Chapter 02

Spermatogenesis and Cycle of the Seminiferous Epithelium

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Abstract

Spermatogenesis is a complex biological process of cellular transformation that produces male haploid germ cells from diploid spermatogonial stem cells. This process has been simplified morphologically by recognizing cellular associations or 'stages' and 'phases' of spermatogenesis, which progress through precisely timed and highly organized cycles. These cycles of spermatogenesis are essential for continuous sperm production, which is dependent upon numerous factors, both intrinsic (Sertoli and germ cells) and extrinsic (androgens, retinoic acids), as well as being species-specific.

Introduction

Spermatogenesis is the transformation of spermatogonial cells into spermatozoa over an extended period of time within seminiferous tubule boundaries of the testis. The seminiferous epithelium (Fig. 1) consists of germ cells that form numerous concentric layers penetrated by a single type of somatic cell first identified by Enrico Sertoli in 1865.[1] The cytoplasm of Sertoli cells extends as thin arms around all the germ cells to nurture and maintain their cellular associations throughout the process of spermatogenesis. Germ cells multiply first by repeated mitotic divisions and then by meiosis, which involves the duplication of chromosomes, genetic recombination, and then reduction of chromosomes through two cell divisions to produce spherical haploid spermatids that differentiate into highly compacted spermatozoa for release into the tubule lumen. To study this complex and lengthy process, spermatogenesis has been organized by several different approaches, including the more popular method of 'Staging' or the recognition of germ cell association in time and the 'phases' of spermatogenesis (mitosis, meiosis and spermiogenesis). This review will examine the stages and their cycle in the production of sperm in several species, but the mouse will receive special emphasis, as it is currently the most commonly used species in research.

Cellular Components-Stages of Spermatogenesis

The seminiferous epithelium consists of only one somatic cell type, the Sertoli cell,[2,3] but many different germinal cell types.[4] The complexity of this epithelium was simplified when Leblond and Clermont[5] were able to divide the epithelium into separate stages, according to the cellular associations observed in each tubular cross section. Stages of spermatogenesis are artificial definitions that are based upon rules established by the investigator. The original stages were defined according to changes observed in the Golgi region of spermatids, an area where the forming acrosomic system can be visualized by the periodic acid-Schiff's reaction (PAS). In the mouse, XII stages are well defined by this method (Fig. 2). However, spermatoge-

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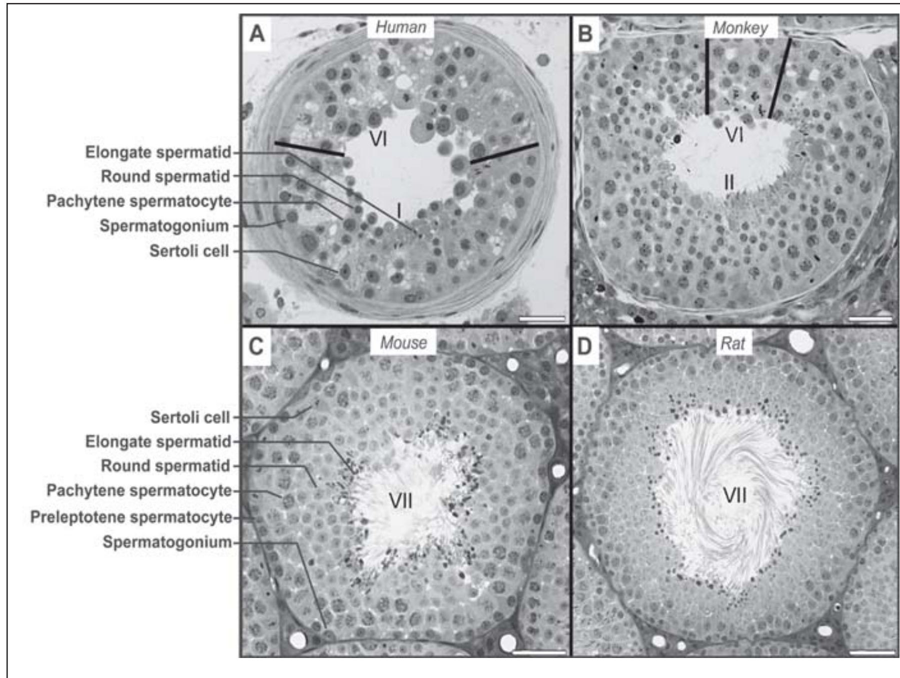

