treatments with secretion of female accessory gland and micropyle cap substance. Int. J. Insect Morphol. Embryol. 14, 381-391.

Engelmann, F. (1970). The Physiology of Insect Reproduction. Pergamon, New York.

Gillett, C. *0* 988). Arthropoda-Insecta. In Reproductive Biology *of* invertebrates. Vol III. Accessory Sex Glands. (K. G. and R. G. Adiyodi, Eds.), pp. 319-471. Wiley, New York.

Gillott, C. (1995). Insect male mating systems. In Insect Reproduction (S. L. Leather and J. Hardie, Eds.), pp. 33-55. CRC Press, Boca Raton, FL.

Hinsch, G. W. (1990). Arthropoda-Crustacca. In *Reproduc* tive Biology of Invertebrates. Vol. IV, Part B. Fertilization, Development, and Parental Care (K. G. Adiyodi and R. G. Adiyodi, Eds.), pp. 121-155. Wiley, New York.

Kasuga, H., Aigaki, T., and Osanai, M. (1987). System for supply of free arginine in the spermatophore of Bombyx mori. Arginine-liberating activities of contents of male reproductive glands. Insect. Biochem. 17, 317-322.

Leopold, R. A. (1976). The role of male accessory glands in insect reproduction. Annu. Rev. Entomol. 21, 199-221.

Mann, T. (1984). Spermatophores. Springer-Verlag, Berlin.

Reger, J. F., and Fitzgerald, M. E. C. (1983). ArthropodaMyriapoda. In Reproductive Biology *of* Invertebrates. Vol. II. Spermatogenesis and Sperm Function (K. G. Adiyoch and R. G. Achyodi, Eds.), pp. 451-475. Wiley, New York.

Sonenshine, D. E. (1991). The Biology *of Ticks. Vol. 1.* Academic Press, New York.

Thibout, E. (1981). Evolution and role of apyrene sperm cells of lepiclopterans: Their activation and denaturation in the leek moth, Acrolepiopsis assectella (Hyponomeutoidea). in Advances in Invertebrate Reproduction (W. H. Clark, Jr., and T. S. Adams, Eds.), pp. 231-242. Elsevier/North Holland, New York.

Spermatogenesis, Overview

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1. The Seminiferous Tubule
11. Phases of Spermatogenesis
Ill. Stages of the Cycle
IV. The Wave

GLOSSARY

acrosomal system A Golgi-derived organelle that forms over the nucleus consisting of a membrane-bound vesicle with dense acrosomal granules that eventually fuse; consists of enzymes necessary for the acrosomal reaction at fertilization.

clonal unit The synchronous group of developing germ cells formed by incomplete cytokinesis during spermatogonial division and held together by intercellular bridges until spermiation.

cycle A complete sequential progression of the cellular associations (or stages) that occur over time is called the cycle of the seminiferous epithelium. The stages follow one another in development over time through an entire cycle, returning to the original stage and repeating this cycle approximately 4.5 times until spermatogonia eventually become spermatozoa and are released.

cytoplasmic ***lobe*** A cytoplasmic protrusion of the late step 19 spermatid in stage VII (rat), containing abundant RNA, mitochondria, lipid droplets, and other unused cellular remnants that are eventually phagocytized by the Sertoli cell.

meiosis A specialized process by which one germ cell produces four haploid spermatids after undergoing two meiotic cellular divisions. A long prophase permits the duplication of chromosomes and genetic recombination before these largest of germ cells rapidly divide, producing secondary spermatocytes after meiosis I and small step I spermatids after meiosis 11.

residual body A large spherical body containing the cytoplasmic remnants of sperm formation which is formed by detachment of the cytoplasmic lobe during sperm release into the lumen. Residual bodies are phagocytized by Sertoli cells in subsequent stages.

seminiferous epithelium Consists of two cell types, a somatic cell, the Sertoli cell, and male germ cells at various steps in development.

