Zagazig Veterinary Journal, ©Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt.
Volume 53, Number 3, p:231-255 September 2025
DOI: 10.21608/zvjz.2025.347486.1275

Tracking the Cellular Journey of Spermatogenesis in Rats

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ARTICLE INFO

Article History:

Received: 25 December 2024
Accepted: 10 March 2025
Published online: 30 September 2025

Key words:

Seminiferous tubules, Spermatogenic cycle, Testes, Male infertility, Endocrine regulation

ABSTRACT

Spermatogenesis in rats is a well-coordinated and complex process that occurs within the seminiferous tubules of the testicular tissue. One complete seminiferous epithelium cycle would last 13 days; the whole process of spermatogenesis is last 56 days in rats and consists of a series of stages, each corresponding to a distinct phase in germ cell development. This process includes the proliferation and differentiation of spermatogonial stem cells, meiosis, and spermiogenesis. Spermatogenesis is regulated by a sophisticated interplay of hormones, primarily controlled by the hypothalamic-pituitary-gonadal axis. Luteinizing hormone stimulates Leydig cells in the testes to produce testosterone, a hormone essential for the maturation of spermatocytes and spermatids within the seminiferous tubules. Follicle-stimulating hormone targets Sertoli cells, which help maintain the structure of the seminiferous epithelium and facilitate the progression of spermatogenesis by supplying nourishment and secreting of inhibin, activin, follistatin, androgen-binding protein, and other regulatory factors that participate in the feedback mechanisms that control gonadotropin secretion. Disruptions in the hormonal balance can lead to impaired spermatogenesis and male infertility. Therefore, a thorough understanding of the 14-stage spermatogenic cycle and its hormonal regulation is crucial for elucidating the mechanisms underlying male fertility. Such knowledge provides valuable insights into male reproductive physiology and can guide the development of therapeutic interventions for fertility disorders.

Introduction

Male fertility depends greatly on the proper progression and successful completion of spermatogenesis. In mammals, spermatogenesis is a complex, highly coordinated, and unidirectional sequence of cellular events that leads to the complete development of germ cells within the seminiferous epithelium of the adult testis [1]. Spermatogenesis aims to produce the most specialized cell type in the body, the spermatozoa. This intricate process ensures the formation of mature, functional sperm through a series of carefully regulated stages, culminating in fully differentiated sperm capable of fertilizing an egg.

The regulation of spermatogenesis is a complex and tightly controlled process, primarily governed by the hypothalamic-pituitary-gonadal (HPG) axis, which orchestrates the hormonal signals necessary for sperm development. Key hormones, such as luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone, are crucial for the maturation of germ cells [2]. Sertoli cells also produce regulatory factors, including inhibin, activin, follistatin, and androgen-binding protein, which help regulate gonadotropin secretion through feedback mechanisms [3].

The interaction between Sertoli cells, germ cells, and hormones is central to the successful progression of spermatogenesis. Any disruption in hormonal regulation can lead to impaired spermatogenesis and infertility [4]. Therefore, understanding the intricate hormonal regulation of the spermatogenic cycle as well as the role of Sertoli cells is essential for advancing knowledge of male reproductive physiology and fertility issues. Insights into the spermatogenic cycle and its regulation in rats are valuable for improving fertility treatments in animal models and have significant implications for human reproductive medicine.

The present review aims to provide an in-depth understanding of the spermatogenic cycle and its hormonal regulation in rats, particularly focusing on the role of Sertoli cells and their interaction with germ cells. Insights into this complex process in rats will help advance fertility research, offering valuable implications for enhancing fertility treatments in animal models and improving human reproductive medicine.

1. Spermatogenesis

This process consists of three distinct phases in which specific morphological and biochemical alterations in the nuclear and cytoplasmic components of the germ cells mark each phase. The process begins with the mitotic proliferation and differentiation of spermatogonia, then the meiotic division of spermatocytes, followed by spermiogenesis, and concluding with spermiation [1] (Figure 1).

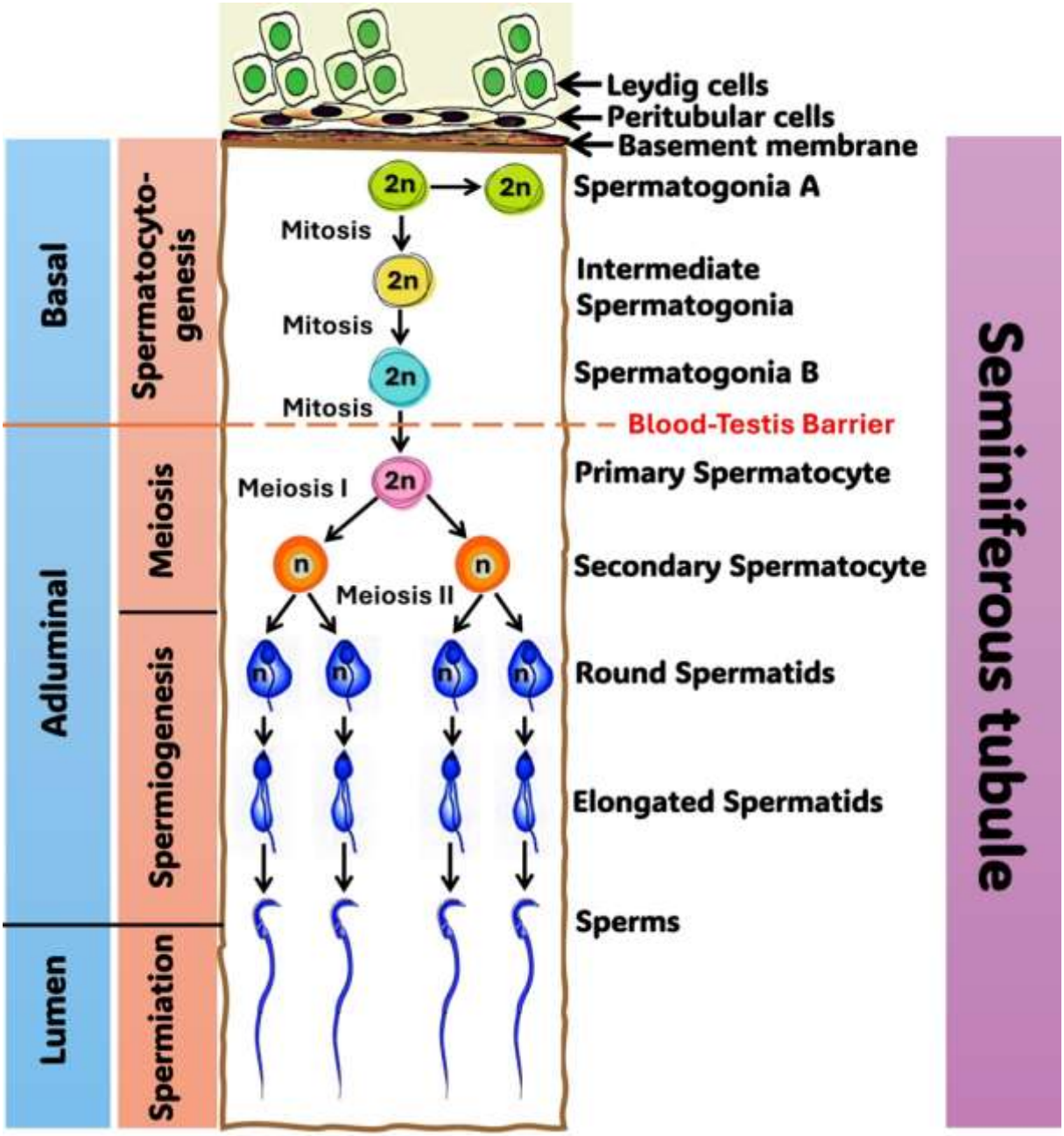

Figure 1. Schematic illustration of spermatogenesis in the rat seminiferous tubules

2-1- Spermatocytogenesis

This phase, often referred to as the "proliferation and renewal" phase of spermatogenesis, is the initial stage where spermatogonial stem cells, exist at the basal membrane of the seminiferous tubules, undergo mitotic division [1]. This process produces identical spermatogonia that either self-renew or differentiate into two diploid primary spermatocytes. The spermatogonial stem cells divide multiple times, generating a series of type A spermatogonia.These type A spermatogonia then differentiate into

intermediate type spermatogonia, eventually developing into type B spermatogonia. Type B spermatogonia goes through mitotic division to form primary spermatocytes [1, 5] (Figure 1). Proper regulation of this balance between self-renewal and differentiation is essential for normal spermatogenesis. Disruptions in spermatogonial stem cells' self-renewal or differentiation can lead to impaired spermatogenesis and male infertility [6].