Figure 1. Seminiferous tubule cross-sections in different mammalian species. In the human (A) and marmoset *Callithrix penicillata* (B), two stages of the cycle or germ cells association (delimited by a black line) are observed, whereas in mouse (C) and rat (D) only one stage is found. Bars = 40 μm in A, B, and C; and 60 μm in D.

nesis is a continuum, which results in transitional areas being observed between two stages.[6] In such cases, a preponderance of cell types can be used for stage identification. PAS staining and higher microscopic resolution is required to identify specific stages. However, for most research purposes, grouping stages into three categories is adequate and much easier for evaluation. For example, it is possible to group Stages I-V as 'early'; Stages VI-VIII as 'middle'; and Stages IX-XII as 'late' (Fig. 2).

Phases of Spermatogenesis

Mitosis

Spermatogonia are diploid germ cells (2n) that divide by mitosis and reside on the basement membrane (Figs. 1, 2). Currently, it is not possible to identify spermatogonial stem cells by routine microscopy, but different types of spermatogonia are recognized as type-A, intermediate, and type-B. In well-studied laboratory rodents, such as rats and mice, four classes of spermatogonia are present: undifferentiated type A spermatogonia [A single (A_s), A paired (A_{pr}), A aligned (A_{al})]; differentiated type A spermatogonia (A_1, A_2, A_3, A_4); intermediate spermatogonia (In); and type B spermatogonia (B).[4,7] In these species, the different spermatogonial classes can be characterized by light and transmission electron microscopy according to the presence and distribution of heterochromatin.[8,9] It has also been suggested that undifferentiated spermatogonia, including A_s or stem cell, are located in niches of the seminiferous epithelium, which are regulated by the Sertoli cell.[8-18]

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Meiosis

B-spermatogonia divide by mitosis forming two preleptotene spermatocytes, cells representing the beginning of meiotic prophase (Fig. 2). These small cells rest on the basement membrane, but leptotene and zygotene spermatocytes become transit and move through the blood-testis-barrier (or Sertoli-Sertoli barrier).[19,20] Preleptotene, leptotene and zygotene spermatocytes are located in specific stages and are identifiable by routine microscopy, although fixation artifact results in the leptotene and zygotene cells appearing to be attached to the basement membrane. Spermatocytes are found in all stages, because meiosis is a prolonged period of spermatogenesis that extends over approximately 14 days in the mouse. Thus, any attempt to isolate specific stages of spermatogenesis for molecular analysis, will include cells of this phase. Spermatocytes are the cells of meiosis and their regulation requires a special focus. Of special note, meiotic cell division occurs in and defines a single stage (XII). In the mouse, stage XII is found in approximately 10% of the seminiferous tubular cross sections and meiotic division is completed in approximately 1 day.[21] This cellular division goes through three categories, all occurring in stage XII: a) meiosis I, the division of 4n cells; b) formation of secondary spermatocytes (2n), which are larger than step 1 spermatids, but rarely are found as the only spermatocyte in a tubular cross section; and c) meiosis II, the division of 2n secondary spermatocytes to form haploid (1n) round spermatids. Studies in rats, buffalos, rams, and pigs revealed a striking increase in size for primary spermatocytes, from preleptotene to diplotene.[22,23] This increase is followed by a dramatic decrease of cell size during spermiogenesis in such a way that, due to changes in chromatin and nuclear condensation, in rats, for instance, before spermiation spermatid nuclear volume reaches only $1/50^{th}$ (~500 to $10 \mu m^3$) of its initial volume.

Spermiogenesis

The transformation of spherical, haploid spermatids (1n) into elongate, highly condensed and mature spermatozoa that are released into the seminiferous tubule lumen is called spermiogenesis (Fig. 2). The differentiation of spermatids proceeds through at least 4 prolonged steps (or phases): Golgi, capping, acrosomal, and maturation. These steps are useful for the identification of specific stages in the cycle of the seminiferous epithelium.