Sertoli cell barrier Once called the "blood- testis-barrier," this tight occluding junction is formed between adjacent Sertoli cells separating basal and adluminal compartments. The barrier separates most germ cells from blood-borne substances and lymph, thus requiring the Sertoli cell to sustain germ cell development.

spermiation A complex process by which spermatozoa are released into the seminiferous tubule lumen after detaching from the Sertoli cell junctional complex.

spermiogenesis Cellular differentiation of the spermatids from a small, nondescript round cell to the spermatozoon that has a highly condensed elongate nucleus, unique acrosomic system derived from the Golgi, and a complex flagellum that is motile.

stages A stage (numbered with Roman numerals) is represented by a defined association of spermatogonia, spermatocytes, and spermatids in a cross section of seminiferous epithelium, at a specific phase in time during spermatogenesis. The acrosomal system of the spermatids is commonly used to identify specific stages in the cycle of the seminiferon,, epithelium.

stem cell Quiescent self-renewing spermatogonia that, with proper stimulation, proliferate in order to renew the germinal epithelium.

steps A unique morphologically identifiable change in the differentiation of a spermatid, based on the acrosomic system formation, sperm head shape, and nuclear condensation. These changes divide spermiogenesis into sequential steps that are numbered with Arabic numbers (e.g., step 1 spermatid).

wave A series of sequential stages in physical space along the length of a seminiferous tubule, formed by the synchronous development of clonal units of germ cells.

Spermatogenesis is the biological process of gradual transformation of germ cells into spermatozoa over an extended **period of time within** the boundaries of the seminiferous tubules of the testis. This process involves cellular proliferation by repeated mitotic divisions, duplication of chromosomes, genetic recombination through cross-over , reduction-division by meiotic division to produce haploid spermatids, and terminal differentiation of the spermatids into spermatozoa. Thus, spermatogenesis can be divided into three phases: proliferation, reduction-division (or meiosis), and differentiation. These phases are also associated with specific germ cell types, i.e., spermatogonia, spermatocytes, and spermatids, respectively.

1. THE SEMINIFEROUS TUBULE

Spermatogenesis occurs within the extensive seminiferous tubular structures of the testis. Seminiferous tubules are lined by the seminiferous epithelium and contain a fluid-filled lumen, into which fully formed spermatozoa are released. The seminiferous epithelium consists of two basic cell types, somatic and germinal cells. The germ cells (Fig. 1) are found at different levels from the base of the tubule to the lumen and are surrounded by cytoplasm of the somatic cell, the Sertoli cell (Fig. 2). The Sertoli cell cytoplasm extends the entire height of the epithelium because the cell serves to nurture the germ cells through their cycles of development. As the germ cells divide and *develop* into different types of cells, they move from the basement membrane region through tight junctional complexes of adjacent Sertoli cells until they reside in the adluminal compartment. The Sertoli-Sertoli cell junctions form the blood-testis barrier, which helps to protect the developing germ cells from potentially harmful blood-borne chemicals. The germ cells develop as a syncytium or clonal unit connected to one another by intercellular bridges after cell division (Fig. 3). *This* unique process of incomplete division ensures synchronous development and permits rapid communication between the *cells. Synchrony of* germ cell development results in large areas of the seminiferous tubule containing vast numbers of cells at the same level of development, the specific identification of which scientists refer to as stages.