2-2- Meiosis

The process by which diploid primary spermatocytes undergo two successive divisions, Meiosis I, and Meiosis II, to produce haploid cells [1]. Therefore, this phase involves genetic recombination and two meiotic divisions that quadruple the cell number. During meiosis I, the primary spermatocytes are divided into two secondary spermatocytes each containing half the chromosome number (haploid). In meiosis II, each secondary spermatocyte divides again, resulting in four haploid spermatids (Figure 1).

2-3- Spermiogenesis

Spermiogenesis is the final stage of spermatogenesis, occurring post-meiotically, where haploid round spermatids undergo a series of intricate morphological, biochemical, and histochemical transformations to become elongated, fully differentiated spermatozoa [7]. This transformation from a round, immature spermatid into a highly specialized spermatozoon, ready for fertilization, takes place in the seminiferous epithelium before the spermatozoa are discharged into the seminiferous tubules' lumen (Figure 1). Once haploid round spermatids are formed, their nuclear and cytoplasmic structures undergo significant reorganization, leading to the development of highly specialized and differentiated spermatozoa. These changes ensure the development of fully functional sperm cells, equipped for fertilization.

Spermiogenesis involves several key steps as reviewed in many literatures [1, 7] including:

(1) Nuclear Condensation: The nucleus of the spermatid undergoes significant condensation, becoming compact and streamlined to fit into the head of the spermatozoon. This helps to ensure that the genetic material is efficiently delivered during fertilization.

(2) Acrosome Formation: The acrosome, a specialized structure that encases the front part of the sperm's head, is generated from vesicles created by the Golgi apparatus. It contains enzymes that are crucial for penetrating the egg's protective layers during fertilization.

(3) Flagellum Development: A centriole elongates to form the tail of the sperm, which provides motility. The flagellum structure, made up of microtubules, begins to form as the spermatid elongates, allowing the spermatozoon to swim toward the oocyte.

(4) Cytoplasmic Remodeling: As the spermatid matures, excess cytoplasm is discarded and phagocytized by Sertoli cells, which provide the growing sperm cells with nourishment and support.

2-4- Spermiation

This process marks the end of spermiogenesis, where the spermatozoa, now fully differentiated, detach from the supportive Sertoli cells, release into the lumen of the seminiferous tubules, and become ready to enter the epididymis for further maturation [7] (Figure 1). Spermiation is a prolonged and intricate

process that unfolds over several days (approximately 82 hours in rats) [8].

Spermiation involves a series of coordinated events, including the removal of excess cytoplasm around the spermatid's flagellum, the projection of the spermatid into the lumen, and the gradual breakdown of specialized adhesion structures. It also includes the formation and degradation of tubulobulbar complexes, which aid in the detachment of the spermatid from the Sertoli cell, allowing the spermatozoa to move freely within the tubule in preparation for their journey to the epididymis [8].

The progression of spermatogenesis phases occurs in a highly controlled manner, both in terms of timing and location. The differentiation of spermatogenic cells within these phases is carefully coordinated within and between the germ cell syncytia found in distinct regions of the seminiferous tubules. This ensures that the various stages of spermatogenesis occur in a highly organized manner to ensure the production of healthy, functional sperm capable of fertilization. Disruptions at any stage can lead to male infertility.

2-5- The duration of spermatogenesis

This duration is specific to each species and tends to remain fairly consistent within a given species. For example, it lasts about 56 days in Sprague-Dawley rats, 34.5 days in mice, 35 days in hamsters, 43 days in rabbits, 49 days in bulls, and 64 days in humans [9].

1- Seminiferous tubules

3-1- Overview

In rats, the seminiferous tubules are long and intricately coiled, with about 30 separate tubules [1]. These tubules are folded in such a way that they extend roughly 12 meters in length for every gram of testicular tissue [1]. The general organization of the seminiferous epithelium is consistent across all male mammals. This epithelium is structured into tubules composed of three main cell types:

(1) Stromal peritubular cells, which reside along the outer boundary of the tubule.

(2) Sertoli cells, highly branched cells emerging from the basement membrane to the lumen of tubule, where they are essential in organizing and supporting the spermatogenic process.

(3) Germ cells, which include spermatogonia, spermatocytes, and spermatids (Figure 1). These germ cells undergo a series of differentiation steps to produce mature sperm. The base of the Sertoli cells is separated from the peritubular cells by a basement membrane.

The tubules are situated within interstitial tissue, which is made up of resilient and flexible connective tissue that includes Leydig cells, blood vessels, mast cells, macrophages, lymphatic vessels, fibroblasts, and nerves.

3-2- Peritubular Cells

The seminiferous tubules are encircled by flat mesenchyme-derived cells known as peritubular myoid cells (Figure 1). They perform several essential functions including regulating Sertoli and Leydig cells through paracrine signaling and providing contractile properties to the seminiferous tubules to facilitate the release and transport of sperm toward the rete testis [1]. Peritubular cells release an androgen-regulated factor that contributes to the onset and sustainment of Sertoli cell differentiation. In addition, these cells secrete other paracrine factors, including

transforming growth factors and insulin-like growth factor I, which help regulate the function of both Sertoli and Leydig cells [10].

3-3- Sertoli cells

Sertoli cells, the somatic cellular constituent of the seminiferous epithelium, are important in germ cell development. In rat seminiferous epithelium, Sertoli cell proliferation stops and their maturity begins between birth and sixteenth day postpartum [11]. This period frame aligns with the onset of spermatogenesis and beginning of the first wave of germ cell maturation [12]. The number of Sertoli cells are strongly correlated with numbers of spermatogonia, counts of spermatid, and rates of spermatozoa production in various species, including rats. Normal Sertoli cell activities are essential for maintaining seminiferous epithelial integrity. Consequently, any disturbance in these functions can compromise spermatogenesis, leading to a reduction in sperm production. Sertoli cells carry out several vital roles, such as offering structural support and nourishment to germ cells (such as supplying lactate as an energy source), assisting in the spermiation of mature spermatids, helping in the transport of developing germ cells, and engulfing degenerated germ cells as well as residual bodies from released spermatozoa. Additionally, Sertoli cells secrete luminal fluid and proteins, establish the blood-testis barrier, and facilitate communication between cells. The blood-testis barrier, essential for spermatogenesis, is created by tight junctions between neighboring Sertoli cells [12]. This barrier is one of the most selective in the mammalian body, effectively separating the seminiferous epithelium into basal and adluminal compartments [12] (Figure 1). The seminiferous epithelium is divided into basal and adluminal compartments by Sertoli cell junctional complexes. Spermatocytogenesis takes place in the basal compartment, where preleptotene primary spermatocytes are generated. After their formation, these primary spermatocytes move through the blood–testis barrier into the adluminal compartment, where meiosis and spermiogenesis take place. The blood–testis barrier protects spermatocytes and spermatids from components of the serum, providing crucial immunological defense for these germ cells. Additionally, the blood–testis barrier is vital for maintaining the unique environment within the seminiferous tubules by preventing the loss of crucial substances like androgen-binding protein, inhibin, and enzyme inhibitors from the luminal compartment [12]. As germ cells mature, they move through the seminiferous epithelium, a process that requires the remodeling of both Sertoli-germ cell junctions and Sertoli-Sertoli cell junctions at the blood-testis barrier.

3-4- Germ Cells

The testicular seminiferous epithelium contains various germ cell types, including spermatogonia, spermatocytes, spermatids, and spermatozoa (Figure 1).

3-4-1- Spermatogonia

They are the stem cells of the male germ cell lineage, located on the basement membrane of the epithelial wall of the seminiferous tubules of the testes, though not directly attached to it. They are essential for the continuous production of sperm, ensuring both the renewal of stem cells and the initiation of spermatogenesis to produce mature sperm throughout a male's reproductive life. Spermatogonia are categorized into three types: stem cell spermatogonia (Type A),

which self-renew and maintain the population of spermatogonia; proliferative spermatogonia (Type B), which undergo mitotic divisions to expand the germ cell pool; and differentiating spermatogonia, which begin the process of differentiation and enter meiosis to become primary spermatocytes [9].