Golgi

Golgi apparatus is very important during the early steps of spermiogenesis,[4,6,24] as the formation of the acrosome is dependent upon this organelle's ability to produce vesicles and granules containing the enzymatic components of the acrosomic system that will cover the developing sperm nucleus. Differentiation of the first three steps of round spermatid formation involves a prominent Golgi apparatus that is identified by PAS staining. Step 1 spermatids have a small, perinuclear Golgi region without an acrosomic vesicle or granule. Subsequent steps 2-3 show proacrosomal vesicles and granules within the Golgi apparatus, with the formation of a single, large acrosomal granule within a larger vesicle that will indent the nucleus (Fig. 2).

Capping

Capping involves steps 4-5 round spermatids, where the acrosomic granule touches the nuclear envelope and the vesicle begins to flatten into a small cap over the nuclear surface. In steps 6-7, the acrosomic vesicle becomes very thin and the granule flattens. Step 8 is the last round spermatid, and the acrosome flattens over approximately 1/3 of the nuclear surface. In late stage VIII, step 8 nuclei begin to change shape.

Acrosomal

Acrosomal steps 9-14 involve migration of the acrosomal system over the ventral surface of the elongating spermatid nucleus (Fig. 2). This migration of the acrosome is completed approximately by step 14 spermatid and is difficult to identify in typical histological sections, due to its presence in different planes of sections and angles or orientation. Thus, recognition of

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Figure 2. Mouse Stages in the cycle of the seminiferous epithelium (I-XII). Layers depicting the cellular associations are drawn with Sertoli cells separating each stage. Along the base are photos of early, middle and late spermatid nuclei, stained with the PAS reaction and hematoxylin. Spermatogonia (A, In, B); spermatocytes (Pl: preleptotene, L: leptotene, Z: zygotene, P: pachytene, D: diakinesis, Mi: meiotic division); round spermatids (1-8); elongate spermatids (9-16). Adapted with permission from Dr. Robert E. Braun. (Figure legend continues on next page.)

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(Continued from previous page.) Stage I. Two generations of spermatids are found in Stages I-VIII, round and elongate spermatids. In this stage, the round spermatid nucleus is smaller than in subsequent stages and contains a typical large central nucleolus. The Golgi is also small and lacks PAS+ granular material.
Stage II. Small PAS+ proacrosomal granules are seen in the center of the Golgi apparatus, which is attached to the nucleus of round spermatids.
Stage III. An acrosomic granule is well formed within the larger round Golgi vesicle, which forms an indentation of the round spermatid nucleus.
Stage IV. The acrosomic granule begins to flatten in this stage.
Stage V. The acrosomic system is clearly defined now and there is a straight line formed by the acrosomic granule lying on the PAS+ dark line that caps the round spermatid nucleus, surrounded by the vesicle. Along the basement membrane, B-type spermatogonia are prominent.
Stage VI. The acrosomic system begins to spread, but remains thick and the granules are distinct. In this stage, B-type spermatogonia undergo mitosis to form preleptotene spermatocytes. Elongate spermatids begin to migrate toward the lumen.
Stage VII. The acrosomic system spreads across the nucleus and becomes thinner, allowing the central acrosomic granule to bulge slightly above the acrosomic vesicle. Elongate spermatids are located at the luminal edge of the tubule, but the cytoplasm covers the sperm head and about 1/2 of the tail.
Early VII. There is more cytoplasm covering the mid region of elongate spermatids and no cytoplasmic lobe has formed. Numerous small preleptotene cell nuclei are found on the basement membrane.
Middle VII. The cytoplasmic lobe begins to form and elongate spermatid cytoplasm no longer covers the midpiece of tail. Large dark granules in cytoplasmic lobes are still absent.
Late VII. The cytoplasmic lobe is well formed and much of it is now between the sperm head and the basement membrane. Dark granules are beginning to appear distinct near the sperm head and sometimes below it. Preleptotene cell nuclei are enlarging as these cells transform into leptotene and chromatin begins to disperse into smaller, finer clumps.
Stage VIII. The acrosome is flattened and forms a cap that covers nearly half of the round spermatid nucleus. Many of the nuclei have migrated to the cytoplasmic plasmalemma and the acrosomic system may be oriented toward the basement membrane. Elongate spermatids are being released into the lumen through a process called spermiation, while excess spermatid cytoplasm forms large cytoplasmic lobes with large dark bodies beneath the head of step 16 spermatids.
Stage IX. Only one generation of spermatids is found in Stages IX-XII, the transition from round into elongate. Cross sections of step 9 spermatid nuclei are oblong, as they begin the elongation process, with the thin PAS+ acrosomic system off center and extending from the apex toward the caudal region of the nucleus. Cytoplasmic lobes fuse into very large residual bodies that are phagocytized by the Sertoli cell and disappear by Stages X-XI.
Stage X. The spermatid head forms a distinct protrusion with a sharp angle. Only the protrusion is covered by the PAS+ acrosome on the ventral side, while the dorsal side is covered to the caudal surface of the nucleus. Pachytene spermatocyte nuclei reach their maximum diameter prior to diplotene phase.
Stage XI. Step 11 spermatid nuclei become thinner, more elongated and begin to stain more intensely, indicating chromatin condensation. Diplotene spermatocyte nuclei become excessively large and begin to lose nuclear envelope as the cells enter diakinesis of meiosis I.
Stage XII. In this stage the most important identifying feature is the presence of meiotic and secondary spermatocytes. Step 12 spermatid nuclei are thinner and nuclear staining is intensely dark throughout except for the most caudal region. PAS+ acrosomic system forms a ventral and dorsal fin over the apical protrusion.