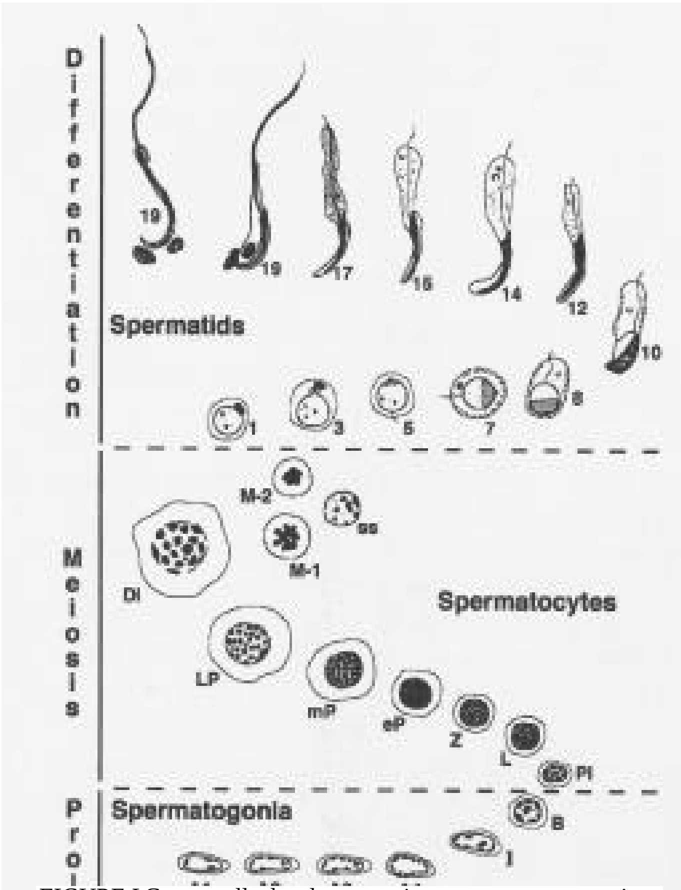

FIGURE I Germ cell development in rat spermatogenesis. The proliferation phase (Prol) includes repeated spermatogonial division from type A spermatogonia (Al-A4) to intermediate (1) and B-type cells. Meiosis is an extended phase that begins after B-type spermatogonia divide to produce preleptotene spermatocytes (PI). Meiotic prophase begins with small leptotene spermatocytes (L). The cells enlarge as prophase continues through zygotene (Z), early, mid-, and late pachytene (eP, mP, LP) spermatocytes. Diplotene cells undergo the first meiotic division (M-1) producing secondary spermatocytes (ss). After the second meiotic division (M-2), haploid cells called spermatids begin the differentiation phase by forming round spermatid steps (1 -7). Round spermatids are slowly transformed into elongated cells (steps 8-19) and finally into spermatozoa that are released.

11. PHASES OF SPERMATOGENESIS

A. Proliferation

Spermatogonia, which constitute the first phase, are the most immature cells and are located along the base of the seminiferous epithelium. **They** prolif

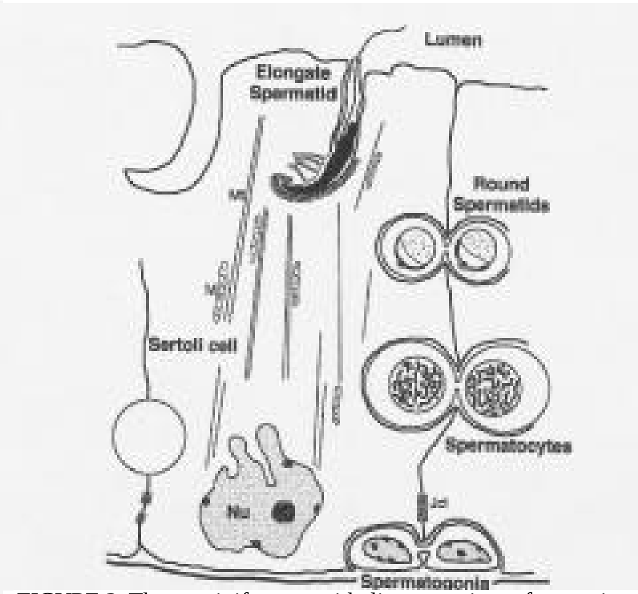

FIGURE 2 The seminiferous epithelium consists of somatic cells, the Sertoli cells, whose cytoplasm surrounds the developing germ cells. Sertoli-Sertoli cell junctions Oct) separate spermatogonia from the adluminal compartment where spermatocytes and spermatids develop. Microtubules (M0 are parallel in the Sertoli cell cytoplasm and help to transport germ cells within the epithelium. M, mitochondria; Nu, nucleus of the Sertoli cell.

