (Reconsider Stage VI)
YES (Probably Stage VII or VIII)
2. Are basophilic granules large at size and located near the nucleus of elongated spermatids?
NO (Reconsider Stage VI)
YES (Probably Stage VII, located beneath the nuclei in Stage VIII)
3. Are large basophilic granules located beneath the ventral curvature of the elongated spermatid head?
NO (Within or adjacent to the ventral curvature in Stage VII)
YES (Consider Stage III)
the round spermatid nuclei located centrally in the cytoplasm?
4. Are
NO (Stage VIII; to one cytoplasmic pole)
YES (Stage VII)

5. Have type B spermatogonia been replaced by small preleptotene spermatocytes? NO (Reconsider Stage VI) YES (Stage VII)
6. Are mitotic figures present?
NO (Stage VII)
YES (Reconsider Stage VI)
Is the round spermatid nucleus covered by an extensive acrosomic head cap (one-third or greater)?
NO (Reconsider Stage VI)
YES (Stages VII or VIII)
- Stage VIII
1. Do the late spermatid nuclei line the luminal border?
NO (Reconsider Stages VI-VII) YES (Consider Stage VII or Stage VIII)
2. Are large basophilic granules located between the curvature of the elongated spermatid nucleus and the round spermatids? NO (Stage VII) YES (Consider Stage VIII)
3. Are the nuclei of the round spermatids located to one pole of the cytoplasm? NO (Reconsider Stage VII) YES (Stage VIII)
4. Have some mature spermatids been released into the lumen? NO (Stages VII-VIII) YES (Stage VIII)
5. Do the nuclei of round spermatids stain less intensely and appear somewhat distorted? NO (Reconsider Stage VII) YES (Stages VIII-IX)
6. Does the Sertoli cell nucleus exhibit a perpendicular orientation that extends nearly halfway to the lumen? NO (Not definitive) YES (Probably Stage VIII)
7. Are a majority of the acrosomic systems in the round spermatids orientated toward the basement membrane? NO (Reconsider Stage VII) YES (Probably Stage VIII)
8. Are knoblike structures formed on the end of lateral acrosomic projections? (More distinct in epoxy) NO (Reconsider Stage VII) YES (Stage VIII)
9. Are mitotic figures present?
NO (Not definitive)
YES (Consider Stage IX)
- Stage IX
1. Are mature spermatids missing from the luminal side of the epithelium? (A few step 19 spermatids may still be attached) NO (Reconsider Stage VIII) YES (Consider Stages IX X)
2. Are large residual bodies seen at various levels within the epithelium? (If disappeared, consider Stage X) YES (Probably Stage IX)
3. Do the spermatid nuclei exhibit asymmetry and flattening?
NO (Reconsider Stage VIII) YES (Probably Stage IX)
4. Are mitotic figures present?
NO (Not definitive)
YES (Stage IX)
5. Does the acrosomic system extend the full length of the flattened nucleus? NO (Reconsider Stage IX) YES (Consider Stage X)
6. Are leptotene more prevalent than preleptotene spermatocytes? NO (Reconsider Stage VIII) YES (Stage IX)
- Stage X
1. Does the elongated acrosomic head cap extend the length of the nucleus?
NO (Reconsider Stage IX) YES (Consider Stage X)
2. Does the head cap of the elongating spermatid form a blunt lip rather than a narrow tubular protrusion at a sharp angle to the nucleus?

NO (Consider Stage XI)

I YES (Probably Stage X)

3. *Is there an angle formed on the ventral side of the acrosomic system and nucleus of the elongating spermatid? (Afore distinct in methacrylate) NO (Reconsider Stage IX) YES (Consider Stages X and XI)*
4. *Is the narrow lateral width of the spermatid nucleus one-half that of step 9 spermatids? (At the acrosomic bead cap position in sagittal section) NO (Reconsider Stage IX) I YES (stage X)*
5. *Have the residual bodies disappeared or relocated along the basement membrane? NO (Reconsider Stage IX) YES (Probably Stage X)*
6. **Are mitotic figures present?**
NO (Not definitive)
ITS (Reconsider Stage IX)

Stage XI

1. *Does the head cap of the spermatid form a narrow tubular protrusion at a sharp angle to the nucleus? NO (Reconsider Stage X) YES (Stage XI)*
2. *Is the narrow lateral width of the spermatid nucleus approximately two-thirds that of step 10 spermatids? (At the acrosomic head cap junction) NO (Reconsider Stage X) YES (Stage XI)*
3. *Are there distinct bundles of elongated spermatids? NO (Reconsider Stage X) YES (Probably Stage XI)*
4. *Does the apex of the spermatid nucleus stain intensely? NO (Consider Stage XI) YES (Consider Stage XII)*
5. *is the apex of the spermatid head long, narrow and nearly straight? NO (Probably Stage XI) Y EI (Consider Stage XII II)*

Stage XII

1. *Doⁿ the apex of the acrosomic system form a blunt tip? (Not always present in plastic sections)*
NO XI if the nucleus is less condensed
(Consider Stage XIII if the nucleus is more condensed)
YES (Stage XII)
2. *Does the apex of the spermatid nucleus stain more intensely basophilic than the caudal portion? NO (Reconsider Stage XI) YES (Probably Stage XII)*
3. *Is the apex of the spermatid head long, narrow and slightly bent? NO (Probably Stage XI) I ES (Probably Stage XII)*

4. *Is the lateral width of the spermatid nucleus thin (two-thirds that of step 11)? NO (Reconsider Stage XI) YES (Stage XII)*

5. *Does the acrosomic head cap bulge slightly from the dorsal side of the nuclear apex? (Rarely seen in epoxy) NO (Consider Stage XIII: the bulge will be more ventral) YES (Consider Stage XII)*
6. *Are large numbers of late diplotene and diakinetid spermatocytes present? (Very large nuclei with dispersed clumps of chromatin and large prominent nucleoli with translucent cores) NO (Reconsider XII) YES (Stage XIII)*
7. *Are mitotic figures present?*
NO (Not definitive)
YES (Probably Stage XII)

Stage XIII

1. *Are large numbers of late diplotene and diakinetid spermatocytes present? (Very large nuclei with dispersed clumps of chromatin and large prominent nucleoli with a translucent cores) NO (Reconsider XII) YES (Probably Stage XIII)*
2. *Does the spermatid nucleus stain intensely basophilic (Highly condensed) NO (Stage XII) YES (Stage XIII-XIV)*
3. *Is the lateral width of the spermatid nucleus very thin (two-thirds that of step 12)? NO (Reconsider Stage XII) YES (Stage XIII or XIV)*
4. *Does the acrosomic head cap extend beyond the apex or bulge from the ventral side of the nuclear apex? NO (Reconsider Stage XII) YES (Stage XIII-XIV)*
5. *is the apex of the spermatid nucleus curved and thin? (Rarely seen in epoxy) NO (Stage XII) YES (Late Stage XIII-XIV)*
6. *Are mitotic figures present? No (Probably Stage XIII) YES (Consider Stage XII or XIV)*

Stage XIV

1. *Are meiotic figures present (meiosis I or II)? ..NO (Stages I-XIII or XIV with secondary spermatocytes) YES (Stage XIV)*
2. *Are secondary spermatocytes present? (nuclear diameter is approximately the size of pachytene spermatocytes and larger than that of the round spermatid) NO (Early or late Stage XIV) YES (Mid Stage XIV)*