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Table 1. Number of differentiated spermatogonial generations and germ cell ratios[1].

Species	Spermatogonial generations	Meiotic index (%)[2]	Overall rate of spermatogenesis[3]
Bull	6 (A_{1-3}, In, B_{1-2})[4]	3.6 (10)[5]	65 (75)
Buffalo	6 (A_{1-3}, In, B_{1-2})	3.4 (15)	74 (71)
Ram	6 (A_{1-3}, In, B_{1-2})	3.1 (23)	37 (85)
Goat	6 (A_{1-3}, In, B_{1-2})	2.8 (30)	91 (65)
Boar	6 (A_{1-4}, In, B)	3.2 (20)	68 (73)
Peccary	6 (A_{1-4}, In, B)	3.2 (20)	74 (71)
Wild boar	6 (A_{1-4}, In, B)	2.7 (33)	29 (89)
Dog	6 (A_{1-4}, In, B)	3.4 (15)	51 (80)
Rat	6 (A_{1-4}, In, B)	3.4 (15)	97 (62)
Mouse	6 (A_{1-4}, In, B)	2.3-3.1 (23-43)	44-84 (67-83)
Gerbil	5 (A_{1-3}, In, B)	2.8 (30)	34 (73)
Capybara	5 (A_{1-3}, In, B)	2.1 (48)	21 (84)
Agouti paca	5 (A_{1-3}, In, B)	3.2 (20)	31 (76)
Dasyprocta sp	5 (A_{1-3}, In, B)	3.0 (25)	28 (78)
Chinchilla	5 (A_{1-3}, In, B)	3.0 (25)	49 (62)
Jaguar	5 (A_{1-3}, In, B)	2.8 (30)	45 (65)
Cat	5 (A_{1-3}, In, B)	2.8 (30)	19 (85)
Rabbit	5 (A_{1-2}, In_{1-2}, B)	3.3 (18)	39 (69)
Marmoset	4 (A_{1-2}, B_{1-2})	3.4 (15)	25 (60)
Man	2 (A_{pale}, B)	1.3 (68)	3.2 (80)

[1] Data from our laboratory and compiled from the literature (see reviews in França and Russell, 1998; França et al, 2002; França et al, 2005). [2] Number of spermatids per each primary spermatocyte. [3] Number of spermatids formed per each differentiated type A_1 spermatogonia. [4] Type A spermatogonia (A); intermediate spermatogonia (In); and type B spermatogonia (B). [5] Numbers in parentheses show the percentage of germ cell loss based on the theoretical yield.

specific stages of spermatogenesis will typically rely on the acrosomal system observed in the round spermatids, rather than in the elongate cells. These spermatid steps also involve condensation of the chromatin, as the chromosomes are packed more tightly and stain more intensely with hematoxylin.

