Charles University

Faculty of Science

Study programme: Special Chemical and Biochemical programmes

Branch of study: Molecular Biology and Biochemistry of Organisms

Petra Faltýnková

Modulation of blood-testis barrier by Sertoli cells

Modulace hematotestikulární bariéry Sertoliho buňkami

BACHELOR'S THESIS

Supervisor: RNDr. Dominik Filipp, CSc.

Prague, 2023

Prohlášení:
Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 02.08.2023 Petra Faltýnková

Acknowledgements

I would like to thank to a supervisor of my thesis RNDr. Dominik Filipp, CSc. for giving me the opportunity to join his laboratory team of immunobiology in the Institute of Molecular Genetics of the Czech Academy of Sciences. I would like to thank him and Ph.D. Jana Petrusová for giving me guidance and advice during writing my thesis. I really appreciate their interest in me and I am looking forward to my master project under their guidance. I would also like to thank to my close relatives and my partner for their support.

Poděkování

Nejprve bych ráda poděkovala školiteli mé bakalářské práce panu doktoru přírodních věd Dominikovi Filippovi CSc. za možnost přidat se k jeho laboratornímu týmu imunobiologie na Ústavu Molekulární Genetiky AV ČR. Ráda bych poděkovala jemu a Ph.D. Janině Petrusové za jejich vedení a připomínky během psaní mé bakalářské práce. Velmi si cením jejich zájmu o mě a těším se na magisterský projekt pod jejich vedením. Také bych ráda poděkovala mým nejbližším příbuzným a partnerovi za jejich podporu.

Abstract

Sertoli cells in testicles are the main building blocks of the seminiferous tubules where they facilitate the process of spermatogenesis. Although, the Sertoli cells were discovered by Enrico Sertoli more than a 150 years ago, there is still much to discover about their development and functions. The Sertoli cells are critical for the development of germ cells by the provision of hormonal, nutritional and physical support as well as by engulfment of their emerged waste. Since such intimate nurturing interactions with developing spermatocytes require a stable and isolated environment, Sertoli cells themselves create a protected endoluminal compartment sealed off by the blood-testis barrier (BTB). The BTB is a junctional complex formed between adjoining Sertoli cells and serves as an impermeable barrier in the paracellular space. However, spermatogonial stem cell initiating the spermatogenesis lies in front of the BTB closure, hence the developing spermatocytes have to pass through the BTB which is tightly regulated to maintain tissue homeostasis. BTB also exhibits immunological functions, because it can either sequester germ cell-specific antigens from the systemic circulation or release them behind the BTB. The aims of my thesis are to provide a brief overview of Sertoli cell functions in spermatogenesis, and then focus on the contribution of Sertoli cell and role of BTB to the maintenance of testicular immune privilege.

Key words: testis, Sertoli cell, cellular junctions, blood-testis barrier, testicular immune privilege

Abstrakt

Semenotvorné tubuly ve varleti jsou tvořeny Sertoliho buňkami, které zde napomáhají procesu spermatogeneze. Ačkoliv byla Sertoliho buňka objevena už před více jak 150 lety, stále je na ní mnoho co objevit, zejména podrobnosti o jejím vývoji a jejích funkcích. Sertoliho buňka doprovází vyvíjející se zárodečné buňky po celou dobu spermatogeneze tak, že jim poskytuje hormonální, nutriční a fyzickou podporu a pohlcuje vzniklý odpad. Tyto intimní vyživovací interakce s vyvíjejícími se spermatocyty vyžadují stabilní a izolované prostředí, Sertoliho buňky proto vytváří chráněný kompartment těsně uzavřený hematotestikulární bariérou. Hematotestikulární bariéra je složitý komplex buněčných spojení vytvořený mezi sousedícími Sertoliho buňkami a tvořící nepropustnou bariéru v paracelulárním prostoru. Nicméně, spermatogoniální kmenové buňky zahajující spermatogenezi se nachází před touto bariérou, a proto musí vyvíjející se spermatocyty překonat hematotestikulární bariéru, což je striktně regulováno, aby byla udržena ve varleti homeostáze. Hematotestikulární bariéře je připisována také imunologická funkce, jelikož buďto izoluje specifické antigeny zárodečných buněk před imunitními buňkami ze systémové cirkulace, a nebo je uvolňuje za bariéru. Cílem mé práce je shrnout funkce Sertoliho buňky ve spermatogenezi, a poté se zaměřit na roli Sertoliho buňky a hematotestikulární bariéry v udržení imunitně privilegovaného stavu ve varlatech.

Klíčová slova: varle, Sertoliho buňka, buněčné spoje, hematotestikulární bariéra, testikulární imunitní privilegium

Table of Contents

1. Introduction and aims of the thesis

Spermatogenesis is a process of generation of male gametes, known as sperm within the male reproductive organs called testes, which are paired organ of men reproductive system responsible for the steroidogenesis as well as spermatogenesis. Male fertility depends on the continuous production of sperm by the testes. It is of note, that infertility affects one in every six couples who are trying to conceive (Thoma et al., 2013). In at least half of all cases of infertility, a male factor is a major or contributing cause (Agarwal et al., 2015; Practice Committee of the American Society for Reproductive Medicine, 2015) and strikingly approximately 40% of these men are diagnosed with idiopathic sterility, i.e. without obvious physiological reason (Sharma et al., 2020).

Normal spermatogenesis essentially relies on Sertoli cells, which possess remarkable qualities, which enable spermatogenesis (França et al., 2016). These cells were firstly described more than 150 years ago (Sertoli 1865; as cited in França et al., 2016) but there is still much to discover in these interesting cells. Sertoli cells form the seminiferous tubules and are the only somatic cells accompanying the development of germ cells throughout the whole spermatogenesis. They are crucial for orchestration of the testicular morphology in ontogeny (Svingen & Koopman, 2013) and preservation of cell junctions while providing hormonal, nutritional and physical support for mitosis and meiosis of male germ cells. Moreover, they create a secluded immune-protected environment for development of spermatocytes and spermatids behind an impermeable barrier. Adjoining Sertoli cells seal the paracellular space by a junctional complex called a blood-testis barrier, thus demarcating tubular compartments (Dym & Fawcett, 1970) and thus forming the immune privilege of the seminiferous tubules, i.e. tissue with tolerogenic immune environment (Streilein, 1993). In the basal compartment of the seminiferous tubule spermatogonial stem cells develop into primary spermatocytes, which eventually enter the adluminal compartment, moving towards the lumen of seminiferous tubule. They undergo the first and the second reductional division, thus ending up as haploid spermatids which develop into spermatozoa (Geyer, 2017; Griswold, 2016). While Sertoli cells can change the composition of junctions according to the tubular regions as well as due to the type of the adjacent cells, they must also tightly regulate the composition of cellular junctions to enable blood-testis barrier transient opening, spermiation and phagocytosis of the dead germ cell (Yan Cheng & Mruk, 2015).

Aim of this bachelor's thesis is to give an overview concerning Sertoli cell junctions, their localisation, specific composition, role during spermatogenesis and regulation. Then, I will discuss the effect of cellular junctions on testicular immune privilege and role of Sertoli cell in the processing of the germ cell-specific antigens generated in the seminiferous tubule.

2. Testicular seminiferous tubules and interstitial space

In mammals, the testicles develop from a bipotential gonadal primordia. The gonadal primordia also called genital ridges are pair tissue structures which appear parallel to embryonic head-to-tail axis approximately at embryonic day E10 in mouse and later develop to a pair of testicles in male or ovaries in female (Svingen & Koopman 2013). Cellularly, genital ridges comprise of proliferating coelomic epithelial cells from mesonephros and primordial germ cells (PGCs) (Nef et al., 2019). All PGCs have the potential to differentiate into a sperm or an oocyte, independently on genetics of the embryo. Their contribution to male or female gametes depends whether or not they are in testicular environment (McLaren A., 1985; as cited in Koopman, 2001). From asymmetrically dividing coelomic epithelial cells develop two somatic cell lineages. The supportive progenitors, which will differentiate to testicular Sertoli cells or ovarian granulosa cells and steroidogenic progenitors which become Leydig cells or theca cells (Nef et al., 2019). The fate of the bipotential gonad to develop into a testicle is set by *sex-determining region of the Y chromosome (Sry)* gene present only in the XY embryo (Koopman et al., 1990). *Sry* gene become expressed in supportive gonadal lineage, later recognized as Sertoli cells (Sekido et al., 2004). A *Sox9* gene, a direct downstream target in *Sry* cascade, drives differentiation of Sertoli cells further orchestrating the formation of testicular cords, later developing into the seminiferous tubules (Svingen & Koopman, 2013).

During embryonal development, the testicular cords form *de novo* (Svingen & Koopman, 2013) synchronously from clusters of Sertoli and germ cells also referred as Sertoli germ cell mass. Sertoli cells start to migrate to the cords periphery (Nel-Themaat et al., 2011), where they form a lining of the tubule. The mass of the testicular cords undergoes remodelling, thus forming loop-like structures which further elongate and buckle. In an adult testis there is around dozen of long seminiferous tubules (Nel-Themaat et al., 2009) filling out the whole volume of the testicle, resembling a tangled lump of spaghetti (Svingen & Koopman, 2013). Moreover, more than a half of the seminiferous tubules branch into more arms and terminations (Nakata, 2019) making the structure even more complicated. All terminations of seminiferous tubules lead to a cavity called *rete testis* (Kulibin & Malolina, 2020), which connects seminiferous tubules with efferent ducts allowing sperm to flow from testicle into epididymis (Figure 1).

