Chapter

Seminiferous Tubules and Spermatogenesis

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Abstract

One of the major concerns of the world health community is the infertility. The definition of infertility according to the World Health Organization (WHO) and the American Society for Reproductive Medicine (ASRM) is the inability of a healthy couple to achieve a conception after one year of regular, unprotected intercourse. Fertility complications affect seven percent of the male. The causes of infertility were divided to non-obstructive and obstructive. But, in almost 75% of male infertility cases are idiopathic with predominance of the genetic abnormalities. Numerical or structural chromosomal abnormalities are considered as genetic abnormalities that occur during the meiotic division in spermatogenesis. These abnormalities get transferred to the Offspring, which affects the normal and even the artificial conception. In the human reproduction, sperm cells are considered as a delivery vehicle for the male genetic material packed in chromosomes, which are composed of nearly 2-meter Deoxyribonucleic acid (DNA) molecule and their packaging proteins. This chapter points to grant a summarized description of individual components of the male reproductive system: the seminiferous tubule and spermatogenesis. Here, we describe step by step the structure of the testis seminiferous tubule and what occurs inside these tubules like cell communication and germ cell development from spermatogonia until spermatozoon. This book chapter is very useful for the biologists and physicians working in Assisted reproduction field to understand the physiology and pathology of spermatogenesis.

Keywords: Seminiferous tubules, Spermatogenesis, Chromatin remodeling

1. Introduction

Testes or testicles appear as a pair of oval-shaped complex organs enclosed in the scrotum and based behind the penis and in front of the anus. They produce male reproductive cells, spermatozoa, and androgens, the male hormones [1]. Each adult testis weights 12 to 19 g, 4.5x 2.5x 3 cm in dimension and is suspended in the scrotum by a spermatic cord. The rete testis at the mediastinum of the testis connects to the head of epididymis, which is opposed to the testis posteriorly [2].

The tunica albuginea, fibrous capsule, covers each testis. The testis is divided by partitions of the tissue from the tunica albuginea into approximately 250 lobes. Three to ten coiled tubules are inside each lobe. These tubules are called

seminiferous tubules containing two different cells population: spermatogenic or germ cells and Sertoli cells surrounded by peritubular myoid cell layer. The stroma between the seminiferous tubules is called the interstitium (interstitial tissue), where located blood and lymphatic vessels, the steroidogenic Leydig cells and other cell types [3, 4].

1.1 Tunica albuginea

Each testis is enclosed in a thick fibrous envelope, formed by collagen fibers impregnated with elastic fibers (5% elastin), called tunica albuginea. Besides, it is formed by two layers: outer longitudinal layer and inner circular layer [5].

Because of her contractile properties (erection), the tunica albuginea has different physiological functions: the preservation of the interstitial pressure inside the testis, the support of the spermatozoa movement from the testis to the epididymis, and the regulation of the blood movement through the testis. On the posterior surface of the testis, the tunica albuginea become thicker to form the mediastinum testis from which Septula testis enter the gland, separating it into almost 250 testicular lobules [6].

1.2 Basement membrane

The basement membrane is a fibrous matrix formed by type IV collagen, glycoproteins and lamin produced by the epithelial cells. It plays a crucial part in keeping up the structural and functional integrity of tissues in the testis [7, 8].

Modified cellular layer structure has been related with extreme function abnormalities of the testis like cryptorchidism, autoimmune orchitis, vasectomy [9].

1.3 Peritubular cells

In human testicular, the outer coat of the seminiferous tubules is formed by several layers of myoid, peritubular cells and extracellular matrix (ECM) proteins. The cells are peritubular myofibroblast-like cells that encompass the seminiferous tubules to maintain its structural integrity and are capable of tubular contractility and sperm transport [10].

These cells, in adults, express markers for smooth-muscle-like cells similar to the smooth muscle actin [11].

Immunohistochemical studies suggested that the cellular phenotypes differ between the outer and inner layers. After stain, the inner layers showed a smooth muscle phenotype after stain with desmin. While the outer layers were stained with vimentin indicating a connective tissue phenotype [12].

A basal lamina separates the spermatogonial stem cells (SSC) and the peritubular myoid cells (PMC). This can indicate a possible cellular interaction between the PMCs and SSC to maintain the SSCs niche, similarly to Sertoli cells [4]. One of the contribution mechanisms is through the production of secreted factors like glial cell line-derived neurotrophic factor (GDNF), that acts in combination with the androgen receptor (AR) [13].

It has also been demonstrated that the PMCs produce colony-stimulating factor 1 (CSF-1) in interaction with specific receptor CSF-1R regulate the SSCs activity [4, 14].

1.4 Leydig cells

Leydig cells called also interstitial cells because they are in the stroma between the seminiferous tubules: the interstitium (interstitial tissue) holding the

tubules together within each lobule. This tissue is activated at puberty through the interstitial-cell-stimulating hormone of the anterior lobe of the pituitary gland [15].

After stimulation through the luteinizing hormone (LH), the production of testosterone via the Leydig cells increases through the regulation of the expression level of steroidogenic enzymes like the 17-β hydroxysteroid dehydrogenase [16].

Testosterone exerts its effects locally by binding to the androgen receptor (AR) within the testis or distantly by binding to androgen binding protein (ABP) which increases its levels in the seminiferous tubules and its carrying to the epididymis [17].

Elevated levels of serum LH, as well as FSH and lowered levels of serum testosterone, suggested Leydig and germ cell failure [18].

2. Structure and function of the seminiferous tubules

The seminiferous tubules are the basic units of the testicles where the SSCs proliferate and differentiate through cyclic events (mitosis, meiosis, postmeiotic spermatid development, and spermiogenesis) to generate spermatozoa in a process called spermatogenesis [19].

In humans, the seminiferous tubules represent about 60% of the total testicle volume and they are about 200 μm in diameter and have a total length of ~600 meters. These seminiferous tubules are composed of the lamina propria (peritubular tissue) with about 80 μm height and the germinal epithelium with about 8 μm thickness [20].

The germinal epithelium composed of large sertoli cells and spermatogonial germ cells. These cells are connected via tight junctions [20].