Maturation

Maturation steps 15-16 appear across Stages III-VIII and show fewer changes in nuclear shape and acrosomal migration. The nucleus continues to condense and the acrosome matures into a thin PAS+ structure that protrudes at the apex but covers nearly all the nucleus, except for that portion connected to the tail.[4] Excess cytoplasm is removed in Stages VII-VIII, resulting in the formation of prominent cytoplasmic lobes and residual bodies, which contain unused mitochondria, ribosomes, lipids, vesicles and other cytoplasmic components.[4,25,26]

The Cycle and Wave of Spermatogenesis

Germ cells within each layer of the seminiferous epithelium change in synchrony with the other layers over time, producing the sequence of Stages described above (Fig. 2). The cells do not migrate laterally along the length of the seminiferous tubule; however, an unusual successive order of the Stages is observed, whereby sequential Stages occur with repetition along the length of the tubules, in a 'wave' of the seminiferous epithelium.[27] That is, at least in the

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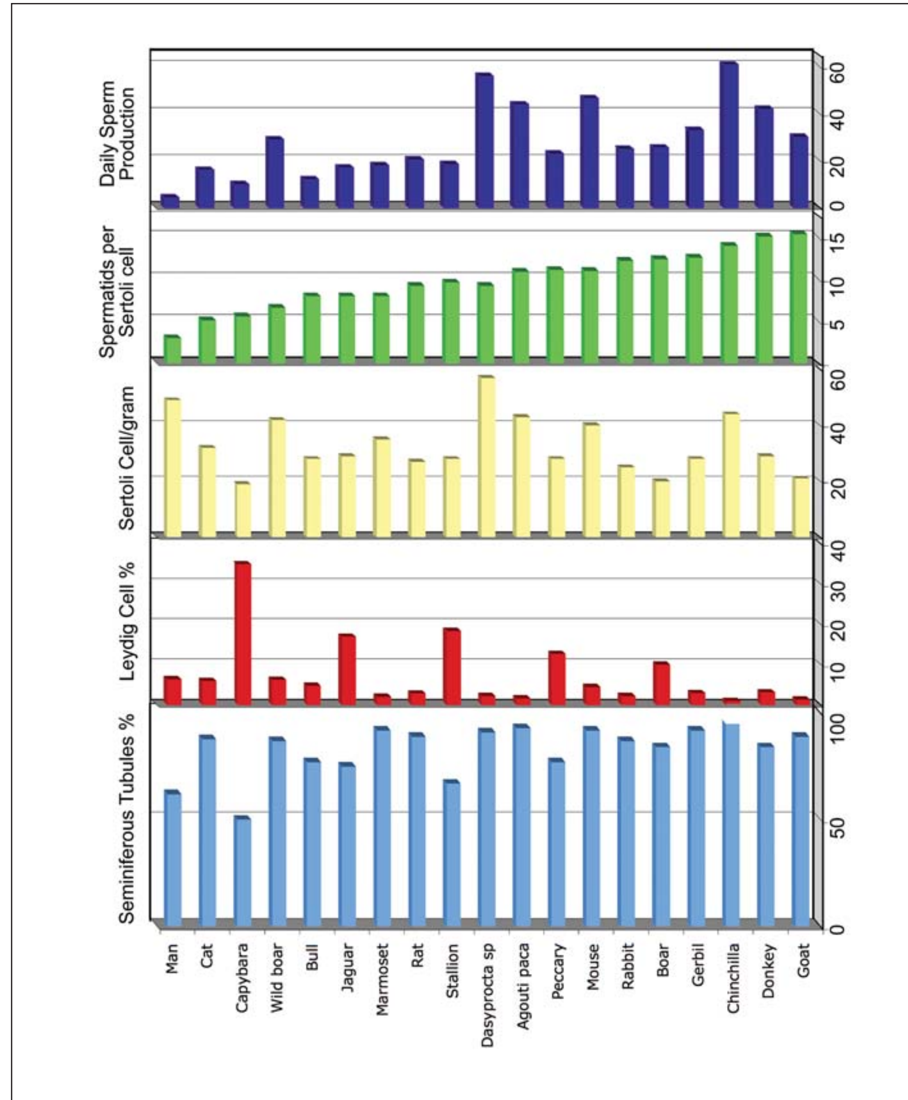

Figure 3. Comparative species testicular data for percentage of seminiferous tubules (%), Leydig cell or interstitial space (%), Sertoli cells (millions)/gram of testis parenchyma, spermatids per Sertoli cell, and daily sperm production per gram of testis (millions).

rodent, Stage I is followed by II, followed by III, etc. through Stage XIV, which is then repeated by Stage I. The Stages are found in ascending order from the rete testis to the center of the seminiferous tubule, where the Stages are reversed.[4] The wave is produced by synchronous development of clonal units of germ cells through a mechanism of biochemical signaling that remains a subject of inquiry.