In adult male, the seminiferous tubule comprises of Sertoli cells and spermatogonia ordered in a rosette-like pattern (Nel-Themaat et al., 2011) and is surrounded by peritubular myoid cells (PMCs), organized in a one (mouse) or more layers (human) (Figure 1 and 2) (Bustos-Obregon, 1976). PMCs resemble smooth muscle cells and generate contractions, thus generating power which transfers spermatozoa in luminal fluid from testes into the epididymis (Clermont, 1958). Sertoli cells and PMCs cooperate to produce extracellular matrix (ECM) proteins (Skinner et al., 1985), which form the basal lamina (also called basement membrane), located between these two cellular types (Hadley & Dym, 1987). The attachment of Sertoli cell to the basal lamina is assured by hemidesmosomes (Yan Cheng & Mruk, 2015). The collagen and laminin containing basal lamina together with peritubular myoid cells form

tunica propria, the edge of the seminiferous tubule and interstitial space (Figure 2) (Hadley & Dym, 1987).

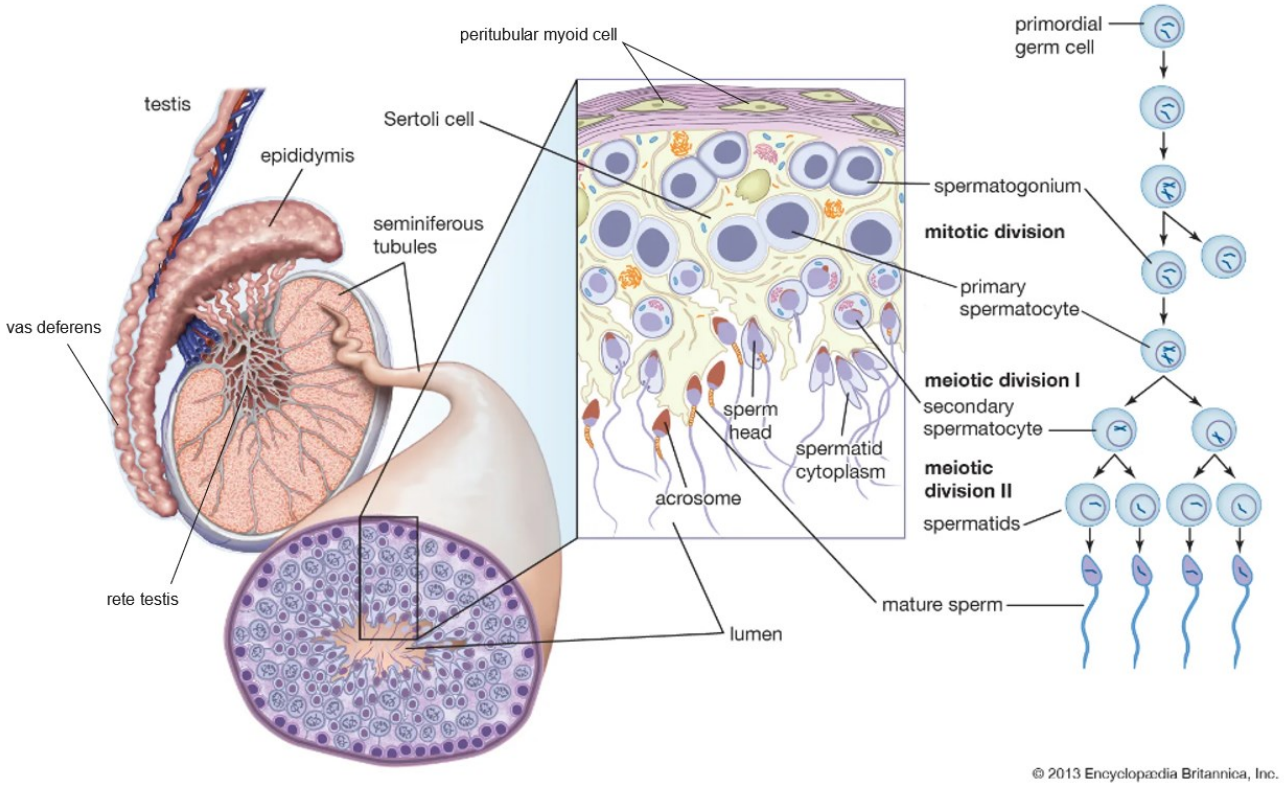

Figure 1. *Process of spermatogenesis appears in seminiferous tubules*

The basic anatomical features of a testicle and a process of spermatogenesis in embrace of the Sertoli cell in the seminiferous tubule are depicted by a scheme edited from Encyclopaedia Britannica (The Editors of Encyclopaedia Britannica, 2023).

The interstitial space is the area outside of the seminiferous tubules within the testis (Figure 2). It is comprised of many cell types such as steroidogenic Leydig cells and their undifferentiated mesenchymal progenitors (Heinrich & DeFalco, 2020), vascular cells, mesenchymal cell such as fibroblasts and numerous types of immune cells (Figure 2) (Gu et al., 2022), which can relocate to this area from the blood vessels or lymphatic vasculature (Oliver & Stukenborg, 2020). Leydig cells are here the only cells with the endocrine function which in the presence of luteinizing hormone (LH) produce testosterone from its precursor cholesterol (Zirkin & Papadopoulos, 2018), which is required for successful spermatogenesis (Holdcraft & Braun, 2004; Walker, 2011). The most abundant immune cell type are macrophages (Hedger, 1997). Their population in the interstitial space is heterogenous with prevailing M2 phenotype (Bhushan & Meinhardt, 2017; Wang et al., 2017). Two subpopulations of testicular macrophages with distinct morphology, phenotype and function were shown recently (DeFalco et al., 2015) - peritubular macrophages localized at the edge of the seminiferous tubule, whereas interstitial

macrophages cooperated with Leydig cells in the open interstitial space (Mossadegh-Keller & Sieweke, 2018). Beside macrophages, the interstitial space under normal physiological conditions contains also leukocytes, monocytes, dendritic cells, NK cells as well as mast cells (Bhushan et al., 2020; N. Li et al., 2012; Meineke et al., 2000).

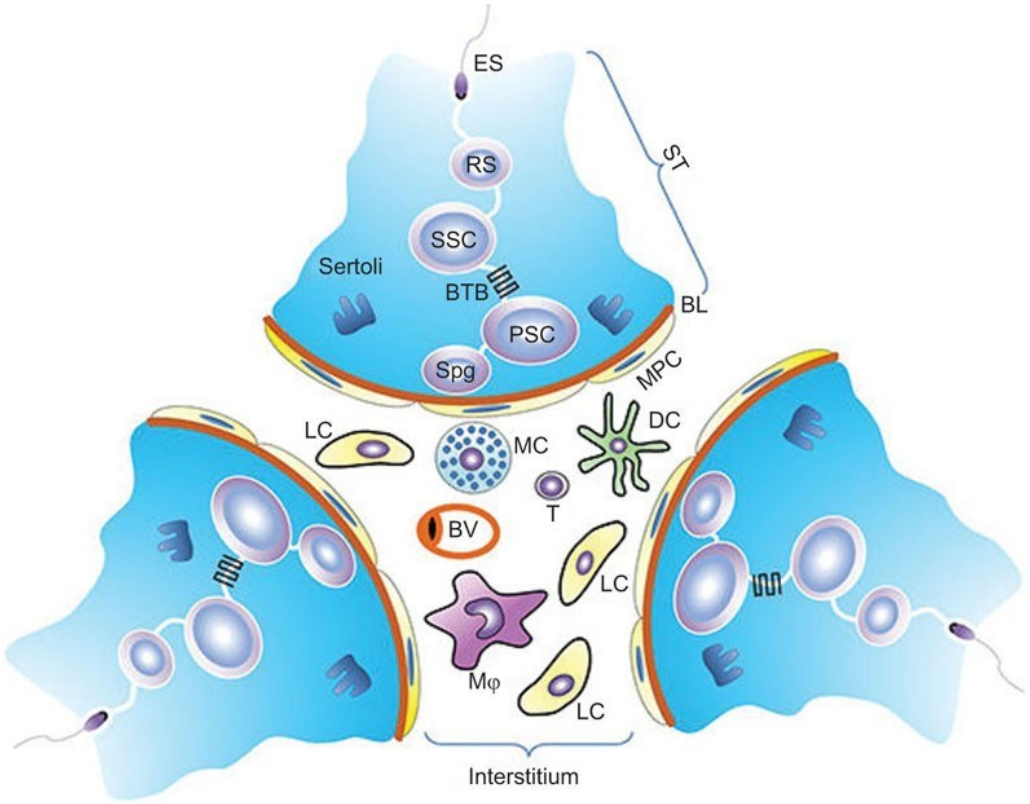

Figure 2. *Interstitial space surrounds seminiferous tubules*

The scheme shows simplified model of rodent seminiferous tubules surrounded by interstitial space with containing immune cells, adopted from (Zhao et al., 2014). Abbreviations: BL – basal lamina, BTB – blood-testis barrier, BV – blood vessel, DC – dendritic cell, ES – elongated spermatid, LC -Leydig cell, Mφ – testicular macrophage, MC – mast cell, MPC -myoid peritubular cell, PSC – primary spermatocyte, RS – round spermatid, SPG – spermatogonia, SSC – secondary spermatocyte, ST – seminiferous tubule.