The stage of the seminiferous epithelium cycle has influence on the architecture of seminiferous tubule sections. In addition, the nerves, lymph vessels and blood vessels do not penetrate the seminiferous tubule and are located only on interstitial tissue [21].

The seminiferous tubules have terminal ends in the mediastinum testis and evacuate via straight tubular extensions called Tubuli seminiferi rect [22].

2.1 Sertoli cells

Enrico Sertoli was the author of the first publication reporting the existence of Seroli cells [23]. Later, numerous reviews in scientific journal and books have been published describing the Sertoli cell morphology and functions, mostly focusing on mammals [24].

In humans, Sertoli cells are crucial for testis physiology [25]. They proliferate during the perinatal and neonatal period, becoming quiescent for several years and having a second peak of proliferation just before puberty [26, 27].

Although, around puberty Sertoli cells stop proliferating and start to differentiate, being therefore able to support full spermatogenesis. The establishment of the Sertoli cell barrier and fluid secretion/lumen formation are clear character of Sertoli cells maturation [23, 25, 28].

Follicle stimulating hormone (FSH) and androgens are considered important factors that regulate Sertoli cell proliferation [25, 29]. In addition, oestrogens, activins, TGF-beta, BMPs, interleukins and TNF- alpha are factors involved by proliferation and differentiation of Sertoli cells [30, 31].

Sertoli cells were identified as 'nurse cells' because they are morphologically reshaped by the developing germ cells and have multitude cytoplasmatic

processes. Each Sertoli cell is "nursing" approximately 30–50 germ cells at four or five diverse stages of their advancement at any given time throughout the epithelial cycle [32, 33].

Structural characteristics of the Sertoli cells varies among species, such as the heavily vacuolated nucleolus present in some ruminants [34], the nucleus localization in the middle of the seminiferous epithelium in monkeys, the presence of Charcot-Bottcher cristaloids in men [23], and the presence and amount of lipid droplets and glycogen in the Sertoli cell cytoplasm [35].

Therefore, the Sertoli cell shape may vary according to the species and the progression of spermatogenesis and the tasks. As the germ cell requirements changed, interactions and metabolic needs change substantially and accordingly, high variations are detected on the Sertoli cell cytoplasm extension, the number of nuclear pores, the presence and translocation of organelles and the protein expression pattern and location across the different phases of spermatogenesis [36, 37].

On the other hand, the Sertoli cells are considered as "epithelial" cells as they are based on a strikingly thick basal lamina, appear a remarkable design (polar-basolateral-apical) with horizontal cell–cell intersections and border on a luminal space [38].

Although Sertoli cells extend from the basement membrane of the seminiferous tubule into the adluminal compartment, the two tubular compartments are isolated by tight and adherent junction complexes between neighboring Sertoli cells, that works as the major component of the blood-testis barrier (BTB). These junctions generate the required chemical environment for fulfillment of meiosis and spermiogenesis [39].

Besides, the molecular character of Sertoli cells changed from keratin IFs to vimentin IFs during their development and maturation. Also, a wealth of special and rather extended forms of adherents junctions connected Sertoli cells and spermatogenic cells instead of the typical epithelial junctions [38].

Functionally, they play a critical role during the spermatozoa development by supporting and organizing spermatogonial germ cells during different stages of spermatogenesis through secretion of androgen-binding protein and interaction with Leydig cells [23, 28]. In addition, they provide the germ cells with a variety of ions, nutriments, carbohydrates, hormones, and growth factors [40, 41].

2.2 The transition region

The seminiferous tubules connect to the rete testis in a region named: Transition region. This region might be a specific area for immature Sertoli cells [27, 42, 43]. Also, transitional region contain a subpopulation of mitotically active Sertoli cells without differentiation, Sertoli cells markers like transcription factor GATA-4 and the androgen receptor [27, 42–44]. It can be assumed that adult Sertoli cells population is not morphologically homogeneous. As the transition region presents modified Sertoli cells that exhibit features that resemble undifferentiated Sertoli cells, with less indentations, smaller nucleolus, and more peripheral heterochromatin [45, 46]. Therefore, the dogma that the adult Sertoli cells population constitutes a terminally differentiated population in mammals has been challenged by several recent studies [23, 42, 47, 48].

In addition, because this transitional region of mammalian testis also contains spermatogonial stem cells, it has been supposed that the transition region might be an area where the seminiferous tubules continue to grow in sexually mature individuals [27, 42, 44]. Other indicated that the transition region is a site where seminiferous tubules are originally formed [49].

2.3 Spermatogonial stem cell's niche

The spermatogonial stem cell's niche (SSCs) microenvironment has a complex regulation that involves the vascular network, macrophages, the basement membrane, peritubular myoid cells, and Sertoli cells. Meantime, the stimulation of SSCs differentiation involves Leydig cells [4, 50–53].

The number of Sertoli cells per testis determines the number of available spermatogonial stem cell niches and, consequently, reflects the magnitude of sperm production capacity. Therefore, this Sertoli cell regulation ensures a proper gem cell homeostasis and regulates the germ cell density observed in the seminiferous epithelium [54].

Depending on the stimulus, a balance between differentiation and self-renewal factors regulates the fate of SSCs that are capable of self-renewal, differentiation and/or entering apoptosis [54].

In mammals, recent studies have demonstrated that the transition region is the closed niche area. The Sertoli cells in this region produce high amount of glial cell-line derived neutrophic factor (GDNF), maintaining the neighboring spermatogonia in an undifferentiated state [44].

Also, the Sertoli cells play a key role in the functional regulation of spermatogonial stem cells niche, where other somatic testicular cells, extracellular matrices and soluble factors actively participate in the complex interaction/signaling with these spermatogonial cells [4].

Several studies have demonstrated that SSCs are usually located in the seminiferous tubules area facing blood vessels of the testis interstitial compartment. It is speculated that FSH, coming from the blood vessels, stimulates Glial cell derived neurotrophic factor (GDNF) synthesis of surrounding Sertoli cells [55].

GDNF and fibroblast growth factor 2 (FGF2) are considered as the most important factors for the regulation of SSCs niche. The GDNF drive SSCs to self-renewal by binding glial cell line derived neurotrophic factor family receptor alpha 1 (GFRA1), a membrane receptor located at the surface of undifferentiated spermatogonia [10, 56, 57].