erate by mitotic division **and multiply** repeatedly **to continually** replenish **the germinal** epithelium. **Spermatogonia are capable** of self-renewal **and thus also produce stem cells that remain along the base as well as committed cells that** are on a one-way tract leading

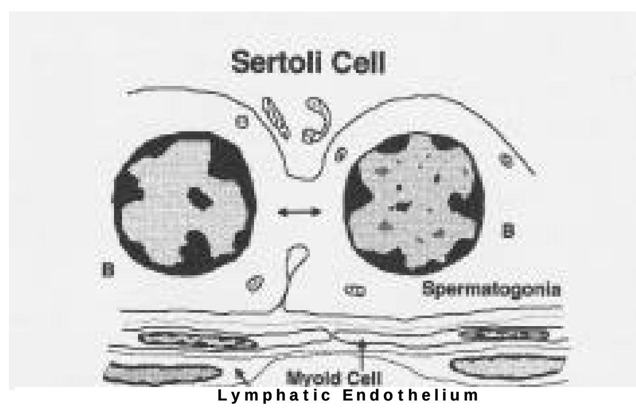

FIGURE 3 Two B-type spermatogonia are connected through the intercellular bridge (double arrow). Sertoli cell cytoplasm helps to hold the bridge in place. Myoid cells have a common basal lamina with spermatogonia and the

to spermatozoa. In most species, the B spermatogonia is the last to divide by mitosis. Its division produces the first cell of the second phase, the preleptotene spermatocyte, which migrates upwards away from the base of the seminiferous tubule and crosses through the Sertoli-Sertoli junction.

B. Meiosis

Reduction-division by meiosis involves numerous types of spermatocytes that range in size from cells smaller than a red blood cell (preleptotene) to very large cells (pachytene) that occupy portions of every cross section of seminiferous tubules. Reduction-division is a biological mechanism by which a single germ cell can increase its DNA content, then divide twice to produce four individual germ cells containing a single strand of each chromosome or half the number of chromosomes normally found in cells of the body. The process of meiosis is extended over a long period of time; therefore, spermatocytes are found in every stage of spermatogenesis, and in some stages two different types of spermatocytes are observed. During meiosis, the changes that take place in the chromosomes are easily recognized (Figs. 1 and 7).

DNA synthesis occurs in preleptotene spermatocytes. Prophase of the first meiotic division may last for nearly 3 weeks, during which time the chromosomes first unravel as thin impaired filaments (leptotene). Homologous chromosomes become paired in the zygotene cell, forming the synaptonemal complex. Pachytene spermatocytes begin as small cells but their nuclei enlarge greatly as the chromosomes become shorter and thicken. Genetic recombination occurs through cross-over between paired chromosomes. Pachytene cells also exhibit an increase in RNA and protein synthesis in preparation for the next phase. Diplotene spermatocytes separate the synaptonernal complexes and the chromosomes are spread apart in the nucleus. In diakinesis the nuclear envelope disappears and chromosomes condense. Both meiotic divisions occurs rapidly, thus limiting these cells to one stage (Fig. 7). Small secondary spermatocytes (2N) are produced by meiosis I which then rapidly divide again by meiosis 11, with unique metaphase formations by the chromatin. Meiosis 11 produces very small haploid (IN) cells called round spermatids that enter the next phase called differentiation.

