Acta Scientific ANATOMY

Volume 2 Issue 12 December 2023

Review Article

Sertoli Cells: Architects of Male Reproductive Function and Clinical Impacts

Akunna GG*, Abah ED and Saalu LC

Department of Anatomy, Benue State University, Nigeria

***Corresponding Author:** Akunna GG, Department of Anatomy, Benue State University, Nigeria.

Received: November 06, 2023

Published: December 15, 2023

Abstract

The male testes are essential for sperm production and hormone regulation, with Sertoli cells as the unsung heroes orchestrating these processes. This review explores the world of Sertoli cells, discussing their structure, functions, and critical role in spermatogenesis. Named after Enrico Sertoli, these cells are pivotal within seminiferous tubules, forming the blood-testis barrier and providing support to developing germ cells. They also play a vital role in hormone regulation, fetal testis development, and various clinical conditions, including testicular tumors, male infertility, and androgen insensitivity syndrome. Understanding the intricate web of Sertoli cell biology not only enhances our comprehension of male reproductive health but also lays the groundwork for innovative diagnostic and therapeutic approaches in the fields of andrology and endocrinology.

Keywords: Sertoli Cells; Testes; Seminiferous Tubules; Leydig Cells; Germ Cells; Andrology; Reproductive Biology; Endocrinology

Introduction

Sertoli cells, often referred to as nurse cells, are a highly specialized type of somatic cell residing within the seminiferous tubules of the testes [1,3,6]. These exceptional cells assume a central role in the male reproductive system, providing indispensable support and regulation for the intricate process of sperm production, known as spermatogenesis [2,7]. Named in honor of the 19th-century Italian physiologist Enrico Sertoli, these cells form the cornerstone of the testicular microenvironment [8,9].

Sertoli cells possess a unique and intricate morphology, characterized by their elongated shape and close association with developing germ cells [3,8]. They take on the crucial responsibilities of structurally shaping the seminiferous tubules, nurturing and safeguarding germ cells, and coordinating the essential hormonal and paracrine signals required for spermatogenesis [3,4,11].

Arguably one of the most complex cell types in the human body [6,13], Sertoli cells exhibit an extraordinary ability to continually adapt their shape and functions to oversee the intricate process of spermatogenesis. The adult Sertoli cell boasts substantial dimensions, exceeding 3000 μm^3 in mice [4,8,19], with a flattened surface in contact with the seminiferous tubule's basement membrane and an extensive cytoplasm featuring numerous lateral and apical processes [7,10].

These cytoplasmic extensions engage with multiple germ cells as they progress from the earliest spermatogonia to the highly specialized elongated spermatids, which ultimately transform into spermatozoa when released from the seminiferous tubules [9,12,14].

Sertoli cells exhibit remarkable dynamism, perpetually adapting to support the development of up to five different germ cell types concurrently, including one to two types of spermatogonia, one to two types of spermatocytes, and one to two types of haploid spermatids, contingent upon the stage of the reproductive cycle [5,18]. The actual number of germ cells per Sertoli cell hinges on various factors such as species, Sertoli cell density within the tubules, prepubertal Sertoli cell development, variations in germ cell apoptosis

across cell types and species, and the influence of the endocrine milieu [6,7,17].

Spermatogenesis is a meticulously organized process, where specific cellular associations or stages group together various phases of spermatogonial, spermatocyte, and spermatid development, with Sertoli cells at each stage exhibiting distinct phenotypic and functional characteristics [1,2,8].

While the importance of Sertoli cells in sperm production has long been recognized [4,9], recent decades have unveiled their significance in other testicular somatic cell functions, including the regulation of steroidogenic Leydig cells [4,12,15]. This review aims to encapsulate how Sertoli cells are pivotal to optimal testicular function and how they dictate its dual outputs: sperm for fertility and androgens for virility.

Comprehending the roles of Sertoli cells is essential for unraveling the complexities of male fertility and reproductive health [1,3,16]. These cells provide indispensable support at various stages of spermatogenesis, fostering a nurturing microenvironment by maintaining pH levels, secreting growth factors, and overseeing nutrient and waste product flow [13,20,21].

Moreover, Sertoli cells act as a protective barrier between the bloodstream and developing sperm, shielding germ cells from the immune system's perception of sperm as foreign invaders [3,16,23]. This immune privilege is vital for the survival of developing sperm. Any disruptions or dysfunction in Sertoli cell activities can result in male infertility and broader implications for reproductive health [5,12,30].

This comprehensive review delves into the multifaceted world of Sertoli cells, illuminating their structure, functions, morphogenesis, and the profound clinical implications of their actions. It explores the latest research findings, shedding light on the role of Sertoli cells in testicular function, both in health and disease. The review covers various intriguing facets of Sertoli cells, including the cellular and molecular mechanisms governing their function, their interactions with other testicular cell types, and the consequences for male fertility and reproductive health.

With an ever-growing understanding of Sertoli cells and their pivotal role in testicular function, this review endeavors to bridge the gap between fundamental science and clinical applications, ultimately providing potential insights into new therapeutic strategies for male infertility and related disorders.

Anatomy of the sertoli cells

Location within the seminiferous tubule

Sertoli cells are critical components of the seminiferous tubules within the testes [1,9,14,26]. They are found within the wall of the seminiferous tubules and extend from the basal lamina to the lumen, creating a barrier that separates the seminiferous tubule into two compartments: the basal compartment (containing spermatogonia and early spermatocytes) and the adluminal compartment (containing more advanced stages of spermatocytes and spermatids [7,17,19,22]. Sertoli cells are aligned along the length of the seminiferous tubules and are essential for the support and nourishment of developing germ cells [16,40].

Specific Locations and arrangement within the tubules are as follows

- **Basement Membrane:** Sertoli cells are situated along the inner surface of the seminiferous tubules and are anchored to the basement membrane. This location provides structural support and a physical barrier within the tubules [8,14,29].
- **Adluminal Compartment:** Sertoli cells extend from the basal lamina to the lumen of the seminiferous tubules, creating two distinct compartments - the basal (or basal lamina-facing) compartment and the adluminal (or lumen-facing) compartment. This division is crucial for the process of spermatogenesis [16,20,23].

Structural components of the sertoli cells

Sertoli cell morphology presents one of the most intricate and three-dimensional structures in the realm of cell biology [4,11,27]. Enrico Sertoli's historic observations were made without the advantages of contemporary laboratory tools like effective fixatives, thin sections of embedded testicular tissues, and commonly used histological stains [2,6,38]. Nonetheless, he managed to describe unique branches of the cell's cytoplasm that support germ cell development and the nucleus featuring a substantial nucleolus, which has now become a pivotal morphological feature for cell recognition [4,18,21].

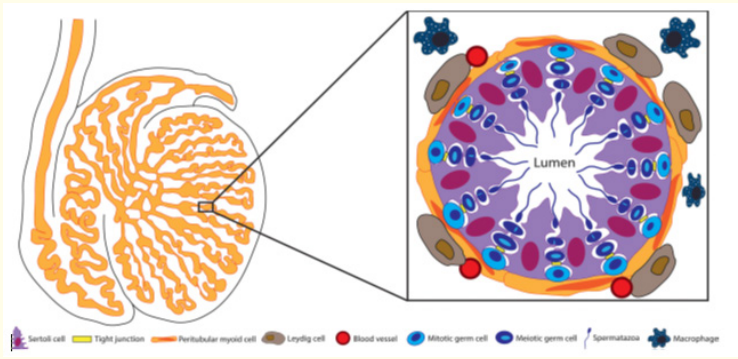

Figure 1: Structure of the Seminiferous tubule, showing the arrangement of Sertoli Cells within the Lumen [28].