3. Sertoli cells - the nurse cell of the spermatogenesis

Enrico Sertoli in 1865 reported about the discovery of branched cells within the seminiferous tubule which were later named as Sertoli cells (Sertoli 1865; as cited in França et al., 2016). He described Sertoli cell's morphology and suggested their supportive function in spermatogenesis. Moreover, he continued publishing about seminiferous tubules among other discoveries during his life and hence started the scientific discussion in the field of Sertoli cell (Patriarca et al., 2019).

Sertoli cell enables the spermatogenesis by embracing all developmental stages of germ cells up to the sperm (Figure 1). This process is highly spatially and temporally organized. The only cellular source of

spermatogenesis is spermatogonial stem cells (SSCs). By definition of stem cells (Slack, 2018), SSCs possess the capacity for self-renewal and differentiation into spermatogonia. At the onset of puberty, a SSCs divide mitotically, going through stages of differentiating spermatogonia, generating the primary spermatocyte (Oatley & Brinster, 2012). From this point spermatogenesis continues by two reduction divisions (meiosis I and II) resulting in four haploid spermatids (Figure 1) (de Kretser et al., 1998; Griswold, 2016), which are small round cells yet without the flagellum. To become a morphologically mature sperm, they enter a process called spermiogenesis (O'Donnell, 2014), which is fully orchestrated by the apical part of the Sertoli cell (L. D. Russell et al., 1989). Spermatogenesis, i.e. the whole process from a SSC up to a motile spermatozoa, repeats periodically in the cycles, described by the cycle of seminiferous epithelium (Figure 3) (Leblond & Clermont, 1952). Leblond and Clermont in 1952 described on a rat 14 stages (I-XIV) of this cycle which describe continuous changes in cellular composition in the tubule through one successive process of spermatogenesis. In mouse are described 12 stages (Figure 3) and in human 6 stages (De Kretser, 1988; as cited in Mruk & Cheng, 2015).

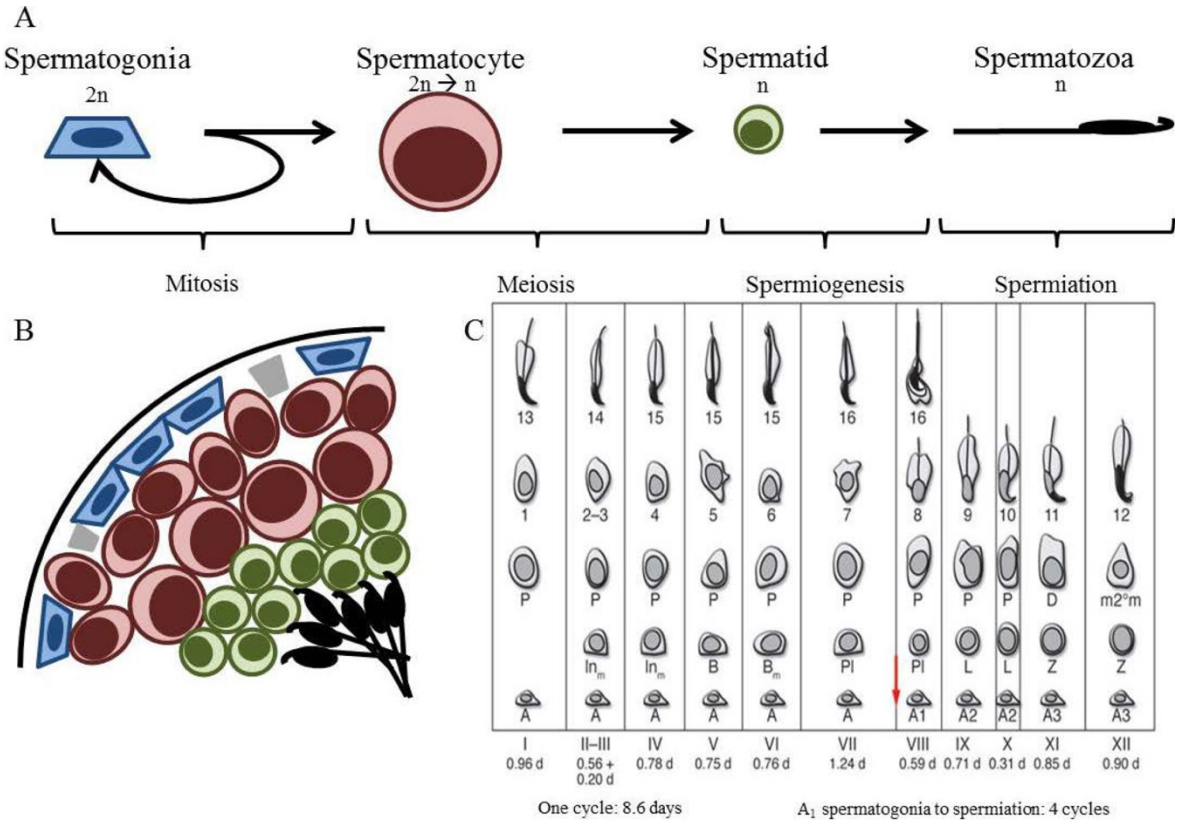

Figure 3. *An overview of murine spermatogenesis; cycle of seminiferous epithelium*

In **A** *are depicted major steps in process of spermatogenesis. From diploid spermatogonia (blue cell) develop spermatocytes (red cells) which by two reduction divisions give rise to haploid spermatids (green cells). Spermatids differentiate into spermatozoa (black flagellated cells) through process of spermiogenesis and are released from the tubule during spermiation.*

Part **B** *shows in simplicity cellular distribution within the seminiferous tubule. Sertoli cell nucleus is in the scheme symbolized by a grey block and Sertoli cell cytoplasm hypothetically surrounds the developing germ cells. Plasma membranes of Sertoli cells were omitted.*

Part **C** *is a diagram showing the cellular associations appearing in murine testicles during twelve stages of cycle of seminiferous epithelium. Red arrow marks a time in the cycle when retinoic acid is required for initiating the differentiation of spermatogonia to spermatozoa.*

Abbreviations of developing germ cell in a chronological manner: A - A spermatogonia, A1 to A4 - A1 to A4 spermatogonia, In_m, intermediate (mitosis) spermatogonia; B – spermatogonia type B; Pl - preleptotene spermatocytes; L - leptotene spermatocytes; Z - zygotene spermatocytes; P - pachytene spermatocytes; D - diplotene spermatocytes; m2°m - secondary spermatocytes. Round and elongating spermatids are labelled as steps 1–16. Adopted from (Kent & Griswold, 2014).

Spermatogenesis starts in human at the onset of puberty and in mouse within a few days after the birth (Drumond et al., 2011). It appears in the asynchronous manner and very fast (Drumond et al., 2011; Snyder et al., 2010) ending up with a high number of apoptotic germ cells (Rodriguez et al., 1997). This phenomenon is not well understood but is generally explained as the control phase of spermatogenesis, prior to a life-long production of sperm. During this time along with the appearance of germ cells, the Sertoli cell changes its transcription profile from proliferative juvenile, which so far facilitated the construction of the tubule, to the mature nurturing state, which changes the Sertoli cell in purpose to support the gametogenesis (Zimmermann et al., 2015). This gene-programme switch also referred as functional maturation of Sertoli cells, changes the cell function and morphology (Sharpe et al., 2003). It is believed that the regulation is made by thyroid hormones with contribution of retinoic acid (RA, an active form of vitamin A) and estrogens (Meroni et al., 2019). Once the Sertoli cell matured, it immensely contributes to the process of spermatogenesis by 1) hormonal and nutrition support and 2) physical maintenance of spermatocytes, hence allowing meiotic progression (França et al., 2016).

Hormonal support of the spermatogenesis means the delivery of testosterone to the germ cells. This hormone is essential for spermatocytes to proceed through the meiosis, to the final spermatid differentiation (Holdcraft & Braun, 2004; Walker, 2011). However, testosterone is produced by Leydig cells in the interstitial space, far from the germ cells, hence Sertoli cell bring the testosterone to the developing gametes (Heinrich & DeFalco, 2020). There is also an effect of the testosterone on the Sertoli cell. Notably, it binds to the intercellular androgen receptor (AR) in Sertoli cell which is then translocated to the nucleus and here it affects the gene expression of the AR-regulated genes (Walker, 2011). In addition, testosterone also induce two other molecular pathways, including kinases and calcium channels. This has an impact on the adhesion of the germ cells to the apical part of the Sertoli cell and remodelling the Sertoli cell junctions. In these processes, it is considered that testosterone plays a major regulatory role (Walker, 2011). Sertoli cells are also involved in the metabolism of retinoic acid (Raverdeau et al., 2012) which is essential for commitment of undifferentiated spermatogonia to meiosis (Figure 3) (Griswold et al., 1989; Snyder et al., 2010). It was shown, that there is a gradient of RA along the seminiferous tubule which maintains the continuity of spermatogenesis (Hogarth et al., 2015). Sertoli cells also provide a lactate (Jutte et al., 1982) and iron (Sylvester & Griswold, 1994) to spermatocytes and spermatids because they are sequestered from the blood vessel supplies by impermeable barrier.

Secondly, Sertoli cells, as the only somatic cell in the seminiferous tubules, provide the spatial support for processes associated with spermatogenesis. By functional maturation, the Sertoli cell becomes columnar, it prolongs into cytoplasmic arms (Solari & Fritz, 1978) and also enlarge its nucleus (Sharpe et al., 2003). Sertoli cells display (just as any other cell) three types of cytoskeletal fibres: microtubules, intermediate filaments referred as vimentin, and actin microfilaments (Hess & Vogl, 2015). While maintaining the shape and function of the cell, cytoplasmic cytoskeleton in Sertoli cells possesses a unique role required for spermatogenesis (Vogl et al., 2008). Specifically, it is important for organisation and function of the inter-cellular junctions between adjoining Sertoli cells or between Sertoli cell and germ cell (Vogl et al., 2008), which holds the developing germ cells in the seminiferous epithelium (Figure 3).