The secretion of GDNF is cyclic and coincident with the differentiation of SSCs to type A spermatogonia that are committed to spermatogenesis (density-dependent regulation), the lowest values of this peptide are found in last development stage near spermiation area [58].

Other important factors produced by Sertoli cells are leukemia inhibitory factor (LIF) and wingless-related MMTV integration site 5A (WNT5A), essential peptides that promote spermatogonial stem cell survival [4, 23].

2.4 Sertoli cell efficiency/spermatogenic efficiency

The key qualitative and quantitative determinants of sperm production are firstly the total number of Sertoli cells in the testicles and secondly their proper interactions with spermatogonial germ cells and the total number of these cells per Sertoli cells (Sertoli cell efficiency) [23, 28].

Sertoli cells show distinct capacities to hold germ cell development that varies among species. Each Sertoli cell can support a relatively fixed, species-specific, number of germ cells.

For instance, whereas chinchilla Sertoli cell can support 14 spermatids, each human Sertoli cell is able to support only 3 spermatids, resulting respectively in a huge difference in sperm production per testis gram per day (~60 vs. 4–4.5 million) between these species [1, 23, 25].

However, spermatogenic efficiency continually reduces and this characteristic is highly associated with the Sertoli cell support capacity, which decreases from around 100–150 (in fish) to 3 (in humans) spermatids for each Sertoli cell [23].

The Sertoli cells size and the space that they occupy in the seminiferous epithelium is another important factor to be considered. Species with reduced Sertoli cells occupancy in the seminiferous epithelium like mice for example (~15%), present higher spermatogenic efficiencies when compared to humans (~40%) [1].

Furthermore, the spermatogenic cycle lengths controlled by the germ cell genotype play a crucial role in determining the efficiency of spermatogenesis [1, 59]. The faster the cell differentiation process from spermatogonia to spermatozoa, the higher the daily sperm output is. If the spermatogenic cycle takes about 9 to 12 days, then the total duration of spermatogenesis (that takes almost ~4.5 cycles) will be 40 to 54 days. Spermatogenesis, in humans, takes a quite long duration (~70 days) [1].

However, germ cell loss particularly in mammalian, which is quite frequent during the spermatogonial and meiotic phases of spermatogenesis (DNA damage), influences significantly the total sperm production [60–63]. Therefore, Sertoli cell efficiency is critical in deciding the frequency of sperm production [1].

2.5 Relationship between germ cells and Sertoli cells

Spermatozoa production and maintenance throughout life is very complex and the fine regulation of spermatogenesis is under tight control and regulation [23, 28, 64–66].

Therefore, interactions among testicular cells, specially between germ cells and Sertoli cells, are crucial to preserve and regulate spermatogenesis in a very coordinated and organized manner, providing all the necessary structural and nutritional support for the developing germ cells. These interactions are important to ensure the development and completion of spermatogenesis [23, 25, 67].

At their different areas/regions, the Sertoli cells present the following contacts and functions with germ cells. In the basal compartment of the seminiferous epithelium, Sertoli cells regulate spermatogonia self-renew and differentiation [68], create contact with spermatocytes on its lateral side and, regulate the meiotic process from the duplication of DNA to the formation of spermatids [69]. In addition, in the adluminal/apical portions, Sertoli cells interact specifically with spermatids, regulate their morphology, controlling spermiation and reabsorb the residual bodies [70].

The germ cells are attached by desmosome-like, ectoplasmic specializations to Sertoli cells. On its basal side Sertoli cells contact spermatogonia through adherens junctions (AJ), guiding their homing, niche, and colonization [24]. At their adluminal aspect, Sertoli cells contact elongated spermatids through ectoplasmatic specialization, organizing the movement of these haploid cells as well as their release during spermiation [24, 71].

Germ cells like spermatocytes and early spermatids are attached by desmosomes and gap junctions. One of the most studied constitutive proteins of the gap junction is connexin 43 [72].

Also, the expression of connexin 43 differs according to the stage of germ cell development, suggesting that a particular group of germ cells can modulate this protein expression in somatic cells [73]. In humans, connexin 43 is observed in Sertoli, spermatogonia and spermatocytes cells, which suggests an accurate communication among these cells [74].

Domke et al. found that the Sertoli cells relate to each other and with the germ cells by special N-cadherin-based AJ-type junctions (Bareae adherents), which in

many regions are characterized by cytoplasmic AJ plaques containing proteins p120 and p0071, plakoglobin, and α- and β-catenin [38].

2.6 Seminiferous epithelial cycle, stages, and wave

The cycle of the seminiferous epithelium refers to all the cellular interactions that occur between Sertoli cells and developing germ cells within the seminiferous epithelium in both adluminal and basal compartment.

Consequently, various cycles of spermatogenesis coincide within the seminiferous epithelium at each given time. The duration of each cycle is 16 days [75]. In humans, the cycle happens in segments rather than simultaneously around the entire periphery of the seminiferous tubule as occurs in some animals (**Figure 1**).

The stages of the cycle of seminiferous epithelium can be differentiated based on meiotic divisions, the arrangement of spermatids in the germinal epithelium and the modification of the spermatid nucleus shape and based on the development of spermatids morphology including the acrosome [77, 78].

The arrangement of stages in the seminiferous tubule of a man is different from that in most other species. The patch work of stages is arranged in a helical pattern, so a single cross section contains cells from more than one stage. Many studies have highlighted the importance of using the seminiferous epithelium cycle (SEC) stages to better understand the kinetics of the seminiferous epithelium (SE) [68, 70].

In most animals there is a wave of spermatogenesis going in an orderly fashion down the seminiferous tubule. In the human, however, there is a mosaic arrangement of the six stages of spermatogenesis [79].

These stages are designed by Roman numerals. Each of these stages has a characteristic spermatid development step. Six stages were identified in cross sections of the seminiferous tubules and were described in the spermatid maturation step and termed as follow: Sa-1 and Sa-2, Sb-1 and Sb-2, Sc-1 and Sc-2. Each one of these stages might be characterized with morphological characteristics (**Figure 1**) [80].