Sertoli suggested that these cells were individual entities, while others contended that they formed a syncytium [5,9,31], sparking a long-standing controversy that remained unresolved until 1955 when electron microscopy finally unveiled cellular membranes and junctional complexes, putting the debate to rest [12,25].

Numerous reviews of Sertoli cell morphology have provided unique insights into the cell's interactions within the seminiferous epithelium, particularly focusing on the following structures: shape of the nucleus, the thin cytoplasmic arms, the intricate physical association with germ cells, the spermatid disengagement complex and the changes observed in these features throughout the cycle of the seminiferous epithelium [8,9,24,31].

These morphological features have contributed significantly to the overall beauty of this 'cellulemadri' or 'mother cell' [19,43]. Sertoli cells are specialized nurse cells with a unique structure [12,29]. They have several structural components that are important for their functions in the testes

- **Cell Shape:** Sertoli cells are elongated, columnar cells that span the entire length of the seminiferous tubules. Their elongated shape allows them to support and nurture developing germ cells [3,8,26].
- **Tight Junctions:** Sertoli cells form tight junctions between one another near the basement membrane, creating the blood-testis barrier. This barrier regulates the movement of substances between the bloodstream and the adluminal compartment [12,15,39].
- **Microtubules and Microfilaments:** Sertoli cells contain an extensive network of microtubules and microfilaments, which are essential for their structural integrity and the support of developing germ cells [18,40].
- **Nucleus:** The nucleus of Sertoli cells is prominent and contains a large nucleolus. It plays a crucial role in regulating gene expression and various processes associated with spermatogenesis [17,26,49].

The Sertoli cell nucleus is one of its most distinguishable organelles [14,20]. It is large, euchromatic, and capable of changing shape throughout the cycle of the seminiferous epithelium, often exhibiting deep invaginations of the nuclear membrane that is surrounded by vimentin intermediate filaments. The nucleolus is very large and stains intensely [48], with three distinct parts (tripartite), with most nucleoli have two satellite chromocenters in rodent species, although three satellite structures are found occasionally in a small percentage of mice [9,17,30,31]. In some species, the satellite chromocenters form donut shapes, but these structures often are out of the plane of section. The nucleus is usually described as residing near the basement membrane [5], however, in some species the nucleus can be located higher in the epithelium near the lumen, as is common in stages surrounding spermiation in rodents. When staining for Sertoli cell nuclear proteins, the more apical nuclei are easily recognized, as seen with the androgen receptor.

However, when a nuclear protein is present in both Sertoli and spermatogonial germ cells, such as the E2f family of transcription factors [46], care must be taken, as stages immediately following

spermiation have fewer spermatogonia and recognition of the Sertoli cells may require an evaluation of nuclear shape as well as the presence of a large nucleolus. Major immunohistochemical markers for the Sertoli cell nucleus that are commonly used for morphology include the following: androgen receptor (AR) [27]; SOX9 (SRY-box containing gene 9) [16]; Wilms tumor protein-1 (WT1) [21]. GATA-binding protein 1 (GATA1) [6]; GATA-binding protein 4 (GATA4) [45]; and cyclin-dependent kinase inhibitor 1B (p27kip1) [3]. Age-specific expression of these markers is an important consideration [18], as SOX9 is strong prenatal but decreases dramatically post birth [9], whereas WT1, is present in the Sertoli cell nucleus throughout all developmental ages and AR shows increasing expression after the onset of puberty [13,21]. GATA4 is expressed throughout development [17,43], and does not vary with the cycle of the seminiferous epithelium in the adult. In addition, GATA4 is not inhibited by the presence of germ cells, which is a problem with GATA1 expression [10,11].

Connection to adjacent structures and cells

Sertoli cells interact with adjacent structures and cells within the seminiferous tubules; they play a crucial role in supporting and nurturing developing germ cells and are closely connected to adjacent structures and cells in the seminiferous tubule [4,8,25]. which are essential for their function

- **Spermatogonia:** Sertoli cells provide a niche for spermatogonia, supporting their self-renewal and differentiation. Sertoli cells provide physical and nutritional support to germ cells through gap junctions and direct contact [16,2029,34].
- **Leydig Cells:** Sertoli cells interact with Leydig cells in the interstitium of the testes, facilitating communication through paracrine signaling to regulate testosterone production and maintain the testicular microenvironment [1,5,18].
- **Myoid Cells:** Myoid cells are located in the peritubular wall of the seminiferous tubules and assist in the movement of sperm through the tubules. Sertoli cells play a role in coordinating this movement [26,35].
- **Blood-Testis Barrier:** Sertoli cells create the blood-testis barrier, which isolates the developing germ cells from the bloodstream, preventing immune system recognition and damage [15,24,28].
- **Sertoli-Sertoli Junctions:** These tight junctions create the blood-testis barrier and are essential for maintaining the microenvironment of the adluminal compartment [3,18,32].

Development of the sertoli cells

The first appearance of fetal Sertoli cells in the primitive gonad defines the initial stage in the development of the embryonic testis [7,19,35]. These cells express the *sry* gene, thus determining the male sex of the gonad [2,21]. As the exclusive source of anti-Müllerian hormone, Sertoli cells are also responsible for inhibiting the development of internal female genitalia [6,20,48]. These cells together with another type of somatic testicular cell, the peritubular cell, are required for formation of the testis cords [11,26,29,40]. The immature Sertoli cell differs extensively from the mature cell with respect to both morphology and biochemical activity [4,19]. The structure of the immature seminiferous tubule (cord) also differs, containing in addition to Sertoli cells, only peritubular and germ stem cells (spermatogonia) [11,15,27,38]. Germ cell differentiation has not yet begun and the seminiferous cords are solid, lacking any lumen [5,21,23].

As puberty approaches, the Sertoli cells become elongated and tight junctions are established between them [12,17,29]. These cells begin to produce seminiferous fluid, which results in the transformation of the testis cords into seminiferous tubules possessing a lumen [30,42,47]. The differentiated, mature Sertoli cell alters its pattern of protein expression, starting to produce, e.g., transferring [23] and the inflammatory cytokine IL-1 [16]. Immature Sertoli cells divide constantly, but this proliferative activity declines in connection with puberty and after tight junctions have been formed, Sertoli cells are considered to no longer exhibit proliferative capacity [9,14,25,33]. These pronounced differences between the immature and mature Sertoli cell indicate that it is as important to understand the differentiation process as to characterize the functional regulation of the mature cells [26,30].

Functions of the sertoli cells

Sertoli cells are key determinants of testis size

Sertoli cells proliferate during the fetal and neonatal periods, ceasing proliferation during puberty when they commence their terminal differentiation into the adult form [12,17]. Some low lev-

el of Sertoli cell proliferation during adulthood may occur under certain circumstances [15,19,28], for example in some forms of human male infertility and in the seasonally breeding Djungarian hamster [20,23]. Adult mouse and human Sertoli cells can resume proliferation in vitro, suggesting they could be arrested proliferating cells rather than terminally differentiating cells [16,27,44]. Nonetheless, it is generally considered that the degree of Sertoli cell proliferation prior to puberty is a major determinant of the size of the adult Sertoli cell population which, in turn, is a major determinant of adult testis size [17,35,40].