Models of spermatogonial renewal in the rat

Researchers proposed that undifferentiated Type A spermatogonia are the only true spermatogonial stem cells. These stem cells either divide into two single cells (As) or into paired Type A cells (Ap). The Ap spermatogonia then divide successively, forming chains of up to 32 aligned Type A spermatogonia (Aal). Although these three types of undifferentiated spermatogonia (As, Ap, and Aal) are morphologically identical and indistinguishable by light microscopy in histologically stained testicular cross-sections, the Aal spermatogonia undergo further differentiation. This process involves morphological changes and the transformation into Type A1 spermatogonia, which initiate a series of four consecutive mitotic divisions [13].

It had been proposed an alternative model of spermatogonial renewal in the rat, suggesting that four sequential stages of spermatogonia Type A (A1 to A4) are recognized within the population of differentiating spermatogonia. Each generation is derived from the previous one via mitotic division. Spermatogonia (A4) subsequently undergo division to form intermediate spermatogonia, which eventually differentiate into Type B spermatogonia [14].

The different types of spermatogonia also vary in their nuclear heterochromatin distribution. Type Type A spermatogonia feature a pale nucleus with a delicate, "dusty" pattern of hetero-chromatin distributed across it [15]. Type B spermatogonia show a more distinct pattern, with packed clusters of heterochromatin accumulated at the nucleus periphery. Intermediate spermatogonia exhibit a more intermediate appearance, with a heterochromatin distribution that combines features of both Type A and Type B cells. [15].

3-4-2- Spermatocytes

Pre-leptotene spermatocytes originate from the last mitotic division of Type B spermatogonia. Following this, these cells proceed into an extended phase of meiosis, lasting about three weeks in rats, during which they progress through the leptotene, zygotene, pachytene, and diplotene/diakinesis stages [1]. Following this, they undergo two meiotic divisions, the first division generates diploid secondary spermatocytes, and the second meiotic division results in the formation of haploid spermatids [1].

At the preleptotene stage, spermatocytes start to migrate away from the basal membrane and pass through the tight junctions between neighboring Sertoli cells, entering the adluminal compartment of the seminiferous tubule. During the leptotene stage, the chromosomes begin to condense, developing into thin, fragile filaments [1]. As the process advances into zygotene, homologous chromosomes converge and align in the nucleus, creating distinct tripartite formations known as synaptonemal complexes. Chromosomes in the pachytene stage underwent further condensation and thickening, with each chromosome splitting into a pair of chromatids joined at the centromere. In rats, this phase is particularly extended for about 12 days [1]. Throughout pachytene,

both cellular and nuclear volumes significantly increase as the chromosomes undergo further condensation and recombination.

3-4-3- Spermatids

The two meiotic divisions of each spermatocyte yield four haploid spermatids. These spermatids then undergo spermiogenesis. The round spermatids closely resemble secondary spermatocytes but with a slight reduction in nuclear size. This reduction is a direct result of the halving of the genetic material during meiosis, signaling the transition from diploid to haploid cells. The development of the acrosomal material, as observed through the periodic acid-Schiff (PAS) staining, is a crucial marker for identifying the stages of spermatid differentiation under light microscopy. Round haploid spermatids are distinguished by the presence of a perinuclear acrosomal vesicle, which becomes visible upon PAS staining [1].

Spermatids progress through four distinct morphological stages: 1) the Golgi phase, 2) acrosome development, 3) tail formation, and 4) maturation [1, 16]. In the Golgi phase, pro-acrosomic granules produced by the Golgi apparatus merge to create a single acrosomal granule. This granule is contained within a vesicle enclosed by a membrane, positioned adjacent to the lumenal side of the spermatid's nucleus. The cap phase begins when a protrusion forms from the acrosomal vesicle, gradually developing into a cap-like structure that covers almost half of the nucleus' circumference [1, 16]. At the same time, the chromatoid body shifts to the opposite side of the nucleus, positioning itself near the two centrioles, where the flagellum will eventually form. The acrosomal area of the nucleus moves towards the seminiferous basement in the acrosome phase. Concurrently, the chromatin condenses, and eventually, the nucleus shapes the sperm head. The spermatid's cytoplasm is reorganized, shifting toward the apical side of the cell, opposite the nucleus. Within this cytoplasm near the lumen, the manchette assembly forms a tubular microtubule structure that attaches to the nucleus and encircles the basal region of the developing tail [1, 16]. This flagellum then elongates, forming the midpiece and tail segments of the spermatozoon, which are essential for motility. Mitochondria, which provide energy for sperm movement after ejaculation, migrate to encase the flagellar fibers, forming a sheath that extends from the midpiece to the annulus, a ring-shaped formation partway down the flagellum [1, 16]. The midpiece is the region that is surrounded by mitochondria. Beyond this midpiece, the nine thick fibers are only encased by the outer fibrous covering, completing the sperm tail structure.

In the final maturation phase of spermiogenesis, immediately prior to the spermatid being expelled to the tubules, its cytoplasmic volume is greatly reduced, and significant cellular restructuring occurs. Excess organelles and mRNA are gathered into a residual body, which is then engulfed by Sertoli cells via phagocytosis during spermiation, marking the completion of the transformation into fully mature spermatozoa.

2- The Spermatogenic Cycle

Spermatogenesis generally takes about 4.5 spermatogenic cycles of the seminiferous epithelium in most mammals [17]. The spermatogenic cycle is a well-organized and controlled process that guarantees the continuous production of sperm. Although the entire process, from spermatogonial stem cells to mature spermatozoa, takes several weeks, different seminiferous tubules are

simultaneously at various stages of the cycle. Understanding this cycle is crucial for research on mammals' fertility, reproduction, and endocrine function.

The coordinated progression of the developed generations of germ cells leads to distinct cell arrangements that follow a precise order. Each complete repetition of this sequence is termed a cycle of the seminiferous epithelium, also known as the spermatogenic cycle, while the individual cellular groupings within it are known as stages. Key morphological changes in the spermatid acrosome, along with other cellular characteristics, serve as important indicators for identifying the stages of spermatogenesis [1]. The presence of these cells helps to distinguish 14 distinct cellular groupings (or stages), which are labeled using Roman numerals from I to XIV (Figure 2).