At the beginning of spermatogenesis, a junctional complex called blood-testis barrier (BTB) is formed between the adjacent Sertoli cells (Figure 3) (Meroni et al., 2019). Assembly of BTB functionally divide the Sertoli cells to apical and basal domain and at the same time BTB partitions the paracellular space into two spatial compartments of the seminiferous tubule - the basal and adluminal compartment (Figure 4) (Dym & Fawcett, 1970). In basal compartment reside undifferentiated spermatogonia including SSCs and preleptotene spermatocytes (Geyer, 2017), while adluminal compartment contains primary spermatocytes (4n), secondary spermatocytes (2n) and round spermatids (n), which elongate into spermatozoa (Figure 4). Sertoli cell branches in adluminal direction into crypts creating the environment for elongating spermatids attachment (L. D. Russell et al., 1989).

Very special function of Sertoli cell essential for spermatogenesis, alongside with the hormonal support and physical maintenance of the developing gametes is that it also engulfs the apoptotic germ cells and excessive cytoplasm from developing spermatids called residual body (Figure 4 and 5) (Breucker et al., 1985; Vogl et al., 1985). This process of phagocytosis is critical for the testicular homeostasis and it is facilitated by the cytoskeleton of Sertoli cell but also by the cellular junctions (L. D. Russell, 1979c; Yan Cheng & Mruk, 2015). The obtained germ cell antigens together with their anchoring devices may be partially recycled, but most of them are degraded in the lysosomes recruited by the microtubules to the basis of the Sertoli cell (Chemes, 1986; Tang et al., 2016). It is of note, that some of the germ-cell specific antigens were found also in the interstitial space (Tung et al., 2017) which points to some specific role of Sertoli cell in egress of obtained molecules. Nevertheless, this yet poorly understood phenomenon will be discussed at the end of this thesis.

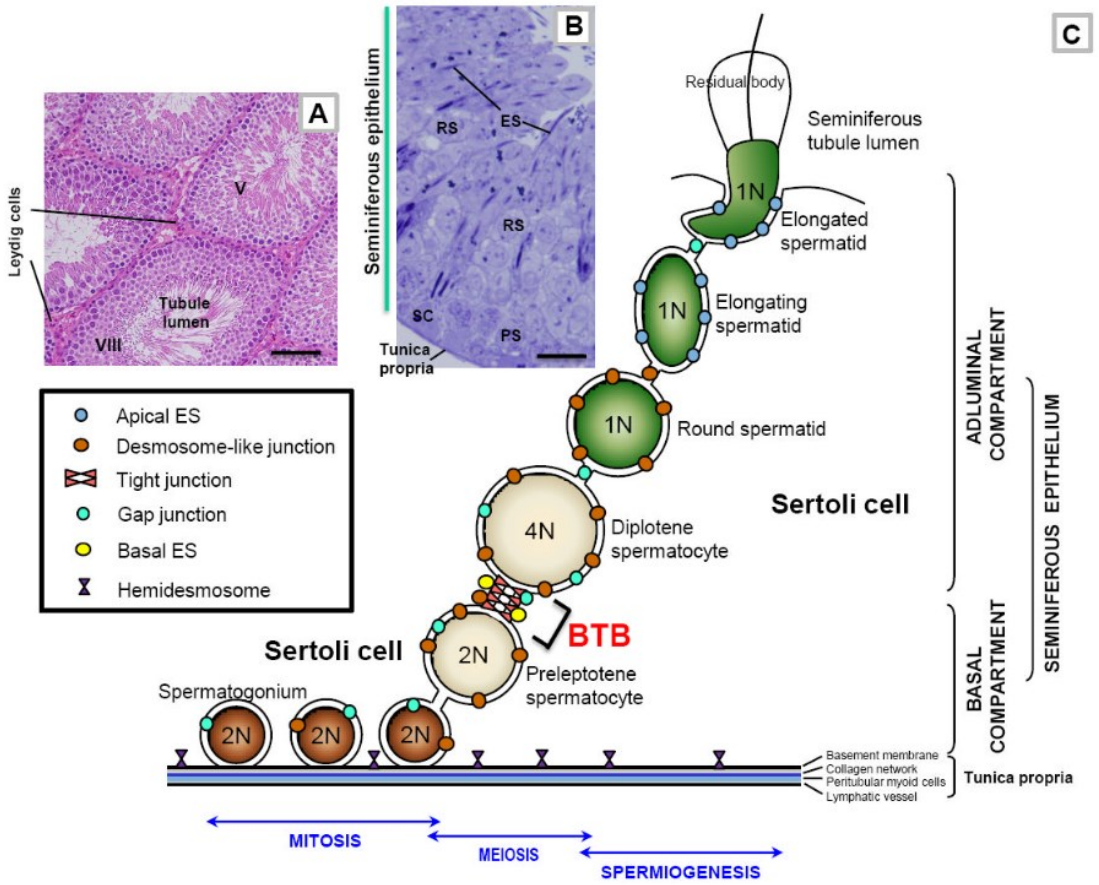

Figure 4. *Sertoli cell junctions and their localisation at the tubular compartments during spermatogenesis.*

A) *A cross-section of adult rat testis showing seminiferous tubules in different stages (V and VII) and Leydig cells in interstitial space. Bar = 80 µm.*

B) *A micrograph of rat seminiferous tubule showing the Sertoli cell (SC) in close contact to developing germ cells. As the epithelium is in stage V, the composition of the tubule is pachytene spermatocytes (PS), round spermatids (RS) and elongating spermatids (ES). Bar = 8 µm.*

C) *A scheme illustrating the localization of each stage of developing germ cell with respect to the Blood-testis barrier (BTB). Additionally, the scheme also shows types of cellular junctions formed by Sertoli cell. 1N (haploid), 2N (diploid), 4N (tetraploid). Taken from (Cheng & Mruk, 2009).*

3.1. Structure and function of the Sertoli cell junctions

At the cytoplasmic periphery and on the cell membrane Sertoli cells form intra-cellular junctions of all types - tight junctions (TJs), gap junctions (GJs) and adherens junctions (AJs) (Hess & Vogl, 2015). Most of these junctions are formed and organised by Sertoli cells along with the developing gametes and sperm (Kopera et al., 2010). Here we describe their structure, function and regulation and their role during each period of spermatogenesis. A special attention is paid to the unique junctional complex forming the blood-testis barrier.

3.1.1. Junctions formed between the Sertoli cell and germ cells

Sertoli cells form intercellular junctions with germ cells through the whole process of spermatogenesis. At the tubular basis, the spermatogonia are connected to Sertoli cell by gap junctions (Decrouy et al., 2004) and also by desmosome-like junction (Figure 4) (L. Russell, 1977a). Both junctions remain associated with the developing germ cell - starting at the spermatogonia and remaining till round spermatid (L. Russell, 1977a). Gap junctions which are responsible for intracellular communication (Pointis et al., 2010), interconnect cells by a channel called connexon formed from proteins connexins (Pointis & Segretain, 2005). The connexin expressed in testes is connexin 43 (Pointis et al., 2010). Desmosome-like junctions function to hold the developing germ cell in the epithelium while it is transported across the entire length of the Sertoli cell (Kopera et al., 2010; L. Russell, 1977a). Their structure is similar to conventional desmosomes, comprised of integral membrane proteins desmoglein and desmocollin from cadherin family. These proteins interconnect Sertoli cells with germ cells and link the cytoskeletal intermediate filaments via adaptor proteins (Holthöfer et al., 2007; Kopera et al., 2010).

When the round spermatid starts to elongate, gap junctions and desmosome-like junctions are fully replaced by apical ectoplasmic specialization (Apical ES, Figure 5) (Kopera et al., 2010). The role of apical ES is to anchor the elongating spermatid to the epithelium while it undergoes morphological changes known as spermiogenesis (E. W. P. Wong et al., 2008). It is of note that ectoplasmic specialization is also a constituent of BTB, where it is called basal ES (Mruk & Cheng, 2015). In fact, the only difference between apical and basal ES is that the apical ES is formed between Sertoli cell and spermatid while basal ES is formed between two adjoining Sertoli cells. In general, ES are structures composed of three layers (Figure 5). This triad is comprised of bundles of filamentous actin (Toyama, 1976) attached to cistern of endoplasmic reticulum on one side and to plasma membrane on the other (Flickinger & Fawcett, 1967). Actin filaments in ES are arranged in discrete bundles of hexagonal shape, and oriented in parallel to the cytoplasmic membrane (Flickinger & Fawcett, 1967; Vogl et al., 2000). From the spatial difference in neighbouring cell ensues, that while at the apical ES the triad is observed only in Sertoli cells and not on the side of the germ cell, the structure of the basal ES is homotypic, i.e. mirrored on both sides of the junction (Hess & Vogl, 2015; E. W. P. Wong et al., 2008).