During embryonic testis development, fetal Sertoli cells aggregate and enclose precursor male germ cells, the gonocytes, to form the testis cords which ultimately become seminiferous tubules in the adult testis [27,49]. Transgenic mouse models demonstrate that defective fetal Sertoli cell proliferation and/or survival at the onset of cord formation reduces cord expansion and lengthening [23,38,41] and causes cord involution and the development of blind-ending tubules that ultimately impact on testicular size and architecture in adulthood [12,16,28]. Ablation of fetal Sertoli cells after cord formation also leads to a major reduction in adult testis size [50]. Other models show that the maintenance of fetal testis cord structure, even after their formation, is essential for normal testis development [23,30]. This process depends on Sertoli cells, including signals from interstitial cells to Sertoli cells, and on the continued expression of key Sertoli cell factors [9,18,24]. For example, members of the transforming growth factor (TGF) β superfamily, such as activin A from the interstitial cells [23] and ECM proteins produced by peritubular myoid cells are just some of the intercellular signals required to maintain fetal Sertoli cells and cord development [23,25].

TGFβ and activins/inhibins Thus, fetal Sertoli cell function, proliferation and survival underpin the formation of the testis cords which, in turn, influences the normal development, size and function of the adult testis [12,19,24].

The proliferation of Sertoli cells after birth and prior to puberty also dictates adult testis size [3,16].

Sertoli cells proliferate continually from birth to puberty in rodents however in humans, proliferation occurs after birth and before puberty, with a period of quiescence in between [14,19,34]. The ablation of Sertoli cells early in neonatal life leads to a major and permanent reduction in adult testis size [26]. Postnatal Sertoli cell proliferation is regulated by endocrine and paracrine factors including activin, estrogen, thyroid hormone and follicle stimulating hormone (FSH); alterations in these factors in the postnatal period cause a permanent change in adult Sertoli cell numbers and size of the testis [6,17,24]. For example, alterations of thyroid hormone function in neonatal rodents have a major influence on the period of Sertoli cell proliferation and entry into the terminally differentiated state, and a corresponding permanent impact on adult testis size [5,16,19].

Once lost during fetal or postnatal development, Sertoli cells cannot increase their proliferation to "catch up" prior to puberty, nor do they appear to be regenerated from stem cells [19,36]. Thus, the proliferation, survival, differentiation and maturation of Sertoli cells prior to puberty is a major determinant of adult testis size [23,29,35].

Sertoli cells as the precursors of spermatogonial stem cells (SSCs)

Sertoli cells are the first somatic cell type to be specified during embryonic development and thus play a key role in initiating and maintaining testis development [7,18,28]. Sertoli cells also regulate germ cell specification to the male pathway [13,25,32]. A key cell fate decision in the embryonic testis is the entry into meiosis for female germ cells or the suppression of meiosis, followed by mitotic quiescence, in male germ cells [35,37,40]. Sertoli cells direct this process in the male by producing CYP26B1 that degrades the meiosis-promoting factor retinoic acid (RA) in the gonocytes [16,21]. to prevent meiotic entry, and producing other factors that suppress gonocyte proliferation and promote entry into mitotic arrest, including androgens, Nodal and Activin, and FGF9 [1,14,28]. Paracrine signals from Sertoli cells, such as TGFβ family proteins, maintain gonocyte mitotic quiescence until birth [36,50].

Spermatogonia are a heterogenous precursor germ cell population containing cells that are capable of renewing their population, the spermatogonial stem cells (SSC), as well as cells that proliferate and ultimately become committed to progression through the spermatogenic cycle [2,29,30]. Defects in undifferentiated spermatogonia can cause sub- or infertility or lead to the development of testicular germ cell tumours [20,22].

After birth, a key event in the establishment of spermatogenesis is the migration of gonocytes to the base of the seminiferous tubules, and their resumption of proliferative activity, to establish the spermatogonial population [11,14,44]. The resumption of proliferation and attainment of the spermatogonial fate after birth is likely achieved by a balance of factors produced by Sertoli cells after birth: the withdrawal of factors that inhibit proliferation, such as TGFβ ligands, and an increase in stimulatory factors, such as GDNF and FGF2 [6,14,25,33]. Migration of gonocytes to the basement membrane occurs simultaneously with the resumption of proliferation [14,23,27]. Sertoli cell factors that regulate gonocyte migration include KIT ligand (KITL) and certain chemokines, whereas Sertoli cell-derived GDNF, FGF2 and CXCL12 stimulate proliferation [23,27,44].

The maintenance of the SSC population is essential for lifelong spermatogenesis and SSCs are maintained within a specialized microenvironment [26,40], called a "niche", that supports their self-renewal [48].

SSCs reside on the basement membrane of the seminiferous tubule in direct contact with Sertoli cells [15,19]. SSC niches are preferentially located adjacent to the vasculature in the interstitium, and local production of factors such as FGFs produced by lymphatic endothelial cells regulates the size and density of the SSC population within the niche [7,13,34,46].

Sertoli cells are a major contributor to SSC niche function and experimentally increasing the number of Sertoli cells causes a corresponding increase in the number of SSC niches [6,12,37]. Sertoli cells produce GDNF, under the influence of Follicle Stimulating Hormone (FSH) from the pituitary and regulated by Notch signaling, which acts on receptors within SSCs to support lifelong maintenance of SSC niches [2,8,19,35]. Other Sertoli cell-derived factors also support maintenance of the niche, including FGF2 and leukemia inhibitory factor (LIF) [17,25]. In the adult, SSC self-renewal appears to occur during certain stages of the seminiferous cycle [16].

While some spermatogonia remain committed to an SSC fate, others become committed to differentiation and meiosis and thus entry into the spermatogenic cycle [30,43]. There is a large body of evidence that retinoic acid (RA) is the primary inducer of this transition in the postnatal testis [46]. A lack of RA-producing enzymes in Sertoli cells results in failure of spermatogonia to commit to entry into meiosis [13,19,42].

While entry into meiosis during the first wave of spermatogenesis requires RA, continued spermatogenesis is also reliant on continual pulses of RA along the seminiferous tubules [1,14,27,39]. There are progressive pulses of RA along the length of the seminiferous tubules as spermatogonia enter into the cycle and these pulses are thought to trigger the cyclic nature of spermatogenesis [8,13,19]. The pulses are generated by the coordinated expression of proteins that synthesize, store and metabolize RA within the seminiferous epithelium; Sertoli cells contribute to this pulse generation and cooperate with other cells to generate the pulse [23,29,47]. The Sertoli cell contribution to the RA-dependent commitment of spermatogonia to entry into meiosis is particularly important during the first wave of spermatogenesis, but becomes less important in adulthood [24,27,43]. This latter observation may explain why spermatogonia populations are less sensitive to a loss of Sertoli cells than later germ cells types [12,25,34].

Ultimately, the Sertoli cells are essential for the establishment of lifelong spermatogenesis by facilitating the specification of germ cells to the male pathway of development, supporting gonocyte and spermatogonial development and mitotic function during fetal and postnatal development, contributing to the maintenance of the SSC niche and supporting the entry of spermatogonia into spermatogenesis, particularly during the pre-pubertal period.

Sertoli cells guide spermatogenesis; dictates meiotic and post – meiotic development and sperm output.