C. Differentiation

The haploid germ cells undergo a prolonged phase of terminal differentiation known as spermiogenesis. The cells undergo dramatic changes, including the following three major modifications: (i) The nucleus elongates and chromatin condenses into a very dark staining structure having unique shapes that are species specific (Fig. 4); (ii) the Golgi apparatus produces a lysosomal-like granule that elaborates over the nucleus to form the future acrosome (Fig. 5). The acrosomic system contains the hydrolytic enzymes required for sperm-egg interaction and fertilization; and (iii) the cell forms a long tail lined with mitochondria in the proximal region and it loses excess cytoplasm, which is discarded first as the cytoplasmic lobe that eventually is phagocytized by the Sertoli cell as the residual body. Recognizable changes in the differentiation of a spermatid are called "steps" of spermiogenesis. In the rat, the first step is the small round step I spermatid produced by meiosis 11. Step 1 occurs in the first stage of the cycle. In all species, the late elongate spermatids, steps 15-19 in the rat, overlap with the younger round spermatids. Thus, in some stages two generations of spermatids are present in the same tubule cross section (Figs. 5 and 7).

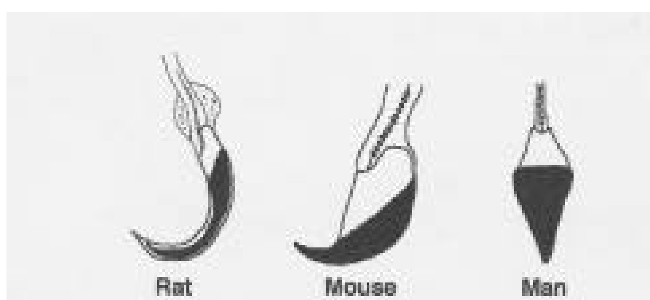

FIGURE4 Heads of newly released sperm from three species illustrating the variation achieved through differentiation of the haploid spermatid. The black areas represent portions of the nucleus covered by the acrosome.