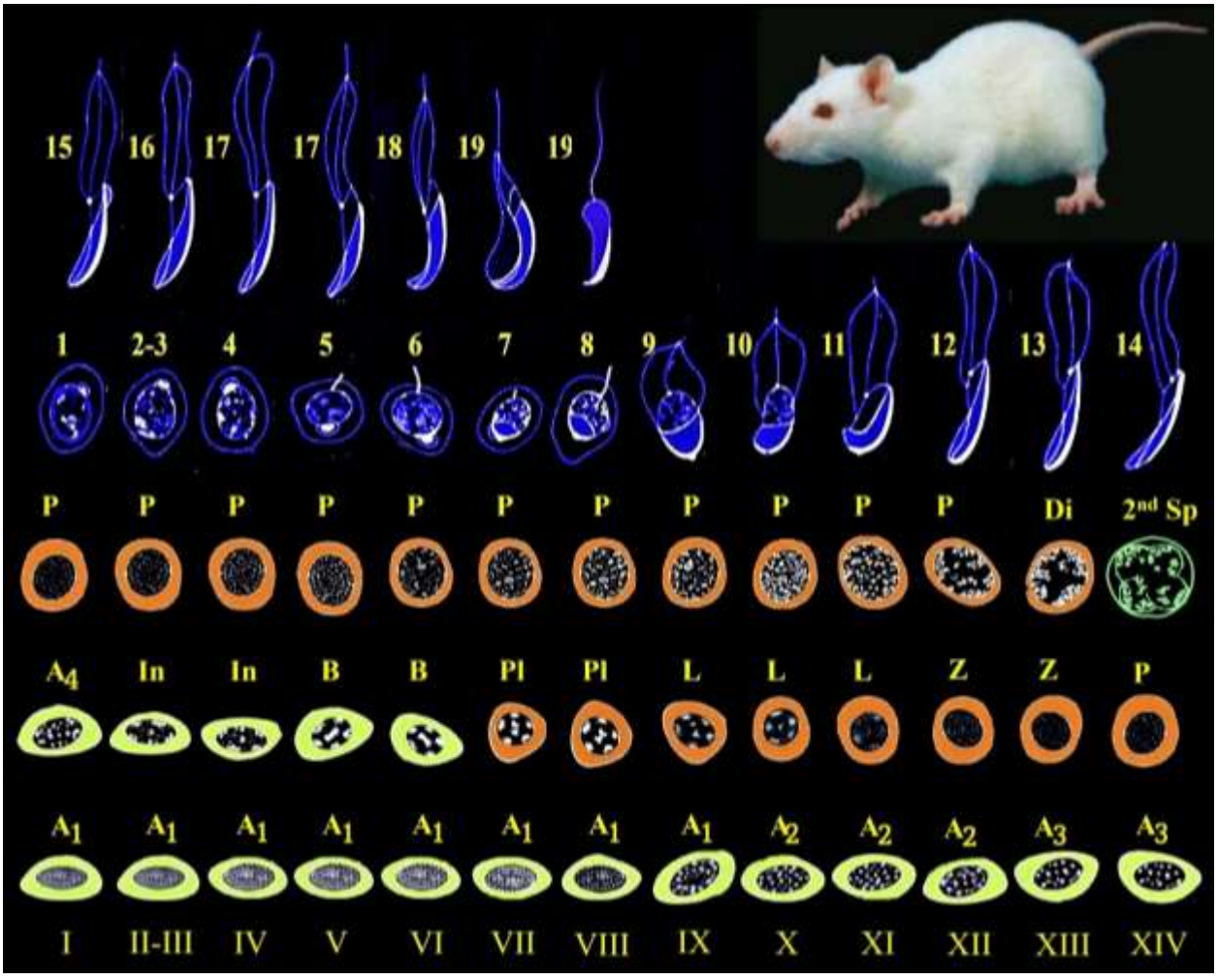

Figure. 2. A schematic representation of the cellular composition in the 14 stages of the spermatogenic cycle within the rat seminiferous epithelium.

Each column represents a stage, numbered with Roman numerals from I to XIV. This framework is primarily based on the morphogenesis of the acrosome of spermatids, progressing along the 19 steps of spermiogenesis (1-19). The various types of germ cells including the spermatogonia [four generations of type A spermatogonia (A1-4), intermediate spermatogonia (In), and type B spermatogonia (B)], followed by the primary spermatocytes [preleptotene (Pl), leptotene (L), zygotene (Z), and diplotene (Di)] and the secondary spermatocytes (2^{nd} Sp) were presented. It is important to note that stage XIV is succeeded by stage I, initiating a new cycle.

The spermatogenic cycle in rats is divided into phases according to the well-established classification scheme of Leblond and Clermont [18], which primarily relies on the morphological development of the spermatid acrosome, easily observed through PAS staining. This scheme identifies 19 distinct steps of spermiogenesis, numbered sequentially from 1 to 19, representing the progressive changes in acrosome formation and other crucial cellular developments during spermatid maturation. The arrangement of these spermiogenic steps determines the 14 stages of spermatogenesis (Figure 2). Stages I-VIII contain two layers of spermatids (one layer of round spermatids and one of elongating spermatids), while stages IX-XIV consist of a single layer of elongating spermatids (Figure 2).

The primary criterion for distinguishing between stages (I-VIII) is the distinct structure of the spermatid acrosome, as observed through PAS staining, while the form and elongation of the nucleus serve as the key characteristic for differentiation between stages IX-XIV [1, 19] (Figure 2). After completing stage XIV, the cycle begins again at stage I, initiating a new cycle. A complete "cycle of the seminiferous epithelium" is represented by the entire series of 14 cellular stages, while the developmental progression from spermatogonia to step 19 spermatid encompasses the process of spermatogenesis.

Species Differences in the Spermatogenic Cycle

The duration of the spermatogenic cycle, and consequently spermatogenesis, remains relatively consistent within a given animal species and strain. The length of each stage can be calculated by dividing the total cycle duration by the percentage occurrence of each stage. The time span of the spermatogenic cycle of the seminiferous epithelium varies across species, with approximately 13 days in rats, 8.6 days in mice, and 16 days in humans [1].

The number of stages within the spermatogenic cycle differs by species, with 14 stages in rats, 12 stages in mice, and 6 stages in humans [1]. Moreover, the frequency of the stages in the cycle of the seminiferous epithelium was determined by Hess et al. [19]. The mean percentage frequencies of each stage in the seminiferous epithelial cycle, as observed in PAS-stained testes of Sprague-Dawley rats, were as follows: 13.7±0.6%, 5.3±0.4%, 2.3±0.2%, 4.9±0.3%, 6.8±0.3%, 7.5±0.4%, 20.9±0.4%, 7.6±0.5%, 3.0±0.2%, 3.2±0.1%, 3.0±0.2%, 8.7±0.2%, 6.2±0.5%, and 6.8±0.3% for stages I to XIV, respectively.

Ultimately, a balance exists between the cycle duration, the total number of spermatogenic cycles, and the capacity for sperm production, which all contribute to the overall sperm count. The length of the

spermatogenic cycle directly influences the sperm production rate. The daily sperm output varies significantly across species [1]. It is approximately 21 $\times 10^6$ sperm/g rat testis, 54×10^6 sperm /g mouse testis, and 3.1- 4.25 $\times 10^6$ sperm /g man testis.

3- Wave of Seminiferous Epithelium

The seminiferous epithelium cycle progresses in a sequential manner across the seminiferous tubule. When examining a specific segment of the tubule at a given moment, the associations of the spermatogenic cycle usually appear in a continuous sequence throughout the tubule, creating a "wave-like" pattern of spermatogenesis in the seminiferous epithelium [1, 20]. This arrangement reflects the continuous and overlapping nature of spermatogenesis, with different stages of germ cell development co-occurring in different tubule regions. Specifically, stage I is followed by stage II, then stage III, and so on, progressing to stage XIV, followed by stage I, starting a new cycle [1, 20]. The stages are organized in ascending order from the rete testis toward the center of the seminiferous tubule, where a reversal site is often located, marking the transition from the end of one cycle to the beginning of the next [1, 20].

As a result, there is a highly organized, spatially, and temporally coordinated progression of stages within the seminiferous epithelium. This wave-like process ensures that spermatogenesis is a continuous and coordinated process, providing a steady output of sperm from the seminiferous tubules. The concept of the wave of seminiferous epithelium is crucial for understanding how sperm production is maintained over time in the testes.

4- Dynamics of the Spermatogenic Cycle

Figures 3-5 represent the findings of our study, which investigated the spermatogenic cellular changes in the seminiferous tubules of rats using Periodic Acid-Schiff staining. In PAS- and hematoxylin-stained cross-sections of control rat testes, the seminiferous tubules were encased by PAS+ basement membranes, with peritubular myoid cells located externally. Interstitial Leydig cells were observed adjacent to the seminiferous tubules. The seminiferous tubules were lined with Sertoli and spermatogenic cells (Figures 3-5). Numerous seminiferous tubules were examined, from the basement membranes to the lumens, to identify all stages occurring during the spermatogenic cycle of the seminiferous epithelium. The 14 distinct stages (I-XIV) of spermatogenesis were identified based on the development of PAS+ acrosomes and head caps (Figures 3-5).

Stage I exhibited type A spermatogonia, followed by primary spermatocytes at the early pachytene stage. Newly formed spermatids at the Golgi phase were also present, along with older spermatids at the maturation stage (Stage 15), characterized by prominent dorsal fins, referred to as immature spermatozoa (Figure 3A).

In Stage II, type A spermatogonia and primary spermatocytes at the pachytene stage were observed, along with newly formed spermatids at the Golgi phase (Stage 2) featuring PAS+ proacrosomic granules. Older spermatids at the maturation stage (Stage 16) showed an extension of the PAS+ acrosome on the dorsal angle of the nuclei, with immature spermatozoa forming bundles toward the basally located Sertoli nuclei (Figure 3B).

Stage III displayed type A spermatogonia, daughter primary spermatocytes, primary spermatocytes at the pachytene stage, newly formed spermatids at the Golgi phase (Stage 3) with PAS+ large solitary acrosomic granules, and older spermatids at maturation stage 16, again forming bundles toward the Sertoli nuclei (Figure 3C).

Stage IV began with the flattening of the acrosomic granule on the nucleus of newly formed spermatids at the cap phase (Stage 4). Type A spermatogonia were observed along the basement membrane, with minimal changes in the daughter's primary spermatocytes. Older spermatids at maturation stage 17, exhibiting a PAS+ acrosome on the nucleus, were deeply embedded in Sertoli cells (Figure 3D).

In Stage V, a recognizable PAS+ head cap appeared as two straight lateral projections of the acrosomic granule on newly formed spermatids at the cap phase (Stage 5). Type A spermatogonia, daughter primary spermatocytes, and older spermatids at maturation stage 17 showed few changes (Figure 3E).