The commitment of spermatogonia to meiosis is a key step in germ cell progression through spermatogenesis [21,38,39]. Meiosis produces haploid germ cells that undergo spermiogenesis to form specialized, elongated spermatids that will be released from the seminiferous epithelium [15,17,20]. Sertoli cells support meiosis, spermiogenesis and spermiation, and progressions through these phases of germ cell development are key determinants of sperm output from the testis [8,12,34].

Meiotic and post-meiotic germ cell development takes place within a specialized microenvironment within the seminiferous epithelium [16,24]. A key determinant of this microenvironment is

the formation of specialized junctions between Sertoli cells which contribute to the so-called blood-testis-barrier [3,17,20,26]. These specialized junctions are comprised of adherens, gap and tight/occluding junctions interspersed with actin-based Sertoli cell cytoskeletal structures and short tubulobulbar complexes [20,24,49].

These junctions form at puberty and prevent the free passage of substances into (and out of) the adluminal part of the seminiferous tubule and can exhibit molecular weight selectivity depending on the presence of particular germ cell types in the epithelium [4,9,39,50]. Sertoli cells express occludin and claudin-11 that are major regulators of these tight junctions, and mice lacking these proteins exhibit defects in spermatogenesis [9,39]. The formation and function of these junctions is regulated by FSH and testosterone acting via receptors within Sertoli cells [6,21,27]. Sertoli cell junctions are also responsive to paracrine signals; for example, retinoic acid stimulates, and activin A disrupts Sertoli cell tight junctions [48].

Cooperativity between RA and activin signaling in Sertoli cells may be an important mechanism by which these junctions are remodeled to allow newly formed spermatocytes to move across the barrier into adluminal compartment [14,24,27].

Sperm output from the testis relies on germ cell development and survival as they progress through spermatogenesis [2,16]. This support is largely orchestrated by Sertoli cells which produce a multitude of factors required by germ cells, such as metabolizing glucose to lactate, the preferred substrate for germ cell glycolysis [19,41,47]. Sertoli cells show extraordinary stage-specificity in mRNA and protein expression as they adapt to the changing needs of the germ cells, and produce non-coding RNAs that also regulate protein expression [12,18,35,46]. As germ cells develop, they adhere to Sertoli cells via different adhesion proteins and junction types, including gap, desmosome and adherens junctions [1,35,46]. As spermatids progress through their elongation phase, they are translocated through the epithelium via microtubules in Sertoli cells linked to a highly specialized junctional structure termed the ectoplasmic specialization [25,29,33]. The number of adult Sertoli cells dictate the numbers of meiotic and post-meiotic germ cells in the testis, highlighting the fact that Sertoli cells are a major determinant of the degree of spermatogenesis in the testis [19,21,47].

Optimal spermatogenic output relies on two main hormones, FSH from the pituitary, and androgens, such as testosterone, produced by the interstitial Leydig cells, and both of these hormones are required for quantitatively normal spermatogenesis [4,25]. Sertoli cells, rather than germ cells, express receptors for FSH and androgen and thus are required to "transduce" these signals to germ cells, however it's important to note that androgen-responsive signaling from other somatic cells such as peritubular cells also support spermatogenesis [13,15,29,46].

Androgen receptor signaling in Sertoli cells is required for the completion of meiosis and to ensure germ cells in early meiotic prophase acquire competence for meiotic division [7,19].

Progression through spermiogenesis also relies on androgen-dependent signals from Sertoli cells [5,11,32,33]. Studies in mice with Sertoli cells lacking androgen receptors reveal many genes and proteins in Sertoli cells that likely participate in the androgen-dependent regulation of germ cell development [35,41,43]. For example, Sertoli cells provide key signals to support germ cell survival as they proceed through spermatogenesis, and the withdrawal of FSH and/or testosterone results in the induction of apoptosis at particular stages of germ cell development, reducing spermatogenic efficiency [17,21,24].

Sperm output from the testis is determined by the number of elongated spermatids produced during spermatogenesis, but also by the ability of these spermatids to be released by Sertoli cells during a process termed spermiation. Sertoli cells perform a "quality control check" during spermiation, whereby sperm with abnormal morphology may not be released [6,14,17,26]. Spermiation is highly sensitive to acute alterations in hormones and disturbances in Sertoli cell signaling pathways, and spermiation, its failure is characterized by the retention and phagocytosis of spermatids within Sertoli cells [20,23,48]. Failure of spermiation is often an acute feature of alterations to Sertoli cell function, and whether Sertoli cells release all elongated spermatids is a key determinant of sperm output from the testis [17,20,37].

Sertoli cells enhance the production of androgens by maintaining the development and functions of leydig cells

It has long been established that Sertoli cells drive germ cell development and sperm output from the testis [1,5,19,28,31]. How-

ever, a new experimental approach involving the specific ablation of Sertoli cells at various stages of testis development defined new roles for these cells as orchestrators of multiple testis functions [24,28,31].

Leydig cells are steroidogenic cells whose major role is to produce androgens, such as testosterone, needed for fertility and virility [25,27,39].In the mouse fetal testis, Leydig cells express CYP17A1 to produce high levels of the androgen precursor androstenedione, which is then converted into androgen (testosterone) in the Sertoli cells by the HSD17B3 enzyme [12,25,29].

The production of androgen within the seminiferous tubules at this time appears to regulate entry of gonocytes into mitotic quiescence [3,17,39]. While Leydig cells and Sertoli cells cooperate to produce androgen in the fetal testis, Leydig cells are the primary site of testosterone synthesis in the adult [11,24].

Fetal Leydig cells (FLC) do not express the sex-determining gene Sry, and thus their differentiation relies on signals from the Sry-expressing Sertoli cells [12,1,25]. Newly-specified Sertoli cells produce factors, such as desert hedgehog (DHH), that support the commitment of interstitial cells to the steroidogenic fate [22,26,49]. Once specified though, fetal Leydig cells become less reliant on Sertoli cells, and remain in the testis when Sertoli cells are lost after E15 in the mouse [3,18,27].

After birth, fetal Leydig cells are gradually replaced by adult Leydig cells (ALC) that gain the ability to produce androgens at the time of puberty [13,28,35]. ALC arise from a subset of de-differentiated fetal Leydig cells that remain after birth and this is directed by Wilms' tumour 1 (Wt1) expression in Sertoli cells [23,24,41]. The ablation of Sertoli cells in mouse testes from postnatal day 2–18 caused a decrease in adult Leydig cell numbers, due to reduced numbers of ALC progenitor cells in the pre-pubertal testis [1,17,19,36]. Furthermore, the number of Sertoli cells that are present during postnatal development predicts the number of Leydig cells in the adult testis [20-22]. Various lines of evidence indicate Sertoli cells produce paracrine factors that maintain ALC progenitors necessary for the development of a quantitatively normal ALC population [23-25].

Once the testis has developed and spermatogenesis is initiated, ongoing support from Sertoli cells is required for optimal Leydig cell function [26-28]. While ALC turnover is low and populations remain stable throughout adulthood, ablation of Sertoli cells from the adult testis causes ALC apoptosis and a marked reduction in ALC numbers [29,30,32].

A partial reduction in adult Sertoli cell numbers causes a corresponding decrease in ALC number and volume, indicating a dose-responsive relationship between Sertoli cells and the maintenance of ALCs [40-42]. Sertoli cell expression of Wt1 and the production of DHH is likely important for the maintenance and function of Leydig cells in the adult testis, although other Sertoli cell paracrine factors are likely required [43-46]. Androgen-dependent paracrine factors from Sertoli cells are also likely to regulate adult Leydig cell differentiation and function [47].