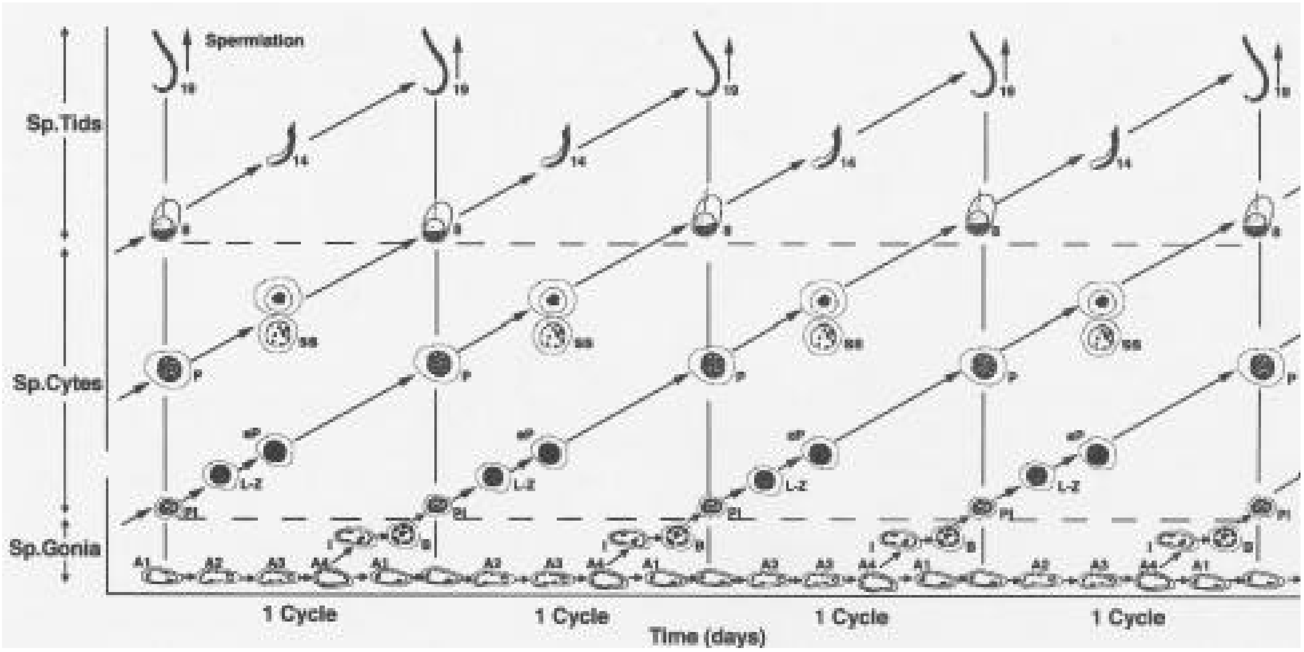

FIGURE 5 Repetitions of the cycle of the seminiferous epithelium are represented in a temporal manner. Each cycle shows the different types of cells and their progeny that would be found in a particular stage of the cycle. The phases of spermatogenesis are represented by the three cell types, spermatogonia (Sp.Gonia), spermatocytes (Sp.Cytes), and spermatids (Sp.Tids). Type A spermatogonia along the first row are self-renewing, but Al-A4 are committed cells in the spermatogenic lineage. Types I (intermediate) and B appear distinctive and are found in greater numbers than the ty e A spermatogonia, The small preleptotene ₍p
spermatocyte begins the extended period of meiosis, with modifications producing leptotene (L), zygotene (Z), early pachytene (0), and pachytene (P) spermatocytes. Meiotic division I produces the secondary spermatocyte (ss). Meiotic division 11 results in the haploid spermatids, of which three are shown: steps 8, 14, and 19. Step 19 spermatids are released

111. **STAGES OF THE CYCLE**

The synchronized process of spermatogenesis allows germ cells to advance (or change) within the seminiferous epithelium. In a general sense, the more mature cells are found away from the basement membrane and in specific associations with the younger cells that will divide and mature in time. This process of epithelial evolution in a synchronized manner over time produces a cycle because there is a beginning, the entrance of spermatogonia into type A mitosis, and an end, the release of new sperm. Spermatogenesis can be split into repeated cycles of the seminiferous epithelium which are defined by the specific cellular associations established at specific points in time. Over a set period of time, these cellular associations repeat themselves, thus establishing the cycle (Fig. 5). When a cellular association exhibits distinguishing morphological features, it is identified as a different stage of the cycle. Stages are recognized by examining cross sections of seminiferous tubules histologically, with a particular focus on the acrosomic system associated with the spermatids. The acrosomic system is stained using the periodic acid-Schiff 's reaction (PAS). The pink PAS stain recognizes the Golgi and acrosomic granule. As the granule flattens against the nuclear envelop the stain picks up the acrosomal vesicle that extends over the nucleus as a cap until finally it forms a very thin layer over the condensed nucleus of the mature sperm (Fig. 6).

The repetitive nature of the cycle is shown in Fig. 5. Although the arrows suggest that the cells move laterally in time, they actually only move upward in the seminiferous epithelium. Over approximately 4.5 cycles the A spermatogonia becomes a spermatozoon that is released, after having gone through six mitoses, two meiotic divisions, and more than 2 weeks of differentiation.

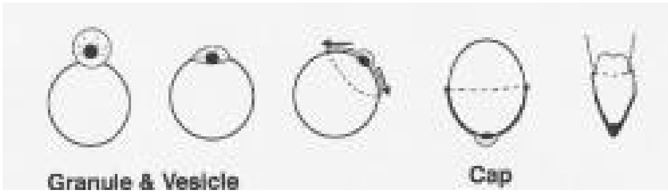

FIGURE 6 The acrosomic system consists of the Golgi apparatus, which produces the acrosomic vesicle, and granules. The granules are small at first, but fuse to form a single large granule that becomes flattened against the nuclear envelop. The vesicle also flattens and spreads across the nucleus (arrows) until a cap is formed that covers nearly one-half of the nucleus. in the mature sperm, the acrosome is tightly bound to the nuclear envelope as a thin covering over a major portion of the sperm head.