Stage VI was characterized by a growing head cap, resembling a small umbrella centered around the acrosomic granules, covering one pole of the nucleus in the newly formed spermatids at the cap phase (Stage 6). Numerous spermatogonia B and a small number of spermatogonia A were observed along the basement membrane, while primary spermatocytes were at the pachytene stage. Older spermatids at maturation stage 18 were migrating toward the lumen of the tubules (Figure 3F).

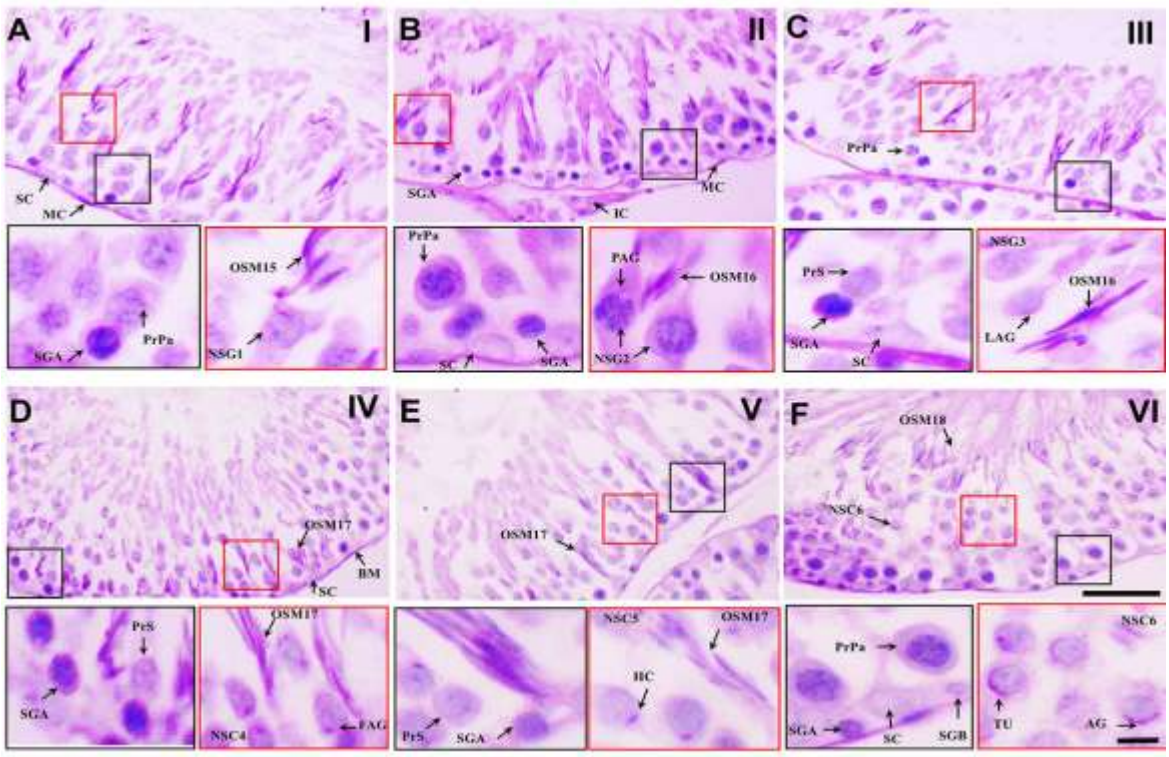

Figure. 3. Representative histological photomicrographs of PAS- and hematoxylin-stained cross section of rat testis (A, B, C, D, E, and F) showing spermatogenic cycle of various spermatogenic stages (I, II, III, IV, V and VI).

(A) Stage I showing flattened myoid cells (MC), Sertoli cells (SC), type A Spermatogonium (SGA), primary spermatocytes at early pachytene stage (PrPa), newly formed spermatids of Golgi phase at stage 1 (NSG1), older spermatids of maturation stage 15 (OSM15) of a prominent dorsal fin. (B) Stage II showing interstitial cells (IC), flattened myoid cells (MC), Sertoli cells (SC), type A Spermatogonium (SGA), primary spermatocytes at pachytene stage (PrPa), newly formed spermatids of Golgi phase at stage 2 (NSG2) with PAS+ proacrosomic granules (PAG), older spermatids of maturation stage 16 (OSM16) with PAS+ acrosome on dorsal angle of nucleus, lost dorsal fin and forming bundles towards the basally located Sertoli nuclei. (C) Stage III showing Sertoli cells (SC), type A Spermatogonium (SGA), daughter primary spermatocytes (PrS), primary spermatocytes at pachytene stage (PrPa), newly formed spermatids of Golgi phase (NSG3) at stage 3 with PAS+ large acrosomic granules (LAG), older spermatids of maturation stage 16 (OSM16) with PAS+ acrosome on dorsal angle of nucleus, lost dorsal fin and forming bundles towards the basally located Sertoli nuclei. (D) Stage IV showing PAS+ basement membrane (BM), type A Spermatogonium (SGA), daughter primary spermatocytes (PrS), newly formed spermatids of cap phase at stage 4 (NSC4) with flattening acrosomic granule on the nucleus (FAG), older spermatids of maturation stage 17 (OSM17) with a PAS+ acrosome on the nucleus and deeply inserted into Sertoli cell (SC). (E) Stage V showing type A Spermatogonium (SGA), daughter primary spermatocytes (PrS), newly formed spermatids of cap phase at stage 5 (NSC5) with a recognizable PAS+ head cap (HC), and older spermatids of maturation stage 17 (OSM17) with a PAS+ acrosome on the nucleus and deeply inserted into Sertoli cell. (F) Stage VI showing sertoli cell (SC), type A Spermatogonium (SGA), type B Spermatogonium (SGB) primary spermatocytes at pachytene stage (PrPa), newly formed spermatids of cap phase at stage 6 (NSC6) with a growing head cap of a tiny umbrella-like (TU) centered by the acrosomic granules (AG), and older spermatids of maturation stage 18 with moving toward the lumen of the tubules (OSM18). The insets showing selected areas of the seminiferous epithelium at higher magnifications. Scale bars (A-F); 50 μm. Insets; 30 μm.

Stage VII began when a well-formed, non-oriented PAS+ head cap covered one-third of the spherical nucleus of newly formed spermatids at the cap phase (Stage 7). Few types A spermatogonia were detected, alongside a new generation of young primary spermatocytes and older primary spermatocytes at the pachytene stage. Older spermatids at maturation stage 19 exhibited a loss of PAS+ head cap and acrosome reactivity. These immature spermatozoa were moving toward the lumen of the tubules (Figure 4A).

Stage VIII was characterized by the orientation of the PAS+ head cap towards the basement membrane of newly formed spermatids. Rare type A spermatogonia were interspersed with numerous resting young primary spermatocytes, followed by older spermatocytes at the pachytene stage. A solid layer of oriented spermatids in the cap phase was observed at Stage 8. Finally, older spermatids at maturation stage 19 completed the elimination of their cytoplasm and moved closer to the lumen of the tubules. The cytoplasmic

remnants were visible as quasi-spherical residual bodies near the lumen (Figure 4B).

After the release of spermatozoa, stage IX begins with the commencement of the flattening and elongation of the spermatids, nuclei. Divided type A spermtogonia were situated along the basement membrane. Next, there were young primary spermatocytes at the leptotene stage, older spermatocytes at the pachytene stage, newly formed spermatids of cap phase at stage 9 with a slight flattening of nucleus, and PAS+ head cap-covered part of the nucleus. A few residual bodies can be seen (Figure 4C).

Stage X begins when the caudal end of the head cap reaches the caudal region of the spermatids, flattened nuclei at stage 10. Type A spermtogonia, growing primary spermatocytes at the leptotene stage, older spermatocytes at the pachytene stage, newly formed intended spermatids of cap phase at stage 10 were identified (Figure 4D).

Stage XI showed type A spermtogonia, primary spermatocytes at the leptotene stage, older spermtocytes at the pachytene stage, newly formed spermatids of cap phase at stage 11 of curved and lengthened nuclei, with lateral flattening covered with PAS+ head cap (Figure 4E).

Stage XII begins when spermatids of cap phase at stage 12 lost most of their curvatures with a pronounced elongation of the nuclei and the apical portion covered by PAS+ acrosomal head cap resembles a triangular wedge. Numerous type A spermatogonia remained associated with a layer of. young primary spermatocytes at the leptotene stage, and old primary spermatocytes at pachytene stage. There were elongated bundles of spermatids between the older spermatocytes (Figure 4F).