Reduced adult Sertoli cell number or function leading to reduced ALC numbers can result in reduced testosterone synthesis and an androgen deficiency phenotype, or functional compensation to maintain testosterone production [48-50].

Signals from Sertoli cells are required to specify the commitment of interstitial cells to a steroidogenic, fetal Leydig cell fate in the early embryonic testis, to maintain postnatal progenitor cells required to generate ALCs, and for the development, maintenance and normal steroidogenic function of Leydig cells in the adult testis [1-4].

Sertoli cells regulate peritubular myoid cells, immune cells and the vasculature of the testis.

Peritubular myoid cells (PMCs) are smooth muscle-type cells that reside external to the basement membrane of the seminiferous tubules and exhibit contractile properties, driven by Ca2 + signaling, to help propel released spermatids towards the rete testis [5-7]. PMCs support SSC, and in response to androgens from Leydig cells, produce factors necessary for Sertoli cell function, germ cell development and fertility [8-10]. Cell ablation studies show that Sertoli cells are required during the fetal and early pre-pubertal period (up to day 18) in mice to maintain PMC differentiation necessary for seminiferous tubule formation, and that the close proximity of PMCs with prepubertal Sertoli cells is required for the maintenance of their smooth muscle identity [31-33]. Sertoli cells may support PMC differentiation by producing extracellular matrix proteins and DHH required for PMC formation [34-36]. Once PMCs

have formed during the neonatal period, they persist in the testis even after long term Sertoli cell ablation, however they show reduced expression of functional markers and there is evidence of fibrosis and calcification around the tubules [37-39]. Thus, Sertoli cells are required for neonatal differentiation of PMCs and to support their normal function in adulthood [17,40].

The testicular vasculature enables cells in the interstitium and seminiferous tubules to access oxygen and nutrients from the circulation, and permits exchange of endocrine factors required to maintain the hypothalamic-pituitary-testis axis necessary for testis development and function [11-14]. Endothelial cell proliferation is a key driver of blood vessel development, or angiogenesis, and various studies have shown Sertoli cells can support endothelial cell proliferation and angiogenesis [15,19]. The importance of Sertoli cells in the maintenance of the testicular vasculature was recently revealed by an adult Sertoli cell ablation model [16-18].

The loss of adult Sertoli cells impeded testicular vasculature function within 30 days, due to reduced numbers of micro-vessels and vascular branches which in turn led to a reduced fluid exchange [5,17]. The presence of Sertoli cells supported micro-vessel development in xenografts, suggesting that Sertoli cells release factors that support angiogenesis [9,15,32]. Reduced vascular function in Sertoli cell-ablated testes impeded fluid exchange in the testis which could reduce endocrine factor exchange; indeed, Leydig cell testosterone production exhibited reduced responsiveness to hCG [3,25,27,39]. It therefore suggests that paracrine factors released by Sertoli cells are required for the ongoing maintenance and. regulation of the vasculature in the adult testis [18,23,35]. Indeed, in vitro studies show that Sertoli cells produce a range of pro-angiogenic factors that can support organization of endothelial cells into capillary formation [47].

The testis is a specialized environment that possesses immunosuppressive properties to prevent infection and recognition of developing germ cells by the immune system; this is important because sperm are first produced at puberty, well after the establishment of the immune system, and could thus be recognized as foreign [19,23,35]. The latter process is achieved via the testis being an immune privileged site and involves local immunosuppressive mechanisms and systemic tolerance [4,15,32]. Sertoli cells produce many immunomodulatory factors that can regulate interstitial immune cell function [27,31].

In transplantation studies, Sertoli cells produce anti-inflammatory cytokines and TGFβ which may protect Sertoli cell grafts from rejection, demonstrating their ability to modify immune responses and contribute to an immunosuppressive environment [26,29,43]. Testicular macrophages and other immune cells in the interstitium also have special properties that contribute to testicular immune privilege and their function is responsive to factors produced by Sertoli cells [35,42,47]. Sertoli cell expression and secretion of proteins that can regulate graft protection or immune privilege is being explored for an ability to support cell transplantation-based therapies [29,34,37]. Sertoli cell-derived immunomodulatory factors require androgens, and a loss of androgen receptor expression in Sertoli cells compromises Sertoli cell tight junction function and alters interstitial immune cells and immune privilege [21,24,35]. It has long been thought that Sertoli cells protect germ cells from immune recognition by sequestration of germ cell antigens within the seminiferous tubules, inside the so-called blood-testis-barrier [12,19,35].

However recent studies have shown that Sertoli cells release many germ cell-specific proteins into the surrounding interstitial fluid and at least some of these proteins may promote peripheral tolerance [3,14,36]. Thus, Sertoli cells may release germ cell-specific proteins to tolerize the immune system to a range of antigens, potentially limiting a more widespread immune response in situations where immune privilege is breached [16,29,31]. Moreso, it is important to note that immune cell infiltration into the seminiferous tubules was rarely observed after chronic ablation of Sertoli cells in adult mice, suggesting that peritubular myoid cells also play an important role in maintaining immune privilege within the seminiferous tubules [21,25,29].

Control/regulation of Sertoli cells

Control of Sertoli Cells in the testes is a crucial aspect of male reproductive function. Sertoli cells play a central role in the regulation of spermatogenesis and various other testicular functions. This control is mediated through a combination of hormonal and biochemical factors: Endocrine Regulation

- **Follicle-Stimulating Hormone (FSH)**: FSH is a key regulator of Sertoli cell function. It binds to its receptor on Sertoli cells, activating the cAMP (cyclic adenosine monophosphate) signaling pathway [4,13,16]. This stimulates the production

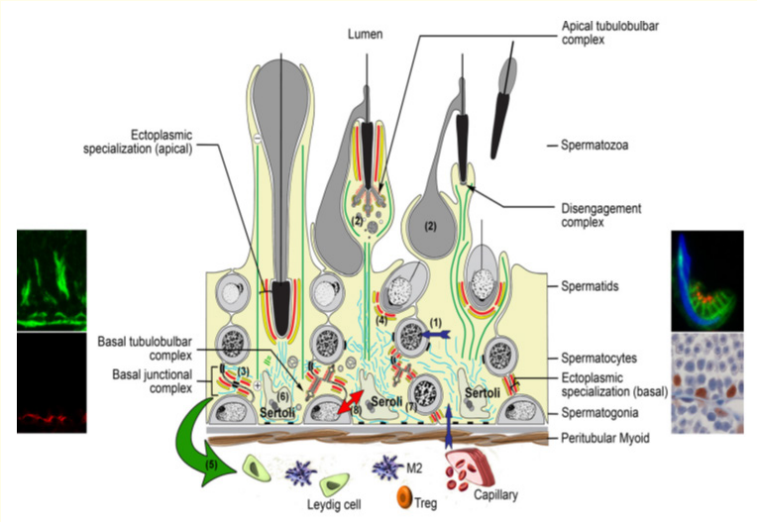

Figure 2: Schematic depiction of Sertoli cell functions during spermatogenesis, including: (1) nutrient transport through junctional complexes; (2) waste management and cytoplasm recycling; (3) blood-testis barrier maintenance; (4) germ cell adhesion and communication; (5) immune reaction inhibition and immune privilege maintenance; (6) endocrine signaling initiation and response; (7) seminiferous epithelium cycle initiation and regulation; and (8) stem cell homeostasis [16].

of proteins, such as androgen-binding protein (ABP) and inhibin, which are essential for spermatogenesis [13].