Recognition of the stages of the cycle is best performed by comparing histological sections to a "staging map" (Fig. 7). In the map, cells progress from left to right, then move up one row and again progress from left to right, In time, the cells are simply changing into the next cell type through cell division or differentiation, and the cells then move through the epithelium toward the lumen. Because the definition of stages is arbitrary, the length of time that the cells remain in a particular stage is variable and ranges from 0.3 to 2.7 days. Thus, the length of time occupied by a stage will determine the frequency in which that stage is found in seminiferous tubule cross sections of the testis (Fig. 8).

IV. THE WAVE

Cells in the stages do not move laterally along the length of the seminiferous tubule. However, there is an unusual ordering of the stages so that the segments of the tubule contain stages in consecutive order. Although there are short reversals of this segmental order, called modulations, the sequential order of the stages and their repetition along the length of the tubules constitutes the "wave" of spermatogenesis in the seminiferous epithelium. That is, stage I is followed by stage 11, which is followed by stage 111, etc. through stage XIV, which is followed by stage 1. The stages are found in ascending order from the rete testis to the center of the seminiferous tubule, where a reversal site is typically found (Fig. 8). The wave is produced by synchronous development of

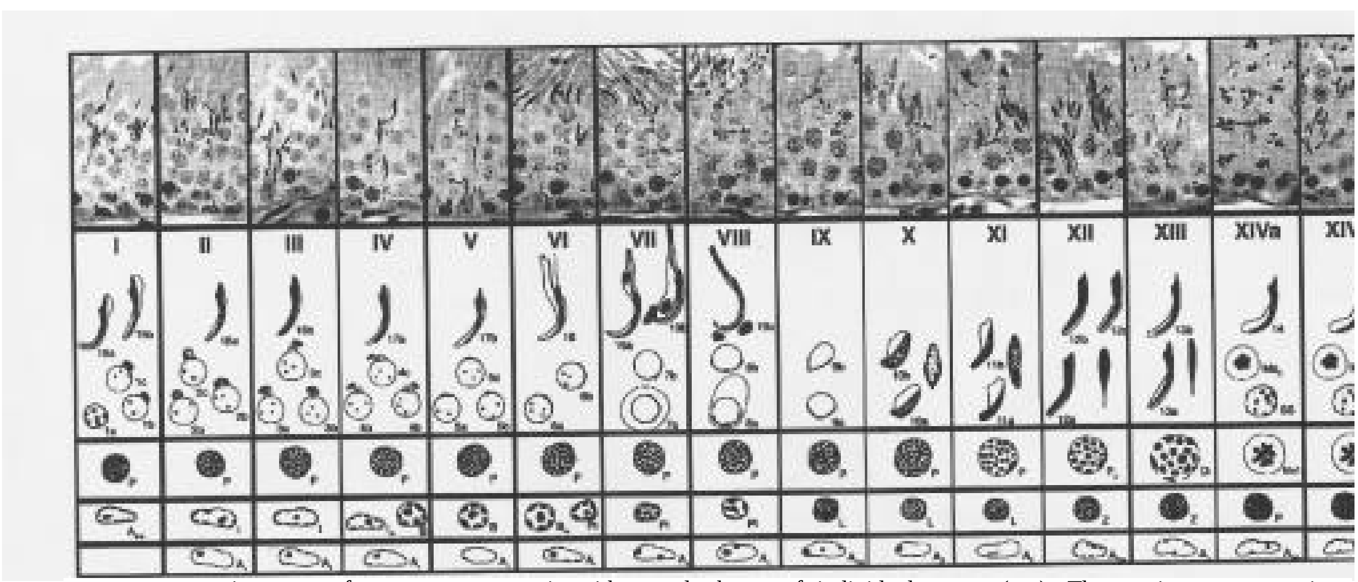

FIGURE 7 A staging map of rat spermatogenesis with actual photos of individual stages (top). The staging map contains illustrations that emphasize the nucleus of all cell types in the cycle of the seminiferous epithelium. Steps of spermiogenesis are split into intermediate steps to demonstrate variations in the morphology within a single stage. Spermatogonia *(Al-4, 1,* B); spermatocytes (PI, preleptotene; L, leptotene; Z, zygotene; P, pachytene; P, diplotene; Di, diakinesis; Mel, meiosis I; Me2, meiosis 11; ss, secondary spermatocyte); spermatids (1-19). S, Sertoli cell; F, acrosomal flag; *G,* Golgi; M, acrosomal margin; *Ac,* acrosomal system; Bg, basophilic granule; Rb, residual body; Nu, nucleus.