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Sperm Production

The precise mechanisms by which spermatogonial stem cells (A_s) and other early proliferative spermatogonia (A_{pr}-A_{al}) transform into differentiating spermatogonia (type A, In, and type B) and simultaneously renew their own population is now a major focus of reproductive biology.[10-12,16-18,28-46] In addition to c-kit and vitamin A, which are important for differentiation of A_{al} into A_1, other important factors are emerging as being involved in the regulation of spermatogonial stem cells. These proteins include the following: GFRα1, PLZF, OCT4, NGN3, NOTCH-1, SOX3, c-RET, RBM, EP-CAM, STRA8, and EE2.[7,10,17,31,47,48]

Spermatogonia give rise to spermatocytes after a fixed number of mitotic divisions that are characteristic of each species,[49] as two to six differentiated spermatogonial generations have been observed in mammals (Table 1). Besides being useful for comparative studies among different species,[49] the precise knowledge of the number of spermatogonial generations is essential for better understanding of regulatory mechanisms of spermatogenesis.[7] Compared to many other well-known self-renewing cell systems in the body, spermatogenesis is thought to have the greatest number of cell divisions during its expansion. For instance, in mice, rats, and pigs, about ten generations of spermatogonia are necessary to form preleptotene spermatocytes from one spermatogonia stem cell ($A_s \rightarrow A_{pr} \rightarrow A_{al4} \rightarrow A_{al8\text{-}16} \rightarrow A_1 \rightarrow A_2 \rightarrow A_3 \rightarrow A_4 \rightarrow In \rightarrow B$); whereas in humans this figure is much lower and estimated to be only 4 mitotic divisions. As will be shown later, both the kinetics and rate of germ cell loss have an impact on the number of sperm produced.

Knowledge of the spermatogenic cycle length is fundamental for determining the spermatogenic efficiency and performing comparative studies among species. The total duration of spermatogenesis based on 4.5 spermatogenic cycles ranges from approximately 30 to 78 days in mammals (8.6-8.9 for each cycle and 39-40 days for total duration in mice) (see reviews by refs. 4,23,50-51), and is under the control of the germ cell genotype, according to a study using xenogenic (rats to mice) spermatogonial transplantation.[52] Also, similar results were found utilizing porcine and ovine testis xenografts.[53] Although strain or breed differences can be found among members of the same species, the length of the spermatogenic cycle has been generally considered to be constant for a given species and is not phylogenetically determined. However, it is suggested in the literature that the temperature and some drugs may influence the duration of spermatogenesis,[54-56] probably altering the cell cycle.[57,58] In most mammals, each spermatogenic cycle lasts around 9 to 12 days, whereas the total duration of spermatogenesis lasts nearly 40 to 54 days. Particularly in humans, the entire spermatogenic process is very long and lasts more than 70 days. As a general pattern for mammals, and probably related to the synchronized development of different germ cell types per seminiferous tubule cross-sections (Stages), each phase of spermatogenesis (spermatogonial, spermatocyte, and spermatid) lasts approximately one third of the duration of the entire process.

Germ cell loss (apoptosis) occurs normally during spermatogenesis in all mammals investigated,[59] playing a critical role in determining total sperm output. However, the greatest influence on germ cell production is the capacity for mitosis, and the number of generations of spermatogonial divisions, which will dictate, at least in part, the number of cells that enter meiosis. Taking into account the number of generations of differentiated spermatogonia and the two meiotic divisions prior to the formation of haploid spermatids, only 2-3 spermatozoa out of 10 are produced from each differentiated type A_1 spermatogonia in most mammalian species (see overall rate of spermatogenesis in Table 1).[23,60] Thus, significant germ cell loss occurs during the spermatogonial phase, called 'density-dependent regulation', primarily during mitotic divisions of type A_2 to A_4 spermatogonia, possibly mediated by the p53 tumor suppressor protein, as well as Bcl-2, Bax and Fas. One possibility, as yet untested, is that the degeneration is a homeostatic mechanism to limit germ cells to the number that can be supported by available Sertoli cells. Apoptosis is also frequent during meiosis (Table 1), especially in humans, and is probably related to chromosomal damage. Also, it should be mentioned that

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missing generations of spermatocytes and spermatids in the seminiferous epithelium, plus apoptosis, contribute to the low efficiency of human spermatogenesis.[60,61]