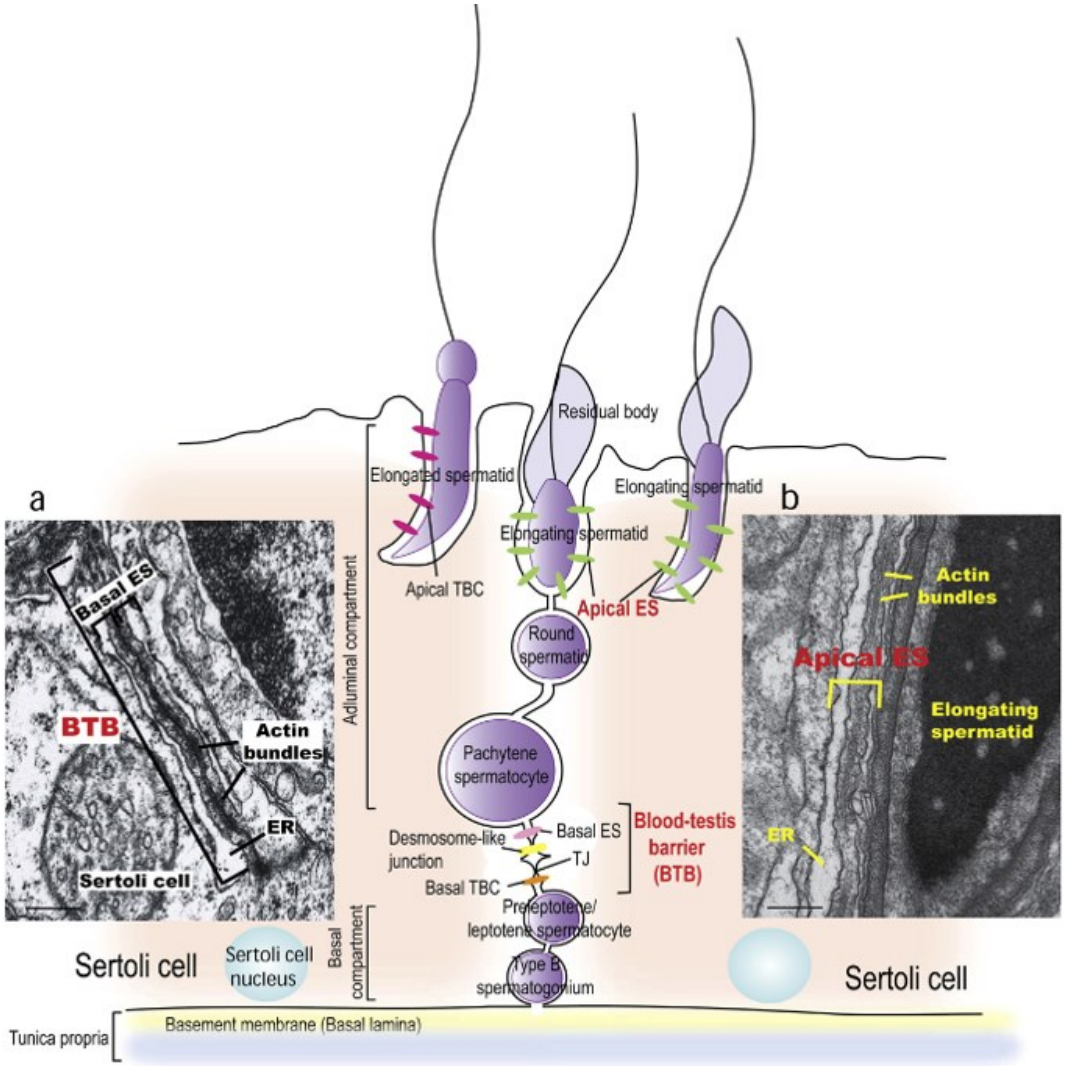

Figure 5. *Ectoplasmic specializations formed by Sertoli cell*

A scheme illustrating position of apical (green) and basal (light pink) ectoplasmic specializations formed by Sertoli cells. Accompanied by two electron micrographs from transmission electron microscope (TEM) showing the ultrastructure of (a) basal and (b) apical ES. Additionally, apical TBC (dark pink) and other components of BTB are also depicted, desmosome-like junctions (yellow), basal TBC (orange), TJ. Abbreviations: BTB – blood-testis barrier, ER- endoplasmic reticulum, ES – ectoplasmic specialization, TBC – tubulobulbar complex, TJ – tight junction. Edited from (E. W. P. Wong et al., 2008).

The ES are adhesive connections and this attribute is ensured by transmembrane adhesion proteins which form functional units with their adaptor proteins (E. W. P. Wong et al., 2008). In particular, these complexes are cadherin-catenin functional units (N- and E-cadherins with α- and β-catenins) (Lee et al., 2003), nectin-afadin-ponsin functional units (nectin-2 and -3 with 1-afadin) (Ozaki-Kuroda et al., 2002), and α6β1-integrin expressed in Sertoli cells with a trimeric laminin-α3β3γ3 ligand on the side of the germ cells (Mruk & Cheng, 2004b; Salanova et al., 1995; Yan & Cheng, 2006). The latter is probably the most important protein complex which maintains the adhesion of the elongated spermatid during the spermiation. While the integral membrane proteins within the functional units interact in the 70-100 Å

narrow intercellular space between Sertoli cell and spermatid (Hess & Vogl, 2015), the adaptor proteins link the integral part to the actin bundles (Hess & Vogl, 2015). Not surprisingly, there are numerous proteins which bind the components of apical ES, such as such α-actinin, espin and fimbrin or Src and ILK kinases, phosphatases and many others, which are involved in restructuring events and cytoskeleton remodelling, (Hess & Vogl, 2015; Mruk & Cheng, 2004b). The apical ES differs from the basal ES in another feature, that is its hybrid character. Apical ES is comprised of proteins conventionally classified to focal contacts (integrins, laminins and associated signalling focal adhesion kinase (FAK)) or typical tight junction (JAMs, ZO-1 or CAR) and gap junction (connexin-43) components (Hess & Vogl, 2015; Mruk & Cheng, 2004b, 2015; E. W. P. Wong et al., 2008). This mixed junctional character presumably gives the apical ES additional functions.

It is important to mention that apical ES holds the elongating spermatid only transiently. Few hours before spermiation, i.e. prior to release of spermatids into the lumen, the apical ES is replaced by apical tubulobulbar complex (apical TBC) which serves as the third and last anchoring junction formed with the developing germ cell (L. D. Russell, 1979a). The apical ES disassembles and it is gradually replaced by apical TBC (Guttman et al., 2004; Hess & Vogl, 2015). Apical TBC is an ultrastructure formed by both Sertoli cell and late elongating spermatid cell membranes creating long tubular protrusions terminated by clusters of vesicles (Figures 5 and 6) (O'Donnell et al., 2011; L. Russell & Clermont, 1976). The tubular structures are surrounded by actin (Vogl, 1989), while the clusters of vesicles are coated with clathrin (Young et al., 2009) and surrounded by Sertoli cell's endoplasmic reticulum (Figure 6) (L. Russell & Clermont, 1976). Apical TBC was shown to internalize the integral membrane proteins of apical ES (Guttman et al., 2004) and is able to bud forming bulbs which enter the endocytic pathway (Lyon et al., 2015; L. Russell & Clermont, 1976). Upon disengagement from the Sertoli cell, the remaining excessive cytoplasm, called residual body (Figures 4, 5 and 6), is engulfed by Sertoli cell (Breucker et al., 1985). Apical TBC might be incorporated in the processes of elimination of spermatid cytoplasm (L. D. Russell, 1979c). It probably also helps in the recycling of apical ES components (Young et al., 2012) which the apical TBC internalizes. The recycled proteins are then reassembled to form a new apical ES for another spermatid at the onset of spermiogenesis (Yan Cheng & Mruk, 2015).

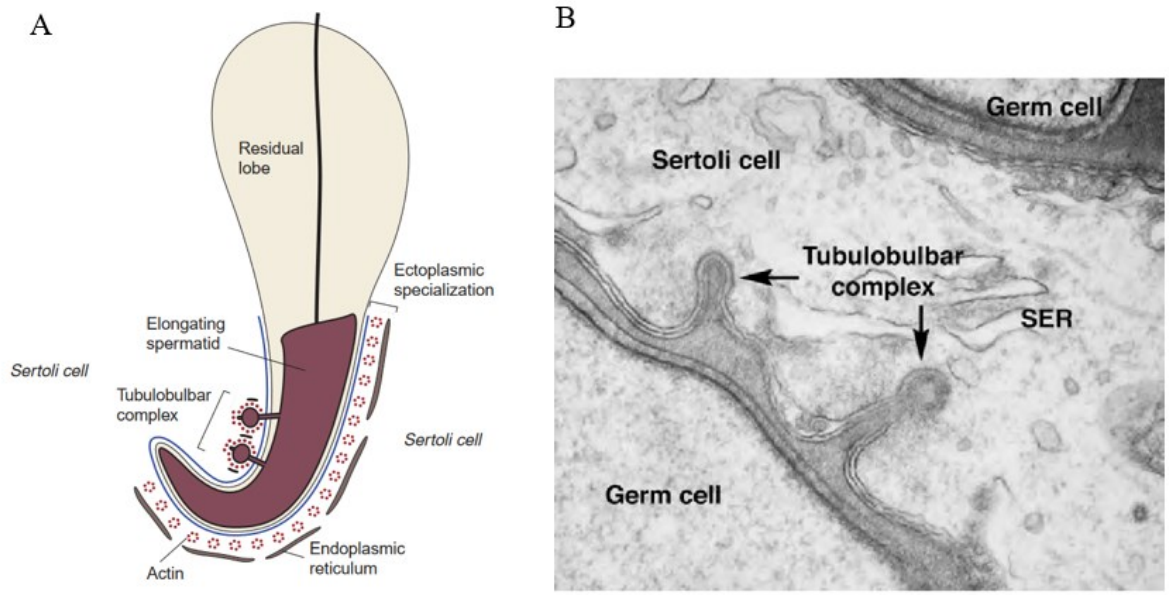

Figure 6. *Apical tubulobulbar complex*

A *Scheme showing the apical tubulobulbar complex appearing at the concave side of the elongated spermatid. Apical ectoplasmic specializations triad is on the convex side of the germ cell and will be replaced by TBC before spermiation. The excessive spermatid cytoplasm, here named residual lobe will be engulfed by Sertoli cell by TBC mediated pathway. Adopted from (Mruk & Cheng, 2004a).*