- **Testosterone:** Testosterone, produced by Leydig cells, acts on Sertoli cells to promote the development of sperm and maintain their function [12,14,23]. It also influences the expression of genes related to spermatogenesis and supports the overall structural integrity of the seminiferous tubules [23].
- **Insulin-Like Growth Factor 1 (IGF-1)**: IGF-1 acts as a local growth factor and influences Sertoli cell proliferation and function, enhancing their supportive role in spermatogenesis [45,47].
- **Estradiol:** Sertoli cells can convert testosterone to estradiol via aromatase enzyme activity. Estradiol may have autocrine or paracrine effects on Sertoli cell function, impacting spermatogenesis [12,43,50].
- **Thyroid Hormones:** Thyroid hormones regulate Sertoli cell activity by affecting the expression of genes involved in spermatogenesis and steroidogenesis [14,16,28].

Biochemical regulation

- **Glucocorticoids**: Glucocorticoids, such as cortisol, can affect Sertoli cell function by modulating the expression of genes involved in testicular function and immune responses [3,15,16].
- **Retinoic Acid:** Retinoic acid is involved in the regulation of spermatogenesis and can influence Sertoli cell differentiation and function [14,17,19].
- **Cytokines:** Various cytokines, including interleukins and transforming growth factor-beta (TGF-β), influence Sertoli cell function and contribute to the immunoregulation of the testes [6,9,37].
- **Glial Cell Line-Derived Neurotrophic Factor (GDNF)**: GDNF plays a critical role in maintaining the integrity and function of Sertoli cells and is essential for spermatogenesis [20,34].
- **Melatonin:** Melatonin receptors are expressed in Sertoli cells, and melatonin can modulate their function, potentially impacting testicular health [46].
- These biochemical factors and hormones work in concert to regulate Sertoli cell functions, ensuring the support and maintenance of spermatogenesis and other testicular activities. The interplay of these regulators is vital for male reproductive health.

Clinical implications

Therapeutic applications of Sertoli cells

Sertoli cells generate and maintain an immunoregulatory environment within the testes conducive to spermatogenesis and germ

cell survival [14,23,48]. In addition, it is well-known that their ability to regulate the immune response is not limited to the testes, which makes them attractive candidates for the treatment of various health issues such as transplantation and autoimmune disease [23,25,36]. For instance, Sertoli cells have been shown to improve symptoms and even reverse the disease state in animal models of type 1 and 2 diabetes [3,6,19] neurodegenerative diseases such as Parkinson's disease [13,36], amyotrophic lateral sclerosis [46], Alzheimer's disease [12], and Huntington's disease [25,29], male infertility [30,41]; and muscular dystrophy [42]. Most of these studies included transplantation of Sertoli cells either alone or with co-grafted cells, tissues, or organs. For cotransplantation, Sertoli cells have been shown to prolong survival of allogeneic pancreatic islets, renal cells and skin grafts, xenogeneic islets, neurons, adrenal chromaffin cells, liver cells and skin grafts, and even provide prolonged survival of allogeneic heart and kidney organs [13,34].

Additionally, Sertoli cells have been found to protect syngeneic islets from autoimmune destruction after transplantation into non-obese diabetic mice [15,18,27] and another study found encapsulated neonatal porcine Sertoli cells could prevent and reverse the onset of autoimmune diabetes in non-obese diabetic mice [19].

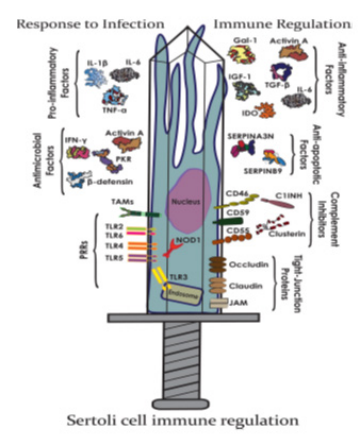

Figure 3: Schema of the Sertoli cell as a double-edged sword for Response to Infections and Immune Regulation [14].

Sertoli cell protection from pathogens

Remarkably, Sertoli cells can also protect against pathogens by initiating an antimicrobial response [13,19,37]. Sertoli cells express a wide range of pattern recognition receptors (PRRs) that can identify and react to a wide range of pathogens [19,37,40]. When PRRs bind to a pathogenic marker, they initiate signaling cascades, like NF-kB, that allow Sertoli cells to express and secrete various antimicrobial molecules such as IL-1b, IL-6, TNF-a, MCP-1, activin A, protein kinase R (PKR), and β-defensin [17,18,40]. This can recruit and activate immune cells such as M1 macrophages and T cells [21,40].

Sertoli cell antimicrobial response and delivery of antibiotics

b-defensins are antimicrobial peptides expressed by Sertoli cells in response to bacterial infections and viral-infected cells [9,13,41]. IFNs are antiviral peptides that also regulate Sertoli cell survival and function [18,34]. IFNs are important in antiviral responses by activating natural killer cells and macrophages [36]. IFNs can also activate and promote the function of inflammasomes within these leukocytes, aiding in clearance of viral infections [35,46]. Regarding adaptive immunity, IFN-g causes T cell polarization toward Th1 proinflammatory phenotype [9]. IFNs are also important in increasing expression of PKR in the presence of infection by viruses [28]. Sertoli cells also express the antiviral peptide PKR in response to stimulation by IFN-g [29]. The role of PKR in combating viral infections is to inhibit translation of viral mRNA and stimulate apoptosis of the infected cell [1,27,39]. Furthermore, PKR also participates in a positive feedback loop with IFNs, amplifying the antiviral response [10,32].

These peptides can recruit immune cells like macrophages in the case of resilient and persistent infection, mounting an effective phagocytic response against pathogens in the testicular interstitium to prevent invasion of pathogens into the seminiferous tubules [20,31,35]. Furthermore, Sertoli cells may be able to influence another antimicrobial and antiviral immune cell, natural killer (NK) cell in case of infection [26]. NK cells are not commonly found in the testes but can migrate there during severe inflammation or infection [50]. NK cells can mount a strong, innate response against viral infected cells and other microbials by secreting proinflam-

matory molecules and through cytolysis [29,34,45]. Interestingly, when NK cells (human, mouse, or guinea pig) were co-cultured with 20-day old rat Sertoli cells, their inflammatory functions were inhibited in a dose-dependent way, but their ability to lyse cells was not affected [31,45,47].

This implies that Sertoli cells may be able to modulate their inflammatory response to prevent collateral damage while still allowing NK cells to fight infection [24,38]. As part of the normal process of spermatogenesis, Sertoli cells phagocytose apoptotic germ cells and residual bodies [37].

These phagocytic abilities also allow Sertoli cells to phagocytose bacteria [15]. However, phagocytosis of Staphylococcus aureus by Sertoli cells differed from macrophage phagocytosis in that Sertoli cells used alpha-defensin to kill the bacteria without eliciting an inflammatory response [13,19]. This was not as effective at killing the bacteria as macrophage phagocytosis [37,41]. Taking advantage of these phagocytic properties, Sertoli cells were loaded with microparticles containing the antibiotic complex ofloxacin and palladium [16,28].