The Sertoli cell has several important roles in spermatogenesis, including the following: support and nutrition of the developing germ cells; compartmentalization of the seminiferous tubule by tight junctions, which provides a protected and specialized environment for the developing germ cells; controlled release of mature spermatids into the tubular lumen (spermiation); secretion of fluid, proteins and several growth factors; and phagocytosis of the degenerating germ cells and phagocytosis of the excess cytoplasm (residual body) that remains from released sperm.[2] The Sertoli cell also mediates the actions of FSH and luteinizing hormone (LH)-stimulated testosterone production in the testis, apparently in a stage-dependent manner.[62] Although it is strongly suggested that FSH plays a major role in the initiation, maintenance and restoration of spermatogenesis in primates, it appears that in most mammalian species testosterone has this important role in maintaining *'quantitatively'* normal spermatogenesis, whereas FSH plays a qualitative role and is not strictly necessary for fertility.[62] Recent investigations of the Sertoli cell specific knockout of androgen receptor (SCARKO) mouse found that spermatogenesis rarely advanced beyond diplotene spermatocytes.[63] Thus, at least in this species, androgens are crucial for late meiosis and spermiogenesis.

The relative mass of seminiferous tissue determines how much space is devoted to sperm production. In general, species whose testes have a high proportion of seminiferous tubular tissue produce more sperm per unit mass (Fig. 3).[23,50,60] Regardless of other factors, the number of Sertoli cells is now well established as being one of the most important determining factors that defines maximum sperm production.[64-73] In all mammalian species investigated, no Sertoli cell proliferation has been observed after puberty. Thus, the perinatal and prepubertal period, when the size of the Sertoli cell population is established, ultimately dictates the magnitude of testis size and sperm production. This occurs because Sertoli cells have differing capacities to support germ cell development and each Sertoli cell is able to support only a relatively fixed number of germ cells in a species-specific manner (Fig. 3).[23,50,60] Thus, animals with more Sertoli cells have more germ cells per testis, and the number of Sertoli cells per gram of tissue combined with the number of spermatids per Sertoli cell is positively correlated with sperm production per gram of testis.

There also appears to be species-specific regulation of the total Sertoli cell population.[66,71,74-84] Volume density of Sertoli cells in the seminiferous epithelium changes considerably in mammals (from ~15% in mice to ~40% in humans) and is inversely related to the efficiency of sperm production. Thus, in contrast to humans, species with a lower proportion of Sertoli cells in the seminiferous epithelium, such as mice, rabbits, rats, hamsters, and pigs are among those with the highest Sertoli cell and spermatogenic efficiencies (Fig. 3).[23]

Daily sperm production per gram of testicular parenchyma is a measure of spermatogenic efficiency in sexually mature animals and is useful for species comparisons. In mammalian species, four to sixty million spermatozoa are produced daily per gram of testis tissue (Fig. 3), and in humans for instance approximately 1,500 spermatozoa are produced with each heartbeat. Usually, species that have shorter spermatogenic cycle lengths have higher spermatogenic efficiency (Table 1; Fig. 3). However, the higher efficiency of spermatogenesis observed in some mammalian species results from the combination of higher Sertoli cell support capacity for germ cells and greater number of Sertoli cells per gram of testis. Data shown in (Fig. 3) for the domestic boar and wild boar illustrate this assumption, because the lower Sertoli efficiency observed for the wild boar is compensated for by the higher Sertoli cell number per gram of testis, resulting in similar daily sperm production per gram of testis in both species. Higher seminiferous tubule volume density (%) in the testis, lower Sertoli cell volume density (%) in the seminiferous epithelium, greater number of spermatogonia generations, and lower germ cell loss during spermatogenesis, also correlate significantly with spermatogenic efficiency.