B *Apical TBC showed by transmission electron microscopy as bulbs on the border of germ cell and Sertoli cell membranes. At the side of the Sertoli cell is visible its endoplasmic reticulum (SER) which surrounds the bulbar part of TBC. Adopted from (Hess & Franca, 2005)*

3.1.2. Blood-testis barrier

Blood-testis barrier (BTB) is a complex ensemble of various types of junctions which are located near the basal membrane between adjoining Sertoli cells. It separates basal and adluminal compartments in the seminiferous tubule (Dym & Fawcett, 1970). BTB restricts the paracellular diffusion of substances such as water, electrolytes, ions, nutrients, hormones, paracrine factors and other biological components between adjoining Sertoli cells (Cheng & Mruk, 2012). This attribute of BTB creates for spermatocytes in meiosis, round and elongating spermatids a protective and strictly regulated environment, behind the impermeable barrier (Cheng & Mruk, 2012). BTB is composed of TJs, basal ES, GJs and desmosome-like junctions (Figure 7) (Dym & Fawcett, 1970; C. Wong & Cheng, 2005). This gathering of different complexes is very unique, and cannot be found in any other somatic cells or blood-tissue barriers (Cheng & Mruk, 2012; Morrow et al., 2010). Moreover, the components of different junctional types in the BTB are often physically and functionally intermingled (Morrow et al., 2010; Vogl et al., 2008; Yan et al., 2007), while in other somatic epithelia these TJs, AJs and desmosome-like junctions are distinctly segregated (E. W. P. Wong et al., 2008). In general, the impermeable barrier in the paracellular space is generated by extracellular domains of integral membrane proteins of BTB (Figure 7) (Mruk & Cheng, 2015). In addition, a huge number of peripheral scaffolding and signalling proteins are linked to integral

membrane proteins of BTB, and mediate communication with actin and tubulin cytoskeleton (Figure 7), thus allowing the their dynamic modulation (Mruk & Cheng, 2015).

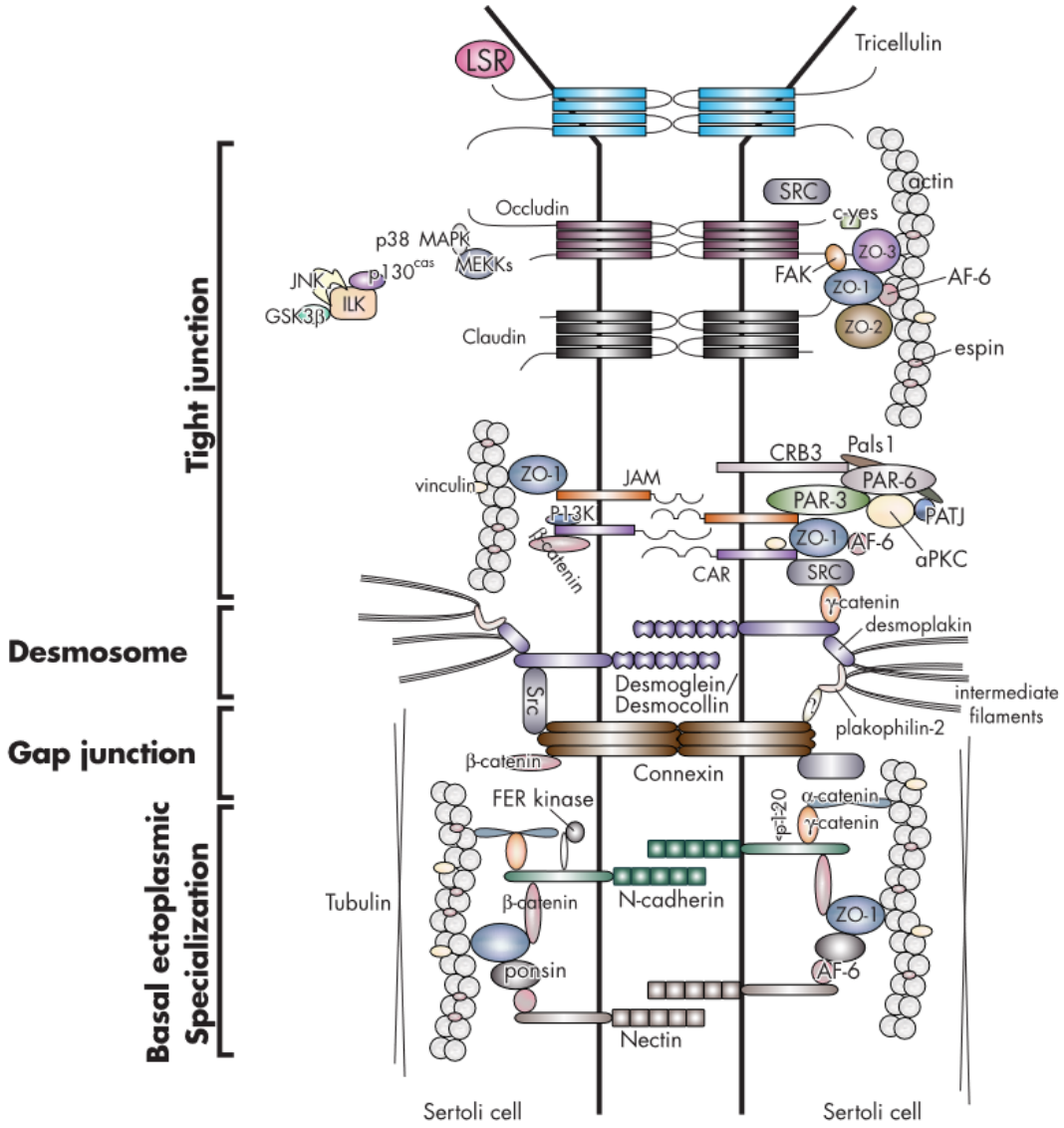

Figure 7. *A scheme depicting molecular composition of Blood-testis barrier*

Note that the concrete order of junctional types varies in literature, moreover physical engagement of integral membrane proteins of tight junctions and basal ectoplasmic specialization is discussed in text, but not highlighted in the scheme. Abreviations of proteins discussed in the text: AF-6 - afadin, CAR- Coxsackie and Adenovirus Receptor, JAM- junctional adhesion molecule, LSR - Lipolysis-stimulated lipoprotein receptor, ZO 1-3 – zonula occludens 1-3. Taken from (Mruk & Cheng, 2015)

A critical structure of BTB are TJs or here referred also as occluding junctions, due to their main component, protein occludin. It forms a belt in Sertoli cell membrane sealing the paracellular space (Van Itallie & Anderson, 2014), which prevents free passage of water, solutes, and large molecules, and in the same time, blocks the movement of membrane proteins and lipids (Mruk & Cheng, 2015). TJs in BTB comprise of integral membrane proteins, mainly claudins and occludins which interact mostly

homo-typically via their extracellular domains (Morrow et al., 2010). These transmembrane proteins are peripherally linked to scaffolding proteins such as zonula occludens 1 to 3 (ZO1, ZO2 and ZO3) proteins, which interact with actin microfilaments through other molecular linkers (Van Itallie & Anderson, 2014; C. Wong & Cheng, 2005).

In addition to claudins and occludin, other known integral membrane proteins of BTB TJs are tricellulin, JAM-A and JAM-B and CAR (Morrow et al., 2010; Mruk & Cheng, 2015). From all these, it appears that indispensable for BTB function are only claudin 3, 5 and 11 and occludin which create the zipper-like sealing between the cells (Chung et al., 2001; Gow et al., 1999). Tricellulin spans the intercellular space in seminiferous tubule (Ikenouchi et al., 2005). It structurally resembles occludin and localizes to tricellular TJs, which usually seal the space between three neighbouring cells (Ikenouchi et al., 2005). Lipolysis-stimulated lipoprotein receptor (LSR) is localized at this tricellular TJs (Masuda et al., 2011). It appears that tricellulin together with LSR functions as a "glue" between claudins and occludins at the tricellular contacts, where claudins and occludins could potentially be only weakly connected and thus form "leaky" spots in the barrier (Masuda et al., 2011). Among integral membrane proteins of BTB are included also Junctional adhesion molecules JAM-A and JAM-B (Gliki et al., 2004) together with JAM family related Coxsackie and Adenovirus Receptor (CAR) (Luissint et al., 2014).

As mentioned above, the integral membrane proteins are linked to scaffolding proteins. Scaffolding proteins mediate their attachment to Sertoli cell cytoskeleton and communication with signalling proteins regulating TJs. These are tight junction plague proteins 1 to 3 (TJP1, TJP2 and TJP3) which are commutable with ZO-1, ZO-2 and ZO-3 (Mruk & Cheng, 2015). All ZO proteins provide the link to other peripheral proteins, such as actin and actin binding proteins (González-Mariscal et al., 2000; Van Itallie & Anderson, 2014). Beside ZOs, cingulin and afadin are another scaffolding proteins, which bind to actin microfilaments and tubulin microtubules (Van Itallie & Anderson, 2014).