The spermatogenic wave is a spatial organization along the length of the tubule occurring at a single moment in time. Morphological examination of cross-sections of seminiferous tubules reveals six typical cellular associations based on the type

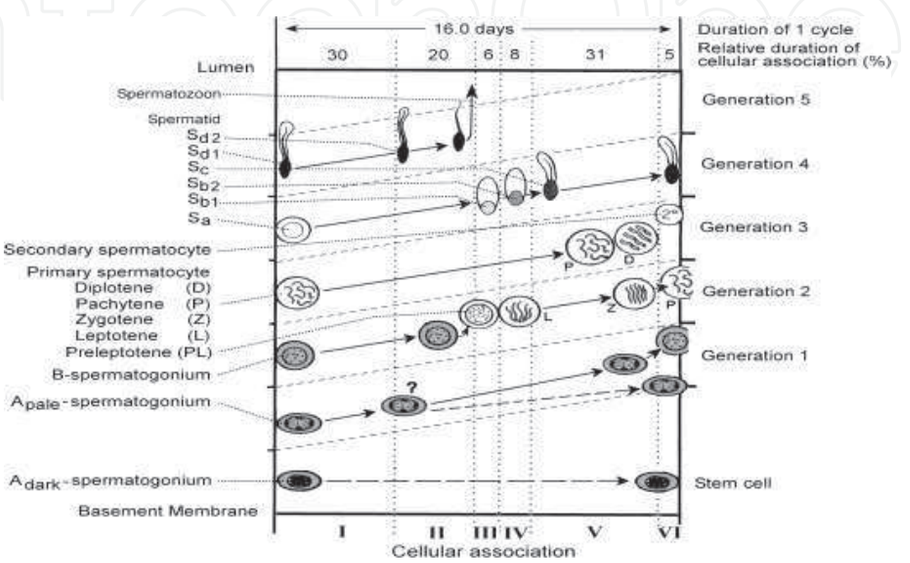

Figure 1.
The cycle of the seminiferous epithelium in humans (adapted from Amann [76]).

and stage of germ cells present in a given segment. A wave contains all 14 stages of the cycle, as well as any modulations that may be present in that segment of the tubule [76].

3. The blood testicle barrier/Sertoli cell-seminiferous epithelium barrier and spermatogenesis

Chiquoine was the first to describe the blood-testis barrier (BTB) or the Sertoli cell seminiferous epithelium barrier [81]. BTB is considered as one of the tightest blood-tissue barriers in the human body. It subdivides the seminiferous tubules epithelium into two compartments: the basal compartment and the apical compartment (**Figure 2**) [71, 82].

BTB is constituted almost exclusively by an inter-Sertoli cell junctional complex located near the basement membrane of the seminiferous tubule's epithelium. Behind the BTB localized the adluminal compartment which is a particular microenvironment that is significantly different from the interstitial space and the systemic circulation [7].

Many researchers have demonstrated that the BTB is established by actin-based tight junction (TJ), gap junction, intermediate filament-based desmosome, as well as basal ectoplasmic specialization (ES) [83–85].

However, different signaling molecules and signal pathways are controlling the BTB functions [83, 86]. Across the seminiferous epithelium in the testis, cellular events are tightly coordinated as shown by various researchers who demonstrate the existence of a local autocrine-based regulatory axis to coordinate these events. During spermiation this axis coordinates the release of spermatozoa at the apical

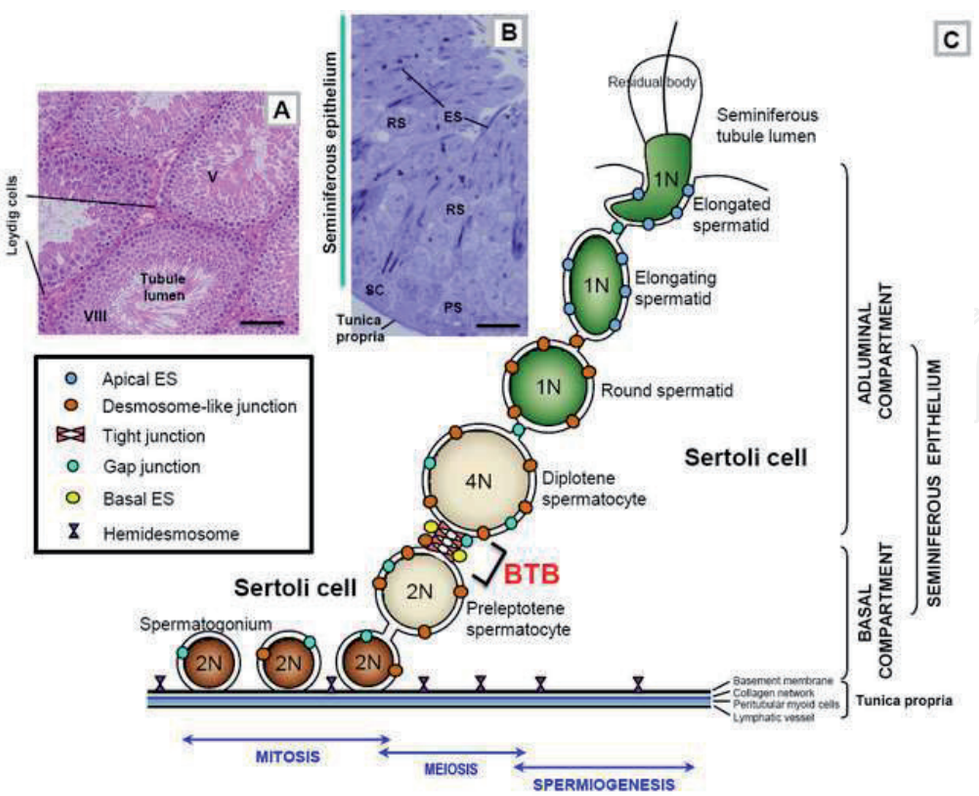

Figure 2.
The position of the BTB in the seminiferous tubules epithelium (B) of an adult mammalian testis (A) and its physiological relationship with developing germ cells during spermatogenesis (PS, pachytene spermatocyte; RS, round spermatid; ES, elongating spermatid) that are tightly associated with the Sertoli cells (SC) (adapted from Cheng and Mruk [82]).