The combination of antibiotic and Sertoli cell antibacterial factors resulted in significant killing of Pseudomonas aeruginosa [36]. Overall, Sertoli cells can act as a double-edged sword capable of producing immunomodulatory factors that not only protect the germ cells from an autoimmune response but that can also fight of pathogens [4,49].

Sertoli cell-related disorders

Sertoli cells play a crucial role in testicular function, and any dysfunction can lead to various clinical conditions [34,46]. Sertoli cell-related disorders can have significant clinical implications, as these cells play a crucial role in testicular function and spermatogenesis. Understanding these disorders is essential for diagnosing and managing various male reproductive health issues. Some of the disorders include

- **Sertoli Cell-Only Syndrome (SCOS):** A condition characterized by the absence of germ cells in seminiferous tubules, leading to male infertility [21,43].
- **Testicular Dysgenesis Syndrome:** A complex disorder involving Sertoli cell dysfunction that is associated with testicular cancer, cryptorchidism, and hypospadias [30,42].
- **Sertoli-Leydig Cell Tumors:** Rare ovarian tumors with both Sertoli and Leydig cell components, which can produce androgens, leading to virilization in females [28,31,39].

Overview of Sertoli cell – related research techniques and tools

Understanding the structure and functions of Sertoli cells, their morphogenesis, and clinical implications involves a range of research techniques and tools. Researchers in this field commonly use the following:

- **Histology and Immunohistochemistry**: These techniques are essential for studying the structural characteristics and protein expression of Sertoli cells within testicular tissue.
- **Electron Microscopy:** Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) help visualize ultrastructural details of Sertoli cells.
- **Molecular Biology Techniques:** PCR, RT-qPCR, and Western blotting are used to investigate gene expression and protein levels in Sertoli cells.
- **Cell Culture:** Sertoli cells can be isolated and cultured to study their physiology and interactions with other testicular cell types.
- **Genetic and Knockout Models:** Genetically modified animal models are used to explore the consequences of altering specific Sertoli cell genes.
- **Proteomics and Transcriptomics:** These techniques provide a broader view of Sertoli cell function by analyzing the complete set of proteins and transcripts in these cells.
- **Functional Assays:** Assessing the role of Sertoli cells in supporting spermatogenesis can be done through various functional assays, such as barrier function studies and hormone production assays.

Current areas of ongoing research on the Sertoli cell

Sertoli cell research is a dynamic field, and there are several current areas of active investigation. Some potential topics to explore in this section of your review article might include:

- **Sertoli Cell Stemness**: Investigating whether Sertoli cells possess stem cell-like properties and their potential role in regenerative medicine [29].
- **Sertoli Cell and Male Contraception**: Research on using Sertoli cell functions to develop male contraceptives [43].

- **Sertoli Cell and Infertility**: Understanding how Sertoli cell dysfunction contributes to male infertility and potential therapies [37].
- **Sertoli Cell-Mediated Testicular Immunology**: The role of Sertoli cells in maintaining immune privilege in the testis [17].
- **Epigenetic Regulation of Sertoli Cells**: How epigenetic modifications impact Sertoli cell function and testicular health [12].
- **Sertoli Cell and Testicular Tumors**: Investigating the link between Sertoli cells and testicular tumors, including potential diagnostic and therapeutic implications [31][43].

Conclusion

Sertoli cells are integral to the testicular microenvironment, providing structural support, nourishment, and a nurturing milieu for developing germ cells. Their functions encompass the regulation of spermatogenesis, hormone secretion, and immunomodulation within the testes. The critical role of tight junctions, gap junctions, and hormonal signaling pathways in coordinating these functions cannot be overstated. Sertoli cells serve as guardians of germ cell development, orchestrating the complex process of sperm production while maintaining an immunoprivileged sanctuary within the testes. The clinical significance of Sertoli cells cannot be understated.

Dysregulation or dysfunction of these cells can lead to a wide array of male reproductive health issues, including infertility, cryptorchidism, and testicular tumors. Understanding the intricate molecular mechanisms governing Sertoli cell function opens up promising avenues for clinical intervention. Targeting Sertoli cells in research and therapeutic approaches may lead to novel treatments for male infertility and other testicular disorders. Emerging technologies such as gene editing and regenerative medicine hold great promise in this regard.

Future research should focus on a more profound understanding of the molecular signaling pathways that underlie Sertoli cell functions. Exploring the genetic and epigenetic factors influencing their differentiation and maintenance could lead to innovative diagnostic tools and therapeutic strategies. Additionally, investigating the potential crosstalk between Sertoli cells and neighboring cell types, such as Leydig cells and peritubular myoid cells, may unveil novel insights into testicular function and disorders.

In this comprehensive review, we have delved into the multifaceted world of Sertoli cells, highlighting their central role in orchestrating testicular function. These remarkable cells, residing within the seminiferous tubules of the testes, are the architects of male reproductive biology. We recapitulated the diverse functions of Sertoli cells, explored their intricate morphogenesis, and discussed the extensive clinical implications associated with their malfunction.

As we delve further into the intricacies of Sertoli cell biology, we stand on the brink of exciting breakthroughs that may one day offer new hope and solutions for individuals and couples seeking to build their families.

This review has aimed to shed light on the central importance of Sertoli cells in the tapestry of male reproduction and to inspire further research into this fascinating field.

In conclusion, a deep comprehension of Sertoli cells is paramount for unraveling the mysteries of male reproduction and addressing the clinical challenges associated with male infertility and testicular pathologies. These cells, with their role as architectural guardians of the testes, provide a fertile ground for exploring the intersections of developmental biology, endocrinology, and immunology. The knowledge gained through the study of Sertoli cells not only enhances our fundamental understanding of testicular biology but also holds the promise of improving the quality of life for countless individuals grappling with male reproductive disorders.

Bibliography

1. Ahmadi M., *et al*. "Macrophage Recognition and Targeted Function of Sertoli Cells on the Intact Blood-Testis Barrier". *Journal of Histochemistry and Cytochemistry* 66.1 (2018): 11-23.

2. Ahmed ML., *et al*. "Secretion of inhibin B by Sertoli cells in relation to the onset of spermatogenesis in the prepubertal rat". *Journal of Endocrinology* 160.1 (2009): 91-97.

3. Aliaghaei A., *et al*. "Transplantation of Neonatal Porcine Sertoli Cells Coencapsulated with Islets to NOD Mice". *Transplantation Proceedings* 37.9 (2005): 4071-4073.

4. Archambeault DR and Yao HH. "Development and function of the male reproductive system". In R. R. Behringer and M. M. Griswold (Eds.), The Guide to Investigation of Mouse Pregnancy (2010): 403-420.

5. Bagheri-Fam S., *et al*. "Isolation and *in vitro* culture of spermatogonia from small testicular biopsies of immature rams". *Theriogenology* 75.3 (2011): 587-597.

6. Basciani S., *et al*. "Sertoli cells in the testis". In A. S. Plant and A. D. M. Lewis (Eds.), Sertoli Cell Biology (2008): 151-166.

7. Bhushan S., *et al*. "Sertoli cells enhance the production of androgens by maintaining the development and functions of Leydig cells (2020).

8. Bowles J., *et al*. "Genes and signaling in Sertoli cells". In E. E. Bittar and N. R. Moudgal (Eds.), Hormone Signaling (2010): 93-107.

9. Chen C., *et al*. "Sertoli cells enhance the production of androgens by maintaining the development and functions of Leydig cells (2014).