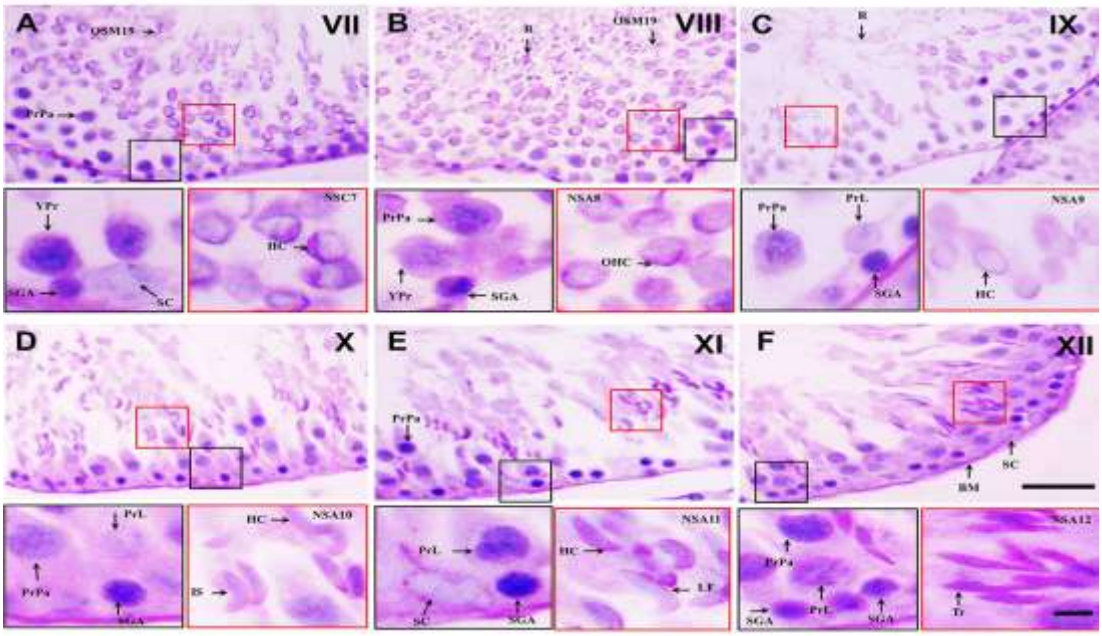

Figure. 4. Representative histological photomicrographs of PAS- and hematoxylin-stained cross section of rat testis (A, B, C, D, E, and F) showing spermatogenic cycle of various spermatogenic stages (VII, VIII, IX, X, XI, and XII).

(A) Stage VII showing Sertoli cells (SC), type A spermatogonium (SGA), young primary spermatocytes (YPr), primary spermatocytes at pachytene stage (PrPa), newly formed spermatids of cap phase at stage 7 (NSC7) with a well-formed non-oriented PAS+ head cap (HC) enclosed one-third of the spherical cell nucleus, older spermatids of maturation stage 19 (OSM19) with losing PAS+ head cap and acrosome reactivity and become closing to the lumen of the tubule. (B) Stage VIII showing type A spermatogonium (SGA), resting young primary spermatocytes (YPr), older spermatocytes at the pachytene stage (PrPa), newly formed spermatids of cap phase at stage 8 (NSC8) with the PAS+ oriented head cap (OHC) toward the basement membrane, a few older spermatids of maturation stage 19 with losing PAS + head cap and acrosome and become closing to the lumen of the tubule (OSM19), quasi-spherical residual bodies (R). (C) Stage IX showing divided type A spermtogonia along the basement membrane (SGA), young primary spermatocytes at the leptotene stage (PrL), older spermatocytes at the pachytene stage (PrPa), newly formed spermatids of cap phase at stage 9 (NSC9) with a slight flattening of nucleus and PAS+ head cap covered part of the nucleus (HC). Few residual bodies (R) were seen. (D) Stage X showing type A spermtogonia (SGA), growing primary spermatocytes at the leptotene stage (PrL), older spermatocytes at the pachytene stage (PrPa), newly formed intended spermatids (IS) of cap phase at stage 10 (NSC10) with flattening nuclei and caudal end of the PAS+ head cap reaches the caudal region of the nucleus (HC). (E) Stage XI showing Sertoli cells (SC), type A spermtogonia (SGA), primary spermatocytes at the leptotene stage (PrL), older spermtocytes at the pachytene stage (PrPa), newly formed spermatids of cap phase at stage 11 (NSC11) of curved and lengthened nucleus, with lateral flattening (LF) covered with PAS+ head cap (HC). (F) Stage XII showing PAS+ basement membrane (BM), Sertoli cells (SC), type A spermatogonium (SGA), primary spermatocytes at the leptotene stage (PrL), primary spermatocytes at pachytene stage (PrPa), newly formed spermatids of cap phase at stage 12 (NSC12) with a pronounced elongation of the nucleus and the apical portion covered by PAS+ acrosomal head cap resembles a triangular wedge, and lost most of its curvature (Tr). The insets showing selected areas of the seminiferous epithelium at higher magnifications. Scale bar (A-F); 50 μm. Insets; 30 μm.

Stage XIII showed type A spermatogonia, young spermatocytes at the zygotene stage, old spermatocytes at the diplotene followed by diakinesis, newly formed spermatids of cap phase at stage 13 with nuclei of slightly shorter, curved, tapering at the apex and PAS+ acrosome caudally along the dorsal edge. The cytoplasm condensed along the tail (Figure 5A).

Stage XIV displayed type A spermatogonia, mitotic division of spermatogonia at prophase, metaphase, telophase, daughter primary spermatocytes, newly formed spermatids of cap phase at stage 14 with contracted and more curved nuclei of high affinity for basic dyes and PAS+ fixed head caps to the dorsal angle caudally (Figure 5B).

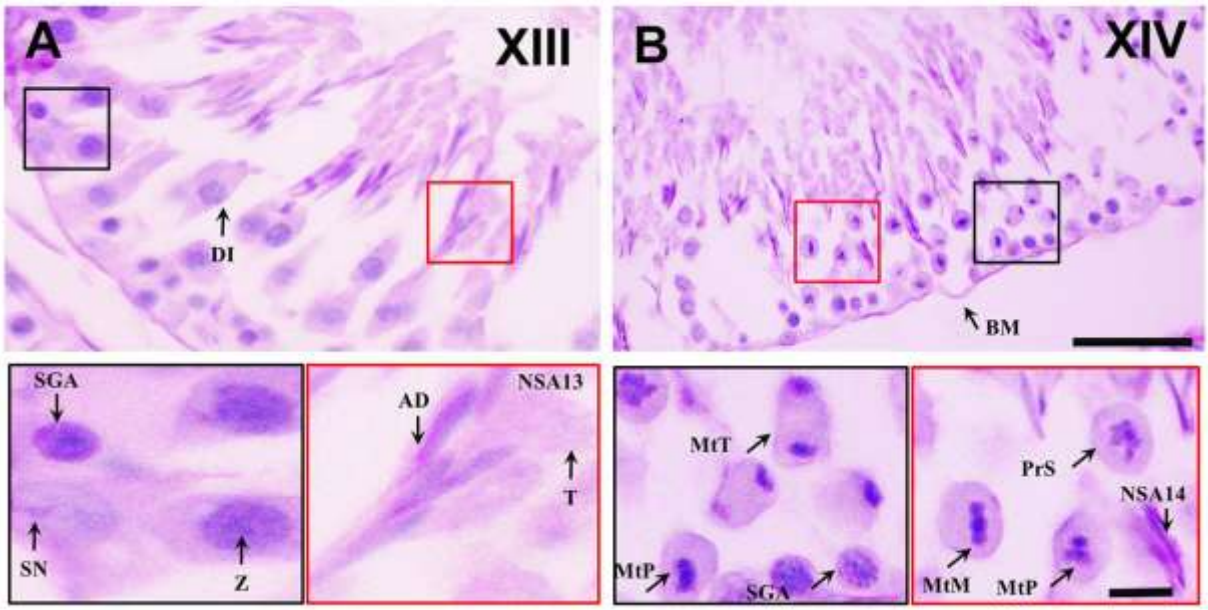

Figure. 5. Representative histological photomicrographs of PAS- and hematoxylin-stained cross section of rat testis (A, and B) showing spermatogenic cycle of various spermatogenic stages (XIII, and XIV).