ES at the luminal edge near the tubule lumen in the adluminal compartment at late stage III, which coincide with the remodeling of the basal ES/BTB to promote and support import of preleptotene spermatocytes that raised in the basal compartment traversing the immunological barrier at stage VIII of the epithelial cycle [1, 21, 87].

There are different types of distinct adherent Junctions (AJs) between the Sertoli cells and spermatogonial cells in the basal part of the Sertoli-Sertoli cells (heterotypic-basolateral junctions), and between Sertoli-Sertoli cells (homotypic), and between the adluminal pockets of the Sertoli cells and the spermatid heads (heterotypic-apical junctions). Therefore, Sertoli cell barrier considered one of tightest barrier in mammals [71].

Tight junctions (TJs) are the main component of the BTB that are found between adjacent Sertoli cells. They divided the seminiferous epithelium into basal compartment harbor spermatogonia and young spermatocytes and adluminal compartments where spermatocytes and spermatids are located [25, 71, 88].

Gap junctions, desmosomes, and two types of adherents junctions, testis-specific (tubulobulbar complexes and ectoplasmic specialization are other components of the sertoli cell barrier [25, 71, 88].

Spermatogenesis takes place stepwise in various segment of the seminiferous epithelium and is associated with extensive Adherent Junctions (AJs) restructuring between Sertoli cells and spermatogonial cells in the basal part of the Sertoli cells (heterotypic-basolateral junctions), and between Sertoli-Sertoli cells (homotypic), as well as between the adluminal pockets of the Sertoli cells and the spermatid heads (heterotypic-apical junctions) [41].

Spermatogonial renewal, differentiation, and cell cycle progression up to the preleptotene spermatocyte stage developed in the basal compartment of the epithelium outside the BTB.

These BTB undergoes reconstruction to allow the transit of preleptotene spermatocytes connected by intercellular bridges as clones at stage VIII of the seminiferous epithelial cycle of spermatogenesis [87, 89].

Therefore, preleptotene spermatocytes are the germ cells that pass the BTB as clones linked by intercellular bridges, that will differentiate into spermatocytes (zygotene and diplotene), to be pursued by two meiotic divisions (meiosis I and II) to form haploid spermatids in the apical compartment behind the BTB [89].

Meiosis I and II, spermiogenesis, and spermiation all take place in a specialized microenvironment in the adluminal compartment behind the BTB [71].

Therefore, the passage across the Sertoli cell barrier is a remarkable achievement, because the spermatocytes are no single cells, but form syncytia in which the cells are connected through cytoplasmic bridges [90].

Smith and Braun have provided critical insights of the molecular mechanism underlying this process. They revealed that an intermediate compartment enclosing the migrating spermatocytes was formed by "new" and "old" TJs above and below the spermatocytes, respectively [91].

Claudin-3 was transiently incorporated into the new tight junctions (TJs) and then replaced by claudin-11. Dissolution of the old TJs released the spermatocytes into the adluminal compartment. Also, when the syncytium moves toward the adluminal compartment, the BTB is opened on the adluminal side and is simultaneously closed on the basal side, at all to prevent the barrier from becoming leaky [91].

Therefore, BTB barrier created to protect germ cells undergoing meiosis from autoimmune reaction. The blood testes barrier was identified as a major barrier between the germinal epithelium and the interstitium of the testis. Also, this barrier established an immune privileged environmental with the seminiferous tubules [23, 42, 92].

By dividing the seminiferous epithelium, the BTB selectively inhibits the passage of many substances included in the general circulation [39, 93].

However, as a barrier, TJs restrict free passage of water, solutes, and ions. As a fence, TJs divide the plasma membrane into basolateral and apical regions, which confers cell polarity [94–96].

Nevertheless, during spermiogenesis, less than 25% of haploid spermatids become spermatozoa and at spermiation, could be liberated into the tubule lumen. The other portion of spermatids undergoes apoptosis [61, 97].

4. Spermatogenesis

Spermatogenesis is a very well-organized temporal process. It included different steps leading to a chronological evolution from totipotent, primitive stem cells (spermatogonial stem cells, SSCs) to a spermatogonium transformed to a specialized cell: Spermatozoon. Also, spermatogenesis is a highly organized process in which the germ cells go through several divisions and intricate differentiation steps, resulting in the production of the spermatozoa (**Figure 3**) [80].

4.1 Germ cell migration and development

Germ cell development is a lengthy and complex process that starts with specification in the early embryo and proceeds through stages of migration, proliferation, epigenetic reprogramming, sex differentiation, and gametogenesis to ultimately produce mature oocyte or sperm [98].

Therefore, Germ cells have a pivotal role in development by transmitting genetic information to the next generation. During germ cell development, epigenetic marks are erased and subsequently re-established during gametogenesis [99].

Embryonic bipotent primordial germ cells (PGCs) are among the first lineages established in early embryonic development, and the successful passage of these dedicated precursors from their birthplace to the developing gonad (gonad primordia) ensures an adequate supply of gametes for reproduction in the adult [100–102].

In the embryo, PGCs migrate from the proximal epiblast to the gonadal ridge, where they are enclosed by Sertoli and peritubular myoid cells, forming the seminiferous cords. In the developing seminiferous cords, the PGCs become gonocytes,