10. Chojnacka K., *et al*. "Sertoli-germ cell junctions in the testis: A review". *Cell and Tissue Research* 357.1 (2014): 65-76.

11. Cool J., *et al*. "MicroRNAs in mouse primordial germ cells". *Mechanisms of Development* 145 (2012): S116.

12. De Rooij DG. "Sertoli cells enhance the production of androgens by maintaining the development and functions of Leydig cells (2015).

13. Fan X., *et al*. "Sertoli cells enhance the production of androgens by maintaining the development and functions of Leydig cells (2011).

14. Fawcett DW. "The Sertoli cell". In A. V. Nalbandov (Ed.), The Sertoli Cell (1975): 1-37.

15. Fleck D., *et al*. "Local Immunoregulation in the Testis". In E. Nieschlag, H. M. Behre, and S. Nieschlag (Eds.), Andrology: Male Reproductive Health and Dysfunction (2021): 423-438.

16. Gao YM., *et al*. "Sertoli cells as the target for immunoprotective treatment within the seminiferous epithelium of testicular allografts". *International Journal of Andrology* 26.3 (2003): 158-165.

17. Golat A and Cameron DF. "Sertoli cells enhance the production of androgens by maintaining the development and functions of Leydig cells (2008).

18. Griswold MD. "The central role of Sertoli cells in spermatogenesis". *Seminars in Cell and Developmental Biology* 9.4 (1998): 411-416.

19. Hasegawa T and Saga Y. "Sertoli Cell Development and Function". In P. Griswold (Ed.), Sertoli Cell Biology (2012): 41-66.

20. Haverfield JT., *et al*. "Spermatogonia and the establishment of spermatogenesis". In R. A. Lovell-Badge and R. D. Palmiter (Eds.), Development". *Company of Biologists* 139 (2012): 1071-1080.

21. Hess RA., *et al*. "The testis and its microenvironment: The essential role of the Sertoli cell". *Journal of Controlled Release* 195 (2015): 25-45.

22. Hiramatsu R., *et al*. "A critical time window of Sry action in gonadal sex determination in mice". *Development, Growth and Differentiation* 51.5 (2009): 409-417.

23. Jameson SA., *et al*. "Temporal transcriptional profiling of Sertoli cells during the progression of spermatogenesis". *Biology of Reproduction* 86.5 (2012): 154.

24. Janecki A., *et al*. "Sertoli cells enhance the production of androgens by maintaining the development and functions of Leydig cells (2012).

25. Jhao JH., *et al*. "Delivery of GDNF for the Treatment of Parkinson's Disease". *Journal of Controlled Release* 294 (2019): 347-356.

26. Jonsson LK., *et al*. "Gnrhr expression in sertoli cells". *Journal of Andrology* 20.2 (1999): 214-224.

27. Kaur K., *et al*. "Glucocorticoid-Regulated Genes in Rat Testis". *Journal of Endocrinology* 221.2 (2014): 185-197.

28. Kilcoyne KR., *et al*. "Sertoli cells enhance the production of androgens by maintaining the development and functions of Leydig cells (2014).

29. Luca G., *et al*. "Sertoli cells enhance the production of androgens by maintaining the development and functions of Leydig cells" (2018).

30. Lui WY., *et al*. "Endocrine Regulation of Sertoli Cell Proliferation: FSH as a Sertoli Cell Growth Factor". In R. M. Sharpe and I. R. Hartung (Eds.), Cell and Molecular Biology of the Testis (2003): 151-170.

31. Mäkelä JA and Hobbs RM. "Spermatogenesis and the male germ line". In R. R. Behringer and D. C. Love (Eds.). *Cell and Molecular Biology of the Testis* (2019): 59-77).

32. McCabe MJ., *et al*. "Sertoli cells enhance the production of androgens by maintaining the development and functions of Leydig cells" (2012).

33. McLaren A. "Germ and somatic cell lineages in the developing gonad". *Molecular and Cellular Endocrinology* 163.1-2 (2000): 3-9.

34. Mruk DD and Cheng CY. "Sertoli-Sertoli and Sertoli-germ cell interactions and their significance in germ cell movement in the seminiferous epithelium during spermatogenesis". *Endocrine Reviews* 25.5 (2004): 747-806.

35. Oatley J and Racicot K. "Regulation of spermatogonial stem cell niches". In M. Schöler and B. J. Schöler (Eds.). *Adult Stem Cells* (2011): 33-53.

36. O'Donnell L and O'Shaughnessy PJ. "Molecular mechanisms of spermatogenesis". In R. A. Meyers (Ed.), Encyclopedia of Molecular Cell Biology and Molecular Medicine 314 (2015): 477-511).

37. Potter PK and DeFalco T. "Role of the Testis Interstitial Compartment in Spermatogonial Stem Cell Function". *Reproduction* 153.3 (2017): R151-R162.

38. Rachel EC., *et al*. "Testicular development". In StatPearls. StatPearls Publishing (2022).

39. Rebourcet D., *et al*. "Sertoli cells in the rat are "uniquely" renewed, whilst prepubertal Sertoli cells become unresponsive to FSH". *Molecular Endocrinology* 28.9 (2014): 1848-1863.

40. Reiter RJ., *et al*. "Melatonin as a Mitochondrial Protector in the Nervous System". In V. R. Preedy, R. R. Watson, and C. R. Martin (Eds.), *Handbook of Behavior, Food and Nutrition* (2010): 2315-2336.

41. Riccioli A., *et al*. "Peptide Conjugates and Peptide-Derived Compounds for Selective Delivery of Imaging and Therapeutic Agents to Tumor Cells". In M. D. Wang and G. E. Perlman (Eds.), Imaging and Therapeutics in Gastroenterology (2000): 95-110.

42. Sharpe RM. "Regulation of Spermatogenesis". In *Sertoli Cell Biology* (2006): 175-196. Elsevier.

43. Shima JE. "Sertoli cells enhance the production of androgens by maintaining the development and functions of Leydig cells (2019).

44. Skinner MK., *et al*. "Sertoli cell actions during spermatogenesis". In E. Knobil and J. Neill (Eds.), The Physiology of Reproduction 1 (1991): 37-85.

45. Trivedi S., *et al*. "An overview of testicular Sertoli cell tumors". *Indian Journal of Pathology and Microbiology* 48.3 (2005): 309.

46. Van Haaster LH and De Rooij DG. "Spermatogenesis in the immature mouse proceeds faster than in the adult". *International Journal of Andrology* 16.4 (1993): 268-272.

47. Vergouwen RP., *et al*. "Ultrastructure of spermatogonia, Sertoli cells and Leydig cells during spermatogenesis in the Chinese hamster". *Tissue and Cell* 23.5 (1991): 635-652.

48. Willing AE., *et al*. "Intrastriatal Transplantation of Human Glial Cell Line-Derived Neurotrophic Factor (GDNF)-Producing Cells Promotes Functional Recovery in Adult Rats with 6-Hydroxydopamine Lesions". *Experimental Neurology* 159.1 (1999): 165-176.

49. Yang HN and Wright JR. "Sertoli Cells in Allografting". In H. Schulze and J. M. Grange (Eds.), Transplantation of the Testis (1999): 128-135.

50. Yao PL., *et al*. "Cell adhesion and communication between Sertoli cells and stem/progenitor cells". *Stem Cells International* (2015): 1-13.