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Regulation of the Cycle

Stages in the cycle of the seminiferous epithelium are established early in the postnatal period. For example, cellular associations suggesting specific stages have been found as early as day 10,[85] which is about the same time that androgen receptors (AR) begin to be expressed in early Sertoli cells,[86] suggesting that Sertoli cells regulate the formation of stages. Transplantation data also support this conclusion. Using the green fluorescence protein mouse (GFP), the same stage of spermatogenesis was observed throughout a single colony, although different colonies were in different stages, 2 months after transplantation.[87] After 3 months, the colonies were much larger and some had merged into a single colony. Most interestingly, these merged colonies exhibited synchronization, as the entire colony became one stage. It was suggested that the transplanted germ cells were probably sensitive to Sertoli cell factors that caused the fused colonies to become one stage.[87]

Another animal model to address the establishment of stages and cycles is the vitamin A deficient rat, which results in an arrest of spermatogenesis, with type A1 spermatogonia differentiation inhibited.[88-91] Resupplementation with retinol re-establishes spermatogenesis, but the seminiferous epithelium throughout the entire testis is synchronized within 2-3 stages. Synchronization was found to be stable for more than 10 cycles of the epithelium[92] and repopulation of the epithelium appeared to be due primarily to the completion of mitotic activity by type A1 spermatogonia, which were arrested in the G2 phase of their cycle.[88] Thus, in this model, it appears that the regulation involves both Sertoli and germ cell responses to vitamin A. Sertoli cells appear to maintain the correct stages, although synchronized, over time, while the germ cells respond to retinol to continue the correct cellular cycle by completion of G2. An investigation of the retinoic acid receptor knockout mouse (*RAR*α-/-) further revealed that vitamin A may be involved in the initial establishment of stages and their long-term regulation, which also appears to be stage-specific, as the first wave of spermatogenesis was arrested at step 8-9 spermatids and preleptotene and leptotene spermatocytes in stage VIII-IX were delayed in the first three waves.[93]

Sertoli cells do appear to regulate the cellular associations or 'stages' within the epithelium; therefore, it has been logical to hypothesize that Sertoli cells may also regulate the 'duration of the cycle of the seminiferous epithelium'. Morphological intimacy between Sertoli and germ cells was first observed in the 19th Century[1] and today we know that up to 50 different germ cells may contact a single Sertoli cell and that a single germ cell can be associated with several Sertoli cells.[94] Based upon this 'Mother cell' concept, early studies using transplant technology[95] hypothesized that if rat germ cells were transplanted into the mouse testis, the rat germ cells may acquire the mouse testis duration of the cell cycle, 8.6 days versus 12.9 days for the rat.[96,97] However, a subsequent experiment demonstrated "the complete domination of rat germ cell genotype in differentiation timing."[52] Thus, it appears that the germ cell determines duration of the spermatogenic cycle, while the resident Sertoli cell is responsible for maintenance of cellular associations or stages through the production and secretion of important factors and providing proper physical and functional environment for spermatogenesis development.[3,98,99]

It has been known for many years that the first wave of spermatogenesis proceeds faster than does the adult seminiferous epithelial cycle. Stage frequency in cross sections is the same on days 13, 23, 30 and in adult mouse testes[100] and the same was found in the rat;[101] however, mean duration of the cycle from 10 to 30 days was approximately 1 day shorter than in the adult rodents.[85,102,103] When germ cells from the GFP mouse were transplanted into the adult testis, the rate of growth was 2x faster during the first 2 months compared to the third month post transplant.[87] It is often pointed out that this reduction in the rate of the spermatogenetic cycle during development is correlated with testicular descent; therefore, it is possible that a higher intra-abdominal temperature may result in acceleration of the cell cycle and mitotic events, as observed in fish (tilapias) maintained at elevated temperatures.[58] Although this explanation may have some credibility, other data suggest that the first wave may be different

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simply because the spermatogonia are filling clonal niches and establishing an epithelial wave. For example, the first wave has a unique regulation that is dependent on a subpopulation of neurogenin 3 (Ngn3) negative spermatogonia that differentiate into the first wave germ cells, while Ngn3+ cells are reserved for stem cells and subsequent waves of spermatogenesis.[28]

Finally, there are numerous studies showing stage and Sertoli cell specific expressions of proteins and it appears that different stages have different dependences upon androgens and FSH, with these factors having a greater influence just before spermiation in stage VII-VIII.[62,104-109] However, understanding the individual contribution of factors to the maintenance of the cycle and stages of spermatogenesis will require careful analysis and interpretation, because disruption of individual factors will often lead to an initial stage-specific and/or cell-specific effect, but the long term consequence is secondary degeneration of the entire process of spermatogenesis.[110,111] This very important aspect of mammalian spermatogenesis is still poorly understood and should be a focus of intensive research in the coming years, mainly because appropriate animal models are now available for dissecting molecular regulation of the cycle of the seminiferous epithelium.

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