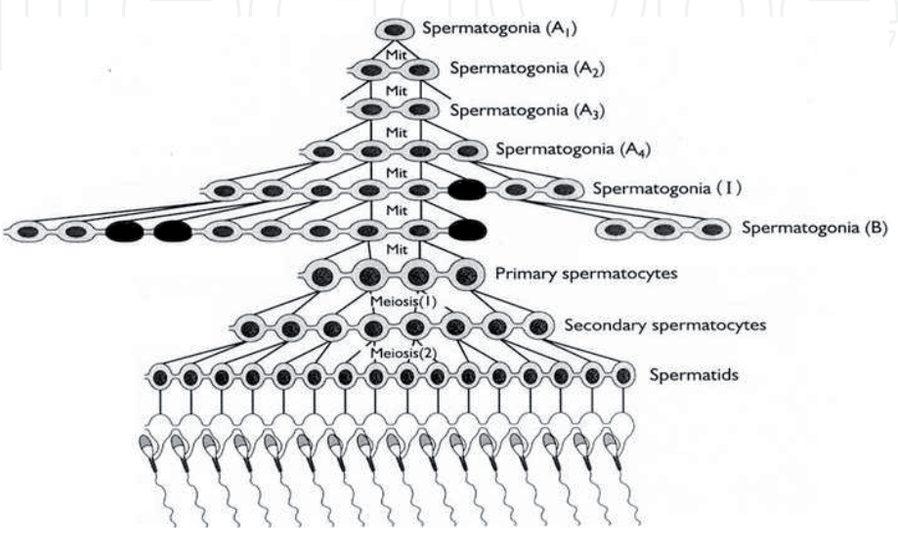

Figure 3.
Different steps of spermatogenesis leading from spermatogonia to spermatozoon.

which home to the basement membrane of the seminiferous tubules, where they differentiate into spermatogonial stem cells (SSCs) and initiate self-renewing divisions [103].

In other words, Primordial germ cells (PGC) take up residence at the testicular tubules during embryonic development, and undergo mitotic proliferation and differentiation, to become the spermatogonial stem cells which stand at the basis of spermatogenesis, starting at the onset of puberty [103]. By the end of PGC migration, around 5% of migratory cells remain outside the gonad and later undergo apoptosis [104].

During their migration however, heterogeneity of cellular behavior is observed due to change in cellular morphology from the time of specification to colonization [100].

SSCs, precursors originate from the PGCs, are usually located in a distinct position inside the seminiferous epithelium, referred to as the spermatogonial stem cell niche [105, 106].

SSCs within their niche either self-renew, remain quiescent, or generate spermatogonia committed to differentiation [4, 51, 107, 108].

Immature (fetal/neonatal) SSC precursors are commonly referred to as gonocytes or prospermatogonia, which are considered quiescent from the time of colonizing the seminiferous cords. This quiescence continues until they re-enter the cell cycle, migrate to the basement membrane, and undergo maturation and differentiation, either to constitute the SSC pool or differentiate into spermatogonia that will later become sperm [109–111].

Therefore, spermatogonial stem cells (SSCs) are the basis of spermatogenesis and maintain the continuous sperm production required for male fertility [107, 112].

More than a decade is required for prepubertal testis development and gonadal maturation in humans, generally characterized by the existence of a juvenile pause and an extended time span of prepubertal development [113–115].

Testicular tissue reactivation at the puberty, called gonadarche (earliest gonadal changes), occurs between 9 and 13 years in human. Before gonardache, there is a period of gonadal dormancy, characterized by low gonadotropin secretion, minimal testosterone secretion, discontinued Sertoli cell proliferation, and variable mitotic activity of germ cells human [116–118].

In humans, the neonatal maturation of the testis in mammals is commonly characterized by an early testosterone peak. The testosterone peak occurs after several months [119, 120].

It is associated with the movement of gonocytes to the basement membrane. Hence, this migration toward the basement membrane can take up to nine months [121]. Some studies have reported some spermatogonial heterogeneity neonatally and the appearance of differentiating spermatogonia prior to puberty [103].

4.2 Spermatogonial stage

The first phase in spermatogenesis is the proliferation and differentiation of spermatogonia.

The fetal spermatogonia develop first into transitional spermatogonia and then to spermatogonial stem cells forming the spermatogonial stem cell niche and located in the basal compartment of seminiferous tubule. These are classified into three categories: dark type (A_{dark}), pale type (A_{pale}) and type B spermatogonia [55].

Type A_{dark} spermatogonia reproduce via mitosis to generate both dark and pale spermatogonia. Throughout adult live, undifferentiated A_{pale} Spermatogonia (A_{pale}) periodically divide, giving rise to B spermatogonia (B). The A_{dark} spermatogonia (A_{dark}) are quiescent reserve cells [80].

Another proliferative spermatogonia type include type A isolated spermatogonia ($A_{isolated}$) which divided to form the type A_{paired} spermatogonia (A_{paired}). After 4 mitotic divisions, 16 cells of type A aligned spermatogonia ($A_{aligned}$) are formed will differentiate into A_1 spermatogonia to be followed by 6 mitotic divisions to form 1024 preleptotene spermatocytes [1].

However, some of type A spermatogonia transform to differentiated type A spermatogonia (A1, A2, A3, A4), intermediate spermatogonia (In) and then type B spermatogonia (B) (**Figure 3**) [1, 112].

It is important to mention that during the different stages of spermatogenesis, the spermatogonia remain connected by intercellular bridges to ensure the synchronization of the germ cell maturation and the biochemical interactions [122].

Besides, it is not yet clear if all cells within the type A spermatogonia pool are true spermatogonial stem cells because different studies have found counts that were significantly lower than the ones originally disclosed. Approximately 1/12–1/15 of the pool appears to be composed of true spermatogonial stem cells [51, 123].

4.3 Spermatocytogenesis

Spermatocytogenesis phase include the meiotic phase, in which primary spermatocyte in the basal compartment undergo meiosis I and meiosis II to give rise to haploid spermatids that are released from the seminiferous epithelium at spermiation area [77, 124].

Mitosis involves the proliferation and maintenance of spermatogonia. The mitotic phase involves spermatogonia (types A and B) and primary spermatocytes (spermatocytes I). Primary spermatocytes are produced by developing germ cells interconnected by intracellular bridges through a series of mitotic divisions. The tight junction barrier supports an early spermatocyte within the basal compartment and all subsequent germ cells within the adluminal compartment.

Type B spermatogonia undergo mitosis to produce primary spermatocytes, secondary spermatocytes, and spermatids. At stage VII of the epithelial cycle, type B spermatogonia differentiate into preleptotene, followed by leptotene spermatocytes, which are the primary diploid spermatocytes that cross the BTB while differentiating into zygotene spermatocytes at stages VIII-IX [125, 126].