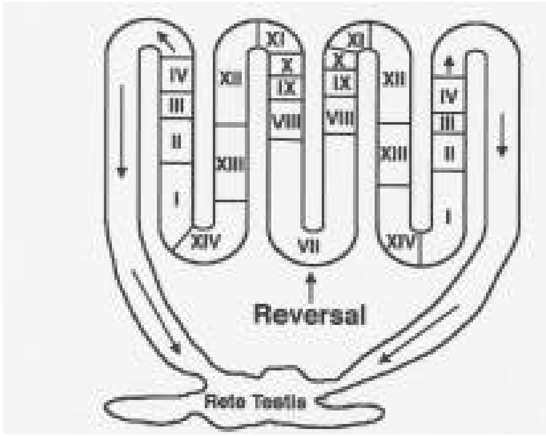

FIGURE 8 The wave of spermatogenesis in the seminiferous epithelium is illustrated with the sequential order of stages, increasing from the reversal site toward the rete testis (arrows).

clonal units of germ cells through a mechanism of biological signaling that is unknown.

Bibliography

Clermont, Y. (1972). Kinetics of spermatogenesis in mammals seminiferous epithelium cycle and spermatogonial renewal. *Physiol. Rev. 52,* 198-236.

Clermont, Y., and Leblond, C. (1953). Renewal of spermatogonia in the rat. *Am. J. Anat.* 93, 475-501.

Clermont, Y., and Leblond, C. P. (1959). Differentiation and renewal of spermatogonia in the monkey, *Macacus rhesus. Am. J. Anat.* 104, 237-273.

Courot, M., Hochereau-de Reviers, M., and Ortavant, R. (1970). Spermatogenesis. *Testis 1,* 339-432.

Dym, M., and Fawcett, D. W. (1970). The blood-testis barrier in the rat and the physiological compartmentation of the seminiferous epithelium. *Biol. Reprod.* 3, 308-326.

Dym, M., and Fawcett, D. W. (1971). Further observations on the numbers of spermatogonia, spermatocytes, and spermatids connected by intercellular bridges in the mammalian testis. Biol. *Reprod.* 4, 195-215.

Hess, R. (1990). 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. *Biol. Reprod.* 43, 525-542.

Leblond, C., and Clermont, Y. (1952a). Spermiogenesis of rat, mouse, hamster and guinea pig as revealed by the "periodic acid-sulfurous acid" technique. *Am. J. Anat.* 90, 167-215.

Leblond, C., and Clermont, Y. (1952b). Definition of the stages of the cycle of the seminiferous epithelium in the rat. *Ann. N. Y. Acad. Sci. 55,* 548-573.

Oakberg, E. F. (1971). Spermatogonial sLem-cell renewal in the mouse. *Anat. Rec.* 169, 515-531.

Percy, B., *Clermont, Y.,* and Leblond, C. (1961). The *wave* of the seminiferous epithelium in the *rat. Ain, J. Anat.* 108,47-77.

Roosen-Runge, E. C. (1962) The process of spermatogenesis in mammals. *Biol. Rev.* 37, 343-377,

Russell, L., Ettlin, R., Sinha Hikim, A., and Clegg, E. (1990). *Histological and Histopathological Evaluation* of *the Testis.* Cache River Press, Clearwater, FL.

Setchell, B. P. (1978). *The Mammalian Testis.* Cornell Univ. Press, Ithaca, NY.

Sharpe, R. (1994). Regulation of spermatogenesis. In *The Physiology of Reproduction* (E. Kn obil and J. D. Neill, Eds.), Vol. 2, pp. 1363-1434. Raven Press. New York.