The basal ES is indispensable part of BTB (Decrouy et al., 2004). Its general structure is comprised of three layers - bundles of actin, cistern of endoplasmic reticulum and plasma membrane, similarly to apical ES. The molecular composition of basal ES is mostly the same as that of apical ES. However, for the basal ES, a predominant adhesive complex is N-cadherin and β-catenin (Lee et al., 2003; Mruk & Cheng, 2004b). Integral membrane proteins of basal ES (N-cadherin) and TJs (occludin) are physically and functionally intermingled and were seen to associate with similar adaptor protein ZO-1 (Yan & Cheng, 2005). These findings suggest that TJs and basal ES together maintain the integrity of BTB. Similarly to the region of germ cell/Sertoli cell interface seen at the apical site of Sertoli cell which assures spermiation (Figure 6), tubulobulbar complexes appear also at the site of basal ES and are referred as basal TBC (Hess & Vogl, 2015). Although, they are smaller and less frequent compared to the apical TBCs (Vogl et al., 2008), they are capable to internalize integral membrane proteins of BTB (Mruk & Cheng, 2015).

Gap junction and desmosome-like junctions are located at the BTB between patches of intermingled TJs and basal ES (Mruk & Cheng, 2015). Gap junctions are here comprised from connexins which form a channel, connexon, similar to that formed at the site of germ cells in adluminal compartment (Pointis & Segretain, 2005). Hence connexin 43 interacts with structural component of desmosome (M. W. M. Li et al., 2009), the term “desmosome-gap junction” is sometimes used instead (Cheng & Mruk, 2010). Moreover, desmosomes interact in addition with TJ components such as CAR and ZO-1 which points to a very complex and interchangeable character of BTB components (Lie et al., 2010).

4. Modulation of Sertoli cell junctions during spermatogenesis

In the previous sections, I focused on the specialized junctions formed by the Sertoli cells with intention to highlight their complexity, importance and exclusivity compared to other somatic cells. However, it is necessary to understand, that these junctions are functionally coupled to the process of spermatogenesis, specific germ cell localization and regulation of their redistribution, and mostly synchronization of these processes which is crucial for developing sperm. Next, I will focus on the communication between the BTB, which is crossed by spermatocytes at the beginning of the gametogenesis, and apical ectoplasmic specialisation which is essential for the final step of germ cell development, the spermiation. Strikingly, these rather timely and regionally distinct processes appear to be highly synchronous. In particular, preleptotene/leptotene primary spermatocytes have to transverse the BTB to enter the adluminal compartment (L. Russell, 1977b) and at the same time, spermatozoa are released to the luminal fluid (Yan et al., 2007) as shown on the Figure 8. These two processes happen on the opposite sides of the Sertoli cells at the window from VIII to XI stage of the cycle of the seminiferous epithelium (Mruk & Cheng, 2015) and both induce restructuring and tight regulation of Sertoli cell’s junctions. A functional axis between apical ES and BTB was postulated (Cheng & Mruk, 2010) and below, I provide an insight into regulation of BTB synchronization with spermiation.

Preleptotene/leptotene spermatocytes migrate across BTB, thus the BTB has to transiently open (Mruk & Cheng, 2015). The passage of spermatocytes includes assembly of “new” BTB with help of assembling claudin 3, under the transported spermatocytes, while the “old” BTB above the spermatocytes disassembles (L. Russell, 1977b; Smith & Braun, 2012). Transported leptotene spermatocytes are thus transiently surrounded by new and old junctions of BTB barrier (L. Russell, 1977b; Smith & Braun, 2012). This mechanism resembles an overflow chamber and ensures that the integrity of the BTB won’t be disrupted (L. Russell, 1977b).

Components from the ”old” disassembled barrier, are internalized and engulfed by Sertoli cell. The process of internalisation is probably facilitated by the newly formed tubulobulbar complexes, via budding of the vesicles into the cytosol of Sertoli cell (Mruk & Cheng, 2015; L. D. Russell, 1979b). Proteins internalized are degraded in lysosomes or recycled in sorting endosomes for repeated use in newly formed junctions (Vogl et al., 2008), and this process is called junctional turnover. The turnover

of the BTB proteins was found to be regulated by testosterone and cytokines such as TNF-α, TGF-β2 and -β3 (Meng et al., 2005; Xia et al., 2009; Yan et al., 2008). Generally, testosterone and cytokines have antagonistic role - while testosterone promote adhesion and integrity of BTB (Meng et al., 2005), cytokines perturb the BTB (Xia et al., 2009). In particular, testosterone is suggested to mediate protein transcytosis and recycling of membrane proteins back to membrane, while cytokines (i.e., TGF-β3) send internalized proteins to endosome- and ubiquitin- mediated degradation. This scenario was experimentally confirmed by using in vitro cultured Sertoli cells growing on Matrigel which enables them to form BTB (Su et al., 2010). When the cells were treated with testosterone, internalized proteins occludin and N-cadherin colocalized with caveolin-1 which regulates transcytosis, and with Rab11, the protein marker of recycling (Su et al., 2010). When Sertoli cells were treated with TGF-β3, the internalized occludin and claudin colocalized with ubiquitin-conjugating enzyme E2 J1, which points to their degradation (Su et al., 2010).

Turnover of junctions is especially important for passing of spermatocytes across the barrier and spermiation. These events occur synchronously and are coordinated by dual effects of biologically active peptidic fragments (H. Li et al., 2020). These bioactive fragments act as local autocrine factors which have an impact on synchronicity of Sertoli cell junction remodelling. Such orchestrated path between two topological sites of Sertoli cell is called apical ES-BTB-basement membrane axis (Cheng & Mruk, 2010; N. Li et al., 2016) and in addition to membranous proteins, the cytoskeleton also plays important part in it (Y. Gao et al., 2016). Remodelling of actin and microtubular cytoskeleton modulates architecture of BTB and is also involved in the proper targeting of proteins into the junctions (N. Li et al., 2016). After disruption of cytoskeleton by glycerol, BTB is disrupted and non-functional (Wiebe et al., 2000). Important player at apical ES is laminin-α3β3γ3, which is one of the integral membrane proteins (Yan & Cheng, 2006). A biologically active F5-peptide can be released from its γ3 chain by the matrix metalloproteinase-2 (MMP-2) (H. Li et al., 2020; Su et al., 2012). Similarly, in the basal lamina are two bioactive peptides, which are released from junctional components. These are NC1-peptide derived from α3 chain of collagen type IV and LG3/4/5-peptide derived from laminin-α2 (H. Li et al., 2020). Both apical and basal active peptides affect the formation of actin and microtubular cytoskeleton of Sertoli cell as is shown in Figure 8 (H. Li et al., 2020). Moreover, there is a cross-talk between the abovementioned testosterone, cytokines and bioactive peptides. Sue et al. showed that TNF-α increased the expression of MMP-9, which is responsible for production of NC1-peptides (Siu et al., 2003). Nevertheless, the whole process concerning the communication axis between apical and basal Sertoli-cell-structures, remain largely uncharacterized and elusive.

It is of note that amongst other regulating factors, proteins with conventional role in inflammation can also affect architecture of BTB. Some of them are TNF-α, activin-A and various interleukins (IL1A, IL6, IL17A) (Pérez et al., 2014; Zhang et al., 2014).

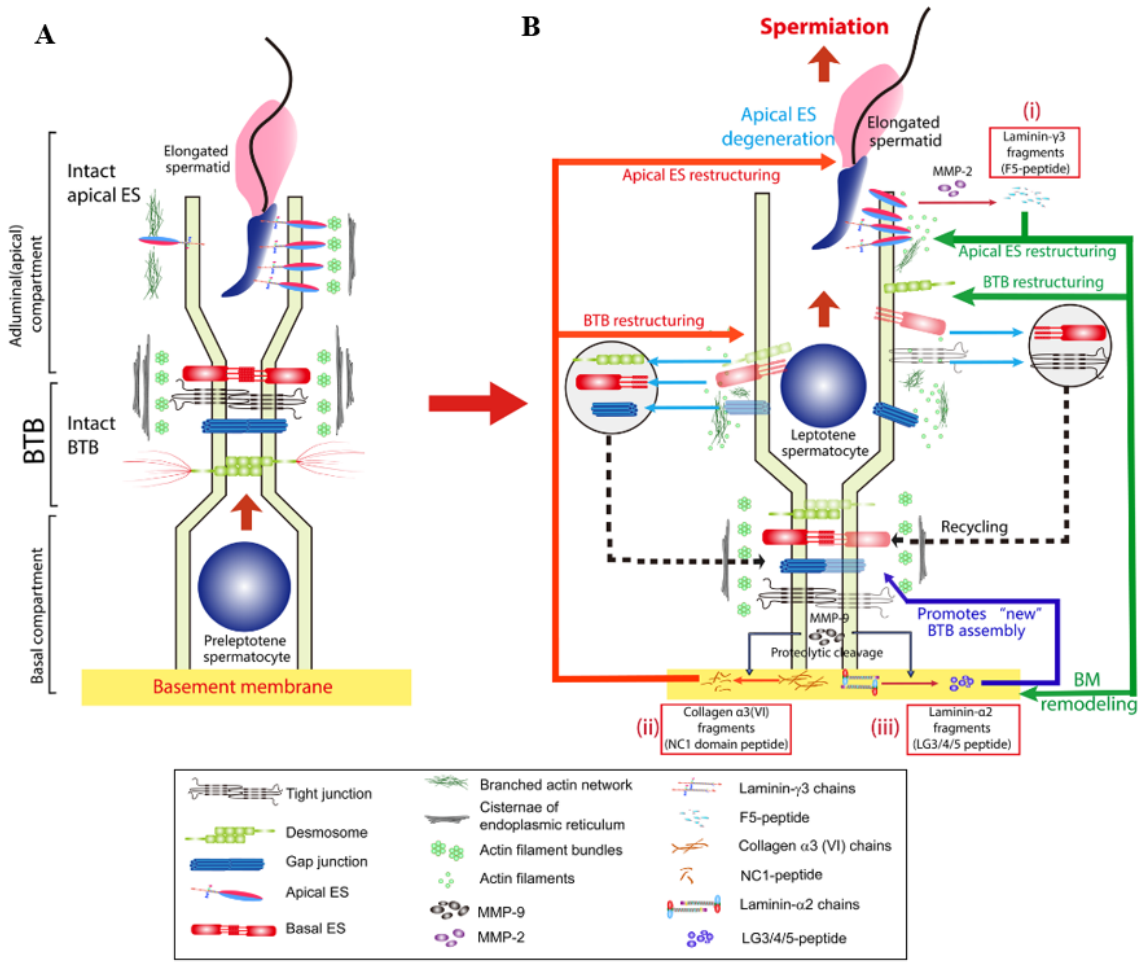

Figure 8. *Effects of bioactive peptides to the BTB and restructuring of apical ES*