Once in the adluminal compartment, spermatocytes undergo two consecutive rounds of meiosis at stage XIV. During the first meiotic division (reduction division), the primary spermatocytes divided to form secondary spermatocytes. The spermatocyte needs almost 26 days to be mature. Spermatocytes type I undergoes a long prophase in the first division, therefore they have the longest life span. The prophase of the second meiotic division is very short, thus secondary spermatocytes have a short life span [80].

4.4 Spermiogenesis

To obtain a hydrodynamic sperm head and to protect the paternal genome from any modifications during his journey through the male and female reproductive tracts, the human sperm DNA, in early spermiogenic phases, undergoes major cellular and nuclear changes [97].

Spermiogenesis is the process of differentiation of the spermatids into spermatozoa with fully compacted chromatin. A process of metamorphosis occurs from a round cell with typical organelles to a highly specialized, elongated cell. Later, the spermatid undergoes a series of morphological changes (Head, midpiece and tail) and their chromatin structure and function change [97].

The spermatid undergoes the Golgi phase, which is marked by the formation of the polarity. The head is at one end covered by Golgi apparatus developed later to acrosome and contain the synthesized proteolytic enzyme. The midpiece is at the other end, in which the mitochondria accumulates and one of the centriole pair elongates to form the tail or flagellum [97].

The post meiotic phase, progressive condensation of the chromatin occurs with inactivation of the genome. The meiotic phase involves primary spermatocytes until spermatids are formed, and during this process, chromosome pairing, crossover, and genetic exchange take place until a new genome is determined. Meiosis consists of two successive divisions to yield four haploid spermatids from one diploid primary spermatocyte. After the first meiotic division (reduction division), each daughter cell contains one partner of the homologous chromosome pair, and they are called secondary spermatocytes (2n). These spermatids are haploid with (22, X) or (22, Y) chromosome and undergo complete differentiation/morphogenesis known as Spermiogenesis [127].

During this process, morphological changes, chromatin structural and functional modifications occur once the process of meiosis is completed (**Figure 4**).

4.5 Chromatin remodeling during spermatogenesis

Sperm cells are remarkably different from somatic cells in their chromatin structure. During spermatogenesis, the majority of histones replace transition proteins and protamine (small highly basic proteins bound to the sperm DNA) [128].

So, in early spermiogenic phases, a major chromatin packaging takes place. The nucleosome-bound DNA configuration will be first destabilized by hyperacetylation of the canonical histones, which will neutralize the positive charge of lysine, reducing their affinity for DNA and by the DNA topoisomerase II (topo II), which will cause double and single DNA strands breaks to reduce the tension of the DNA [129].

The chromatin in the elongated nucleus became ten times more compacted than the chromatin in the nucleus of a somatic cell through progressive modifications (**Figure 5**) [130].

Post-translational modifications of the proteins facilitated the transition histone-protamine: Acetylation, ubiquitination, and phosphorylation of histone H4, phosphorylation and dephosphorylation of the transition proteins. (Adapted from Braun [130]).

Sperm chromatin is tightly packaged by protamines, while up to 15% of the histones remain in the mature human spermatozoa [131], these retained histones within the sperm nucleus possibly have a contribution on sperm function [132].

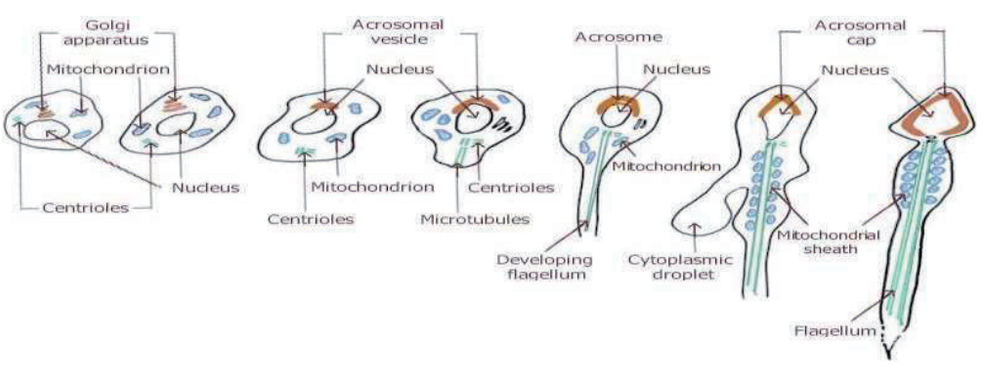

Figure 4.
Developmental changes in the spermatid [127].