(A) XIII stage showing pale stained Sertoli nucleus (SN), type A spermatogonium (SGA), young spermatocytes at the zygotene stage (Z), old spermatocytes at the diplotene followed by diakinesis (DI), newly formed spermatids of cap phase at stage 13 (NSC13) with nucleus of slightly shorter, curved, tapering at the apex and PAS+ acrosome caudally along the dorsal edge (AD). The cytoplasm condensed along the tail (T). (B) XIV stage showing PAS+ basement membrane (BM), type A spermatogonium (SGA), mitotic division of spermatogonium at prophase (MtP), metaphase (MtM), telophase (MtT), daughter primary spermatocytes (PrS), newly formed spermatids of cap phase at stage 14 (NSC14) with contracted and more curved nucleus of high affinity for basic dyes and PAS+ fixed head cap to the dorsal angle caudally. The insets showing selected areas of the seminiferous epithelium at higher magnifications. Scale bars (A-C); 50 μm. Insets; 30 μm.

5- Hormonal Regulation of Spermatogenesis

7-1- Role of Hypothalamic-Pituitary-Gonadal (HPG) axis

The regulation of spermatogenesis is a highly complex and finely tuned process, controlled by a network of key factors that ensure the efficient production of sperm. At the core of this regulation is the hypothalamic-pituitary-gonadal (HPG) axis, which orchestrates the release of key hormones involved in spermatogenesis. Gonadotropin-releasing hormone (GnRH), produced by the hypothalamus, stimulates the anterior pituitary to release luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The development and maintenance of spermatogenesis, therefore, rely on the precise production of gonadotropins, which regulate testicular function and support spermatogenesis [2]. LH and FSH are regulated through feedback mechanisms involving testosterone and inhibin B, respectively. These feedback

systems ensure hormonal balance and proper function of the male reproductive system.

In adult rats, the cessation of gonadotropin secretion, through hypophysectomy, GnRH receptor antagonist treatment, or active immunization against GnRH, results in the interruption of spermatogenesis, although differentiating spermatogonia proceed into meiosis, with subsequent stages of spermatogenesis being halted [21].

FSH directly regulates spermatogenesis by acting on Sertoli cells, whereas LH indirectly regulates the process by stimulating the Leydig cells to produce testosterone. Both gonadotropins play a pivotal role in modulating Sertoli cell factors.

Follicle-stimulating hormone (FSH)

FSH targets Sertoli cells, enhancing their supportive role in spermatogenic progression while working synergistically with testosterone. This interaction promotes germ cell development and survival, thereby boosting the spermatogenic capacity of the testes [4]. The primary role of FSH in spermatogenesis is believed to stimulate the proliferation of Sertoli cells and to provide essential survival and differentiation signals to the developing germ cells in rats. By binding to its receptors on Sertoli cells, FSH triggers cellular responses that support the growth and function of Sertoli cells, which in turn create an environment conducive to the survival, maturation, and differentiation of germ cells [2]. FSH also stimulates Sertoli cells to produce androgen-binding protein and form the blood-testis barrier. Recombinant FSH has been demonstrated to partially restore spermatogenesis in gonadotropin-deficient rats by enhancing the number of spermatogonia and facilitating their progression to the round spermatid stage [21]. However, spermatid elongation was not achieved with FSH treatment alone, suggesting that other factors, particularly testosterone, are crucial for the complete maturation of spermatids and the full restoration of spermatogenesis [21].

In rats, FSH regulates the stem cell factor (SCF) and glial cell line-derived neurotrophic factor (GDNF) production by Sertoli cells [22]. These factors play a central role in promoting the survival, self-renewal, and differentiation of spermatogonial stem cells [22, 23]. SCF interacts with its tyrosine kinase receptor, c-KIT, on spermatogonia A1-A4, as described by Dym et al. [24]. This interaction plays an essential role in spermatogonial proliferation and differentiation [25]. Meanwhile, GDNF promots the self-renewal of the spermatogonial stem cells and prevents their premature differentiation [22]. Together, SCF and GDNF are essential for maintaining a healthy pool of spermatogonia and ensuring robust spermatogenesis [22, 23].

Testosterone

The primary male sex hormone is primarily produced in the Leydig cells of the testes, under the stimulation of luteinizing hormone (LH). Testosterone plays a crucial role in initiating and maintaining spermatogenesis. Additionally, testosterone acts locally within the testes, influencing the function of Sertoli cells, Leydig cell maintenance, and the regulation of feedback mechanisms involving the hypothalamic-pituitary-gonadal axis. These intratesticular effects are vital for normal male rat fertility and reproductive health.

Testosterone is important for the differentiation of spermatocytes into spermatids. Furthermore, its production influences the development of peritubular and Sertoli cells, which in turn supports the maturation and development of germ cells within the seminiferous tubules. Studies by O'Donnell et al. [26] and Sofikitis et al. [27] showed that the reduction in testosterone levels in the testes impairs the transition of round spermatids between steps 7 and 8 of spermiogenesis, preventing the completion of spermatid elongation in rat models. This disruption is likely due to the loss of adhesion between spermatids and Sertoli cells, which is crucial for their development and support [28]. Furthermore, rats with lowered intratesticular testosterone levels display step 8 round spermatids in the epididymal cavity [27]. Additionally, lower intratesticular testosterone concentrations have been shown to trigger apoptotic death of germ cells [28]. As a result, the absence of testosterone causes round spermatids to disconnect from Sertoli cells, causing them to be shed into the seminiferous lumen, where they are later engulfed by Sertoli cells through phagocytosis.

Collectively, testosterone and gonadotropins play a crucial role in determining germ cell fate. The absence or depletion of these hormones leads to the induction of germ cell apoptosis [29].

In rats, the regulation of LH and GnRH through feedback inhibition is mainly controlled by the circulating levels of testosterone. Elevated testosterone concentrations in the bloodstream signal to the hypothalamus and pituitary gland, reducing the secretion of GnRH and LH. This negative feedback mechanism helps maintain homeostasis in the endocrine system by regulating testosterone production within optimal levels, thereby ensuring proper spermatogenesis [2].

Kisspeptin

Kisspeptin signaling via its G-protein-coupled receptor 54 (Gpr54) plays an essential role in maintaining normal fertility in all mammalian species. It is the most powerful activator of (GnRH) secretion [30]. In rats, kisspeptins bind to the GPR54 (Kiss1R) receptor, activating the hypothalamus to release GnRH [31]. This subsequently triggers the release of gonadotropins [31]. The increase in testosterone in male rats is primarily induced by kisspeptin-mediated activation of GnRH, which leads to the release of LH [32]. In Kiss1 knockout male rats, testosterone and LH levels are significantly reduced postnatally [32].

7-2- Role of Sertoli cell hormones

In rat reproductive physiology, inhibin, activin, and follistatin are involved in regulating FSH synthesis and secretion, as well as in the maturation of spermatogonia. Inhibin and activin are related peptides that have opposite effects, while follistatin, a structurally different peptide, likely exerts its effects by regulating the actions of inhibin and activin.

Inhibin

Sertoli cells produce and release inhibin in response to FSH, which in turn controls FSH production at the pituitary gland through a negative feedback mechanism. Inhibin B is the predominant form produced in male rats [33]. In rats, inhibin concentration is positively associated with the quantity and activity of Sertoli cells [34]. Inhibin has an inhibitory action on fertility. Inhibin has been shown to decrease the number of spermatogonia *in vivo* in the testes of adult hamsters and mice [35] and to inhibit DNA synthesis in seminiferous

tubular segments from adult rats *in vitro* [36]. It inhibits the multiplication of spermatogonia in male tests [37]. Inhibin B is therefore considered a potential marker for Sertoli cell damage and disruptions in spermatogenesis in the rat [38].

Studies by Dandekar et al. [39] revealed the presence of specific receptor binding sites for inhibin in rat spermatids. Additionally, the significant amounts of active inhibin found in these spermatids suggest that they may be an important source of inhibin [39]. It has been confirmed that the inhibin levels vary depending on the stage of the rat seminiferous cycle. The synthesis of inhibin B subunits (α and βB) and the inhibin B protein is primarily regulated by circulating FSH but also exhibits a cyclical production pattern. The subunit mRNA levels are highest during stages XIII–IV and lowest during stages VI-VIII [34]. Moreover, i*n vitro* findings by Gonzales et al. [40] demonstrated that the inhibin production in the seminiferous tubules fluctuates during the seminiferous cycle of rats. Inhibin levels were highest during stages II-VI and lowest during stages VII-VIII.