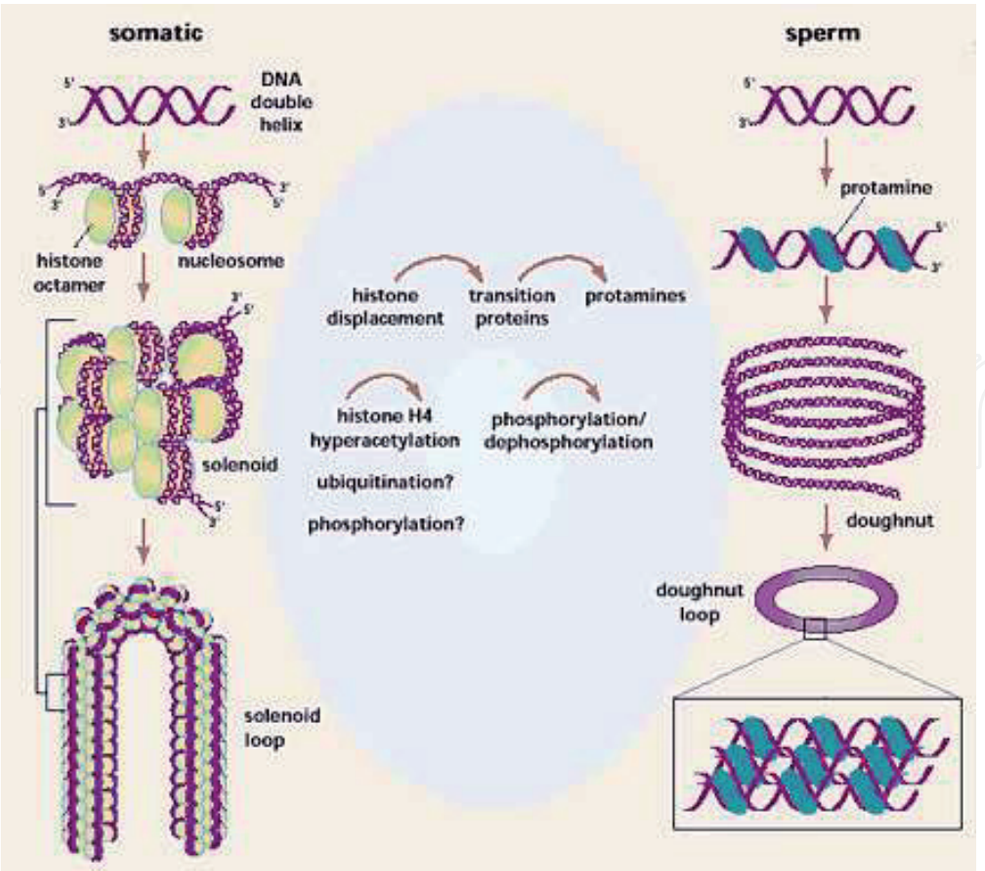

Figure 5.
A representation of the difference in the chromatin packaging between a somatic cell (left) and a sperm (right). The chromatin converted from a nucleo-histone structure (solenoid loop) into nucleo-protamine structure (toroid: Doughnut loop).

It concerns mainly the transcription sites of genes in sperms that are important for the preservation of the paternal genome epigenetics for their later expression during the early embryonic development [133]. The regulatory sequences [134], microRNA clusters, Transcription factors, paternally imprinted genes [132], the centromeric and telomeric DNA [135], retroposons [136], matrix associated regions [137], genes that produces rRNA, are transcribed at the final stages of spermatogenesis [138].

In fact, there are imprinted genes in the male genome, epigenetic changes in the DNA and nucleoproteins that edit the chromatin to make it ready for the control of the embryonic growth and development [139] and step by step the chromatin will be genetically silenced in the spermatozoa [140].

Also, after spermatocytogenesis, the chromatin structural changes will be more obvious when two smaller more basic proteins (10–20% lysine and arginine) named "Transition proteins" TP1 and TP2 are synthesized and deposed at the mid-stage of spermatids formation (**Figure 5**).

TP2 (13KDa) appears in step 1 and TP1 (6,2KDa) appears in step3. At this time, most of the core histones are eliminated, and the chromatin structure becomes more condensed. As their name indicate these proteins stay only for a short period of time attached to the DNA [141].

The transition proteins are then replaced by sperm-specific nuclear basic proteins (protamines), which are a synthesis in the last spermatid stage and play a vital role in the condensation and stabilization of sperm chromatin [142].

Humans sperm contain two protamines, protamine 1 (P1) and protamine 2 (P2), both are expressed in roughly similar quantities with a mean P1/P2 ratio of approximately 1.0 [143].

The sperm protamine 1 (P1) (51 AA) is the first to be synthesized as a mature protein [144]. The protamine 2 (P2) is formed as a precursor which is twice size as P1 (101 residues) and undergoes cleavage by proteolysis after its deposition onto sperm DNA to eliminate a short fragment of peptide [145]. Nanassy et al. suggested a clinical value of the protamine ratio between 0.54 and 1.43 for a fertile, normozoospermic man [146]. Any change in the ratio P1/P2 or between histone and protamines in the human sperm will be associated with the low compaction of human chromatin, which results in DNA fragmentation, lower fertilization rates, and reduced pregnancy rates [143, 147, 148]. Finally, a mature spermatid frees itself from the Sertoli cells and in a process called spermiation and enters the seminiferous tubule as a spermatozoon.

4.6 Hormone regulation of spermatogenesis

Spermatogenesis is controlled through several hormones. The first control is through a neurological pathway; the gonadotrophin-releasing hormone (GnRH) secreted by the hypothalamus stimulate the adenohypophysis to secret the luteinizing hormone (LH) and the follicle-stimulating hormone (FSH). The LH stimulates the Leydig cells to produce the Testosterone, and the FSH assists the Sertoli cells to support the spermatozoa during the different phases of spermatogenesis. Beside FSH and LH there are other hormones which plays crucial roles during spermatogenesis like the prolactin, and the growth hormone (**Figure 6**) [80].

Besides, anti-Mullerian hormone (AMH), which promotes the regression of Müller's ducts as the male foetus develops produced by Sertoli cells [149, 150]. In addition, inhibin and activin secreted by Sertoli cells, Activin increases the FSH levels needed for semen production. Whereas Inhibin regulate FSH secretion by the hypothalamus and helps maintain testicular homeostasis [149]. Sertoli cells only syndrome is characterized by the exclusive presence of Sertoli cells (without germ cells) in seminiferous tubules, making spermatogenesis impossible [151].

Furthermore, Leydig and Sertoli cells produce reproductive steroid hormones. Leydig cells secrete several different types of androgens, including dihydrotestosterone and testosterone, which modulate the development and maturation of spermatozoa [152].

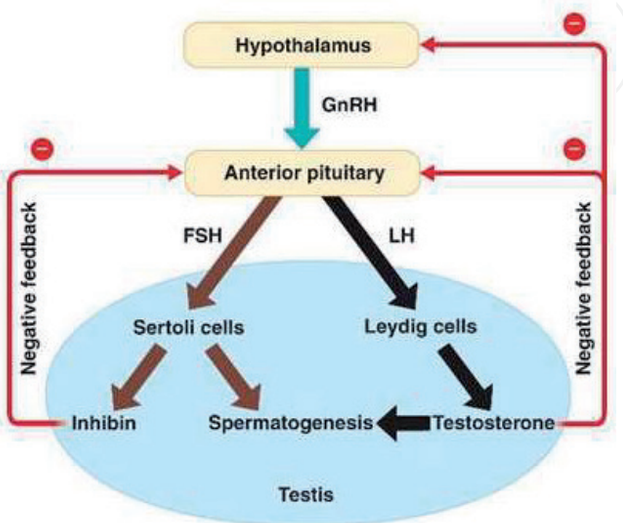

Figure 6.
Hormonal regulation of spermatogenesis.

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