Over a 48-hour culture period, the release of inhibin was highest during stages XIII-VI and lowest during stages VII-VIII. Additionally, the rat seminiferous tubules showed the greatest inhibin response to FSH at stages IX-XIII, with the weakest response observed at stages VII-VIII [40].

Activin

The activins, including activin A (βAβA), activin B (βBβB), and activin AB (βAβB), are known to stimulate FSH secretion from the pituitary gland [41]. Activin A is the isoform having greater physiological significance [41]. In addition to their broad biological functions across various tissues, activin A is locally produced in the rat testis, where it exerts paracrine and autocrine control of Sertoli cell activities [42]. Sertoli cells are the primary source of activin A in rats [42], though the less abundant peritubular and Leydig cells also make significant contributions [43]. Additionally, it has been demonstrated that activin A is released by in vitro anterior pituitary cells of rats, while activin B is the predominant isoform produced in the rat pituitary gland [44].

Researchers have identified the presence of both type I and type II activin receptors in Sertoli cells, primary spermatocytes, and round spermatids in rats [45]. The highest expression level was observed in late primary spermatocytes during stages XIII-XIV and in early round spermatids during stages I-IV of the seminiferous tubule cycle in rats [46].

Activin A helps coordinate the seminiferous epithelium cycle and acts as a mediator between various stages of developing germ cells. Mather et al. [47] demonstrated that activin A and is essential for stimulating and regulating spermatogonia proliferation in rat Sertoli and germ co-cultures *in vitro*. Moreover, activin A promoted the reassembly of Sertoli and germ cells in vitro in rats, even in the absence of basement membranes and peritubular cells. It also stimulated DNA synthesis in intermediate spermatogonia in stage III-IV and preleptotene spermatocytes in stage VII during the rat cycle of seminiferous epithelia; in a dose-dependent manner [36]. Furthermore, Meinhardt et al. [48] established that Activin A affects the development of meiotic germ cells and can specifically maintain the condensed mitochondrial morphology observed in

germ cells after the leptotene stage of the first meiotic prophase in male rats. Activin A increased the number of gonocytes without affecting the number of spermatogonia in rat testes, according to Meehan et al. [49].

Activin A plays a vital role in regulating the function of Sertoli cells and the development of germ cells in the mature testes. A rise in activin A production by Sertoli cells coincides with spermiation at stage VIII of the seminiferous epithelium cycle, mainly triggered by the phagocytosis of residual cytoplasm from spermatids [50]. During this crucial phase, activin A impacts the proliferation and maturation of spermatogonia and spermatocytes and regulates the reorganization of tight junctions between Sertoli cells [50]. Taken together, activin is crucial in regulating rat spermatogenesis and maintaining the functional activity of Sertoli cells and the blood-testis barrier.

Follistatin

The high-affinity activin-binding protein, follistatin, is additionally generated by Sertoli cells [49]. Similar to inhibin, follistatin was identified for its ability to suppress the biosynthesis of FSH in rat anterior pituitary cultures [51]. Follistatin mRNA has been identified in Sertoli cells and different germ cells throughout the testis, including type B spermatogonia, primary spermatocytes (except during the late leptotene and early zygotene stages), and spermatids at steps 1 to 11 in rats [52]. This extensive presence of follistatin and its ability to neutralize activin's effects, suggests that it may play a role in regulating various testicular functions mediated by activin. Follistatin was found to block the ability of activin A to induce the aggregation of Sertoli cell monolayers, however, it did not inhibit the activin-induced stimulation of spermatogonia in co-culture with Sertoli cells in rats [53].

Androgen-binding protein

In the testes androgen-binding protein (ABP) is synthesized in Sertoli cells. Approximately 80% of ABP is secreted in the seminiferous tubule, with the remaining 20% entering the bloodstream [54]. In the rat testis, it is located in both the basal and luminal regions of the seminiferous tubules and the secretory granules of Sertoli cells [55]. ABP secretion by rat seminiferous tubules varies by stage, with the highest secretion at stages VIII-XI and the lowest at stages IV-V [56]. ABP protein binds testosterone, promoting its transport through the seminiferous tubule fluid to the epididymis, which is essential for initiating and maintaining spermatogenesis [1]. Additionally, FSH and testosterone could regulate the synthesis and release of this protein into the luminal and interstitial spaces of rat testes [57].

Insulin-like growth factors

Insulin-like growth factors (IGF-I and IGF-II), secreted by Sertoli cells, regulate both of Sertoli and Leydig cells function through autocrine actions and may have a paracrine role in the seminiferous epithelium cycle. IGFs regulate spermatogonial proliferation and stimulate premitotic DNA synthesis in rat germ cells during spermatogenesis [58]. IGF-I signal is primarily mediated by the IGF-I receptor (IGF-IR). In postnatal rats, IGF-IR immunoreactivity was weak in pachytene primary spermatocytes and Leydig cells, whereas it was strong in Leydig cells and elongated spermatids [59]. Furthermore, it has been shown that the IGF-I peptide is identified in spermatogenic, Sertoli, and Leydig cells in immature rats, and spermatocytes in

adult rats [60]. Leydig cell–Sertoli cell interactions are crucial for regulating intratesticular IGF-I. In hypophysectomized rats, testicular IGF-I levels decrease but are restored with FSH and testosterone treatment [61]. Therefore, FSH and testosterone promote spermatogenesis and spermatid maturation by stimulating IGF-I secretion from Sertoli cells [61].

Conclusion

Understanding the spermatogenic cycle and its hormonal regulation in rats provides essential insights into the complex processes that govern male fertility. The intricate interplay between hormones such as LH, FSH, and testosterone, and the vital role of Sertoli cells in promoting germ cell development, highlights the finely tuned nature of spermatogenesis. Disruptions in any aspect of this regulatory network can lead to infertility, underscoring the importance of maintaining hormonal balance for successful sperm production. The insights gained from studying spermatogenesis in rats not only advance our knowledge of male reproductive physiology but also offer valuable implications for improving fertility treatments in animal models. Furthermore, these findings can inform clinical practices in human reproductive medicine, ultimately contributing to better outcomes for individuals facing infertility challenges. Future research into the pathways of spermatogenesis as well as its hormonal regulation is crucial in developing novel therapeutic strategies to address male infertility.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

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الملخص العربي

تتبع الرحلة الخلوية لتكوين الحيوانات المنوية في الفئران

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يعتبر تكوين الحيوانات المنوية في الجرذان عملية معقدة ومنسقة بشكل جيد وتحدث داخل الأنابيب المنوية في أنسجة الخصية. تستمر دورة تكوين الحيوانات المنوية داخل الأنابيب المنوية حوالي 13 يومًا في الجرذان وتتكون من سلسلة من المراحل، كل منها يتوافق مع مرحلة مميزة في تطور الخلايا الجرثومية. تتضمن هذه العملية تكاثر وتمايز الخلايا الجذعية المنوية، والانقسام الاختزالي، وتكوين الحيوانات المنوية. يتم تنظيم تكوين الحيوانات المنوية من خلال تفاعل متطور للهرمونات، ويتم التحكم فيه بشكل أساسي عن طريق هرمونات محور الغدة النخامية والغدة التناسلية. يحفز الهرمون المنشط للجسم الأصفر LH الخلايا البينية في الخصيتين لإنتاج هرمون التستوستيرون، وهو هرمون ضروري لنضج الخلايا المنوية والحيوانات المنوية داخل الأنابيب المنوية. كما يستهدف الهرمون المنشط للحوصلة خلايا سرتولي، والتي تساعد في الحفاظ على تركيب النسيج الطلائي المبطن للأنابيب المنوية وتسهيل تطور ونمو الحيوانات المنوية عن طريق توفير التغذية وإفراز هرمونات الإنهيبين inhibin والأكتيفين activin والفوليستاتين follistatin والبروتين الرابط للأندروجين androgen-binding protein والعوامل المنظمة الأخرى التي تشارك في آليات التغذية الراجعة والتي تتحكم في إفراز هرمونات الغدد التناسلية. يمكن أن تؤدي الاضطرابات في التوازن الهرموني إلى ضعف تكوين الحيوانات المنوية والعقم عند الذكور. ولذلك، فإن الفهم الشامل لدورة ومراحل تكوين الحيوانات المنوية والمكونة من 14 مرحلة وتنظيمها الهرموني أمر بالغ الأهمية لتوضيح الآليات المنظمة لخصوبة الرجال. توفر هذه الدراسة رؤى ذات قيمة في فسيولوجيا التكاثر لدى الذكور ويمكن أن تساهم في تطورعلاجات اضطرابات الخصوبة لدي الذكور.