A *A part of a scheme depicting the arrangement of junctional compounds before transition of the spermatocyte throung BTB.*

B *In this part of the scheme are visualized effects of (i) F5 peptide a laminin-γ3 fragment (green lines) and (ii) NC1-peptide from collagen α3 chain which both promote apical ES disassembly and BTB remodelling to allow passage of spermatocytes across the barrier or spermiation, respectively. Also are depicted effects of LG3/4/5 peptide (iii) from laminin-α2 chain, which promotes assembly of "new" BTB behind the transported spermatocyte. Abbreviations: MMP-2 - matrix metalloproteinase-2, MMP-9 - matrix metalloproteinase-9. Adopted and edited from (H. Li et al., 2020).*

5. Testes are immune privileged tissue

Testes are anatomical sites of immune privilege (Barker & Billingham, 1978). Commonality of all immune privilege tissues, central nervous system, corneal stroma of eye, pregnant uterus, as well as testicles (Streilein, 1993) is that in these places are antigens tolerated without triggering an inflammatory response (Streilein, 1995). There appears a question, why the site of spermatogenesis must be protected from the host immunity. Since the process of spermatogenesis become active only during puberty when central tolerance is firmly established (Meinhardt & Hedger, 2011), it is believed that the generated germ cell-specific antigens (Lemaire et al., 1992; Yuan et al., 1995) may provoke the immune response

(Diekman et al., 2000). Auto-inflammation causes so-called autoimmune testicular orchitis, which is accompanied by production of anti-sperm antibodies and cause sterility (Schuppe et al., 2008). To avoid this, Sertoli cells via BTB sequester these antigens in the adluminal compartment. Moreover, tightly regulated and closed BTB prevents the entry of immune cells and antibodies from the interstitial space to the adluminal compartment, where germ cells differentiate and mature (Cheng & Mruk, 2010; Mital et al., 2011). Nevertheless, as mentioned previously, there are spermatogonia and preleptotene/leptotene spermatocytes which are localized in the front of BTB closure, and thus are possibly visible for immune cells (Mital et al., 2011). Interestingly, several studies showed egress of germ cell-specific antigens to the interstitial space (O'Donnell et al., 2021; Tung et al., 2017). Those antigens were identified in a healthy adult testicular interstitial fluid (TIF) in mice and men and also in their blood plasma (O'Donnell et al., 2021). Based on these new observations, germ cell-specific antigens were categorized as either sequestered or non-sequestered in Sertoli cells. The non-sequestered antigens egress from the seminiferous tubule to the interstitial space (Tung et al., 2017). These antigens originate predominantly from nuclei and cytoplasm of prophase I spermatocytes, round and elongating spermatids, but can be also derived from sperm acrosome and flagellum which are generated behind the BTB (O'Donnell et al., 2021) Interestingly, it was shown that the transport of non-sequestered germ cell-specific antigens to the interstitial space is facilitated by residual bodies, i. e. via engulfment by Sertoli cells (Tung et al., 2017). While the mechanism of this antigen-generating function of residual bodies mediated by Sertoli cells remains elusive, it clearly points out that blood-testis barrier may not be the only factor maintaining the immune privilege in testicles.

Sertoli cells create immunomodulated environment in and outside of the seminiferous tubule (Fijak & Meinhardt, 2006; Mital et al., 2011). This tolerogenic microenvironment is maintained actively by the modulation of residing immune cells to be more tolerogenic and anti-inflammatory (Mellor & Munn, 2008). To achieve this, Sertoli cells secrete a variety of immunomodulatory factors such as activin A and TGF-β which precondition this microenvironment as anti-inflammatory, which adequately change the profile of immune cells (Washburn et al., 2022; Zhao et al., 2014). The most abundant immune cells in the interstitial space are macrophages, which in this anti-inflammatory environment are prone to M2 type immune responses with enhanced IL-10 secretion (Wang et al., 2017). Sertoli cells limit the number of effector T lymphocytes which are rather pushed into Th2 developmental lineage (Washburn et al., 2022), and on the other hand actively contribute to the generation of T-regulatory lymphocytes (Tregs) which also depends on the presence of IL-10 together with TGF- β1 (Zhou et al., 2008). Sertoli cells also dampen expression of MHC II on dendritic cells (DCs) making these professional antigen presenting cells more tolerogenic (J. Gao et al., 2016; Rival et al., 2007).

Nevertheless, immune privilege cannot limit the immune response to pathogens or infection. Thus, Sertoli cells are armed with pattern recognition receptors (PRR) such as Toll-Like Receptors (TLRs) which recognize evolutionary conserved metabolites/structures of pathogens as their ligands (Riccioli

et al., 2006). Such recognition of a pathogen by Sertoli cells leads to the secretion of pro-inflammatory cytokines IL-6, TNF-α, IL-1β, monocyte chemoattractant, chemokine MCP-1, along with the expression of ICAM-1 adhesive molecules on the surface of Sertoli cells, which then cooperatively facilitate lymphocyte infiltration (Cudicini et al., 1997; Riccioli et al., 2006; Washburn et al., 2022). Based on type of the invading pathogens, Sertoli cells respond by the secretion of either antimicrobial peptide β-defensins or anti-viral interferons (Sang et al., 2005; Washburn et al., 2022). These substances collectively facilitate the polarization of macrophages towards the M1 phenotype, and evoke the migration of pro-inflammatory effector T-cells to the tissue (Washburn et al., 2022). Once the infection disappears, Sertoli cells cease the production of anti-inflammatory factors, thus re-establishing the tolerogenic environment. Taken together, the immune state within the testis is maintained, tuned and thus master regulated by Sertoli cells, and the disruption or failure of the immune privilege leads to chronic inflammation and even infertility (Schuppe et al., 2008).

6. Discussion and conclusion

The main idea of my work was to provide an overview of Sertoli cell functions in spermatogenesis and investigate the current advancement in this field. Since the initial discovery of Sertoli cells in 1865, various and many new functions have been continually attributed to these cells. Firstly, Sertoli cells act in ontogenetic development, where they contribute to the formation of seminiferous tubules. At the onset of puberty, the first differentiating germ cells appear and provide the signal for Sertoli cells to change from immature proliferative to mature columnar cells, which are ready to nurture developing gametes for generation of vital sperm. To enable this, Sertoli cell provides needed support to germ cells in form of nutrition and hormones but also by disposal of cellular waste by endocytosis. What is interesting on these unique cells is that they also create immunologically protected environment, via isolation of the secluded compartment by the blood-testis barrier. This is facilitated by formation of junctions, the composition of which is in general very complex, largely testes-specific, and not yet fully described. Moreover, the uniqueness of these junctional structures is their plasticity by remodelling, which occur with stable periodicity (not just occasionally), and this process is highly synchronized and coupled to passing germ cell development and maturation, and thus creates homeostatically regulated and protective microenvironment. However, the exact mechanism(s) how such modulations are controlled, maintained and spatio-timely coordinated we just become to unravel. The suggested models, described in this work, still seems to be very simplified with many questions remaining to be answered. This suggests that in the future, new factors with additional roles will be discovered and added to already complex regulatory network operating on the level of Sertoli cells. One of the reasons why to study the Sertoli cell junction restructuring is not only the development of novel contraceptive for men but also strikingly increasing number of infertile men in recent decades, worldwide. Since these various types of junctions are essential for intact nurturing function of Sertoli cells critical for the generation of sperm,

it is obvious that many patients diagnosed with idiopathic sterility may suffer from some type of deficiency in formation and plasticity of these structures.

The currently most interesting new function of Sertoli cells, from my point of view as an immunologist, is their ability to generate and release the germ-cell-specific autoantigens to the interstitial space in a controlled manner. The fate of these autoantigens and their effect on the development and maintenance of immune privilege in testicles is tremendously interesting, yet largely overlooked question. While the contribution of Sertoli cells and BTB structural components to immune privilege was solidly investigated and described, this discovery raises many new questions. Firstly, is Sertoli cell deciding which antigens will be sequestered and which will be transported out of the seminiferous tubule, to the interstitial space? Secondly, since Tregs were found to be crucial in preventing autoimmune orchitis, what is the Sertoli cell contribution in modulation of Tregs activity? Does the egress of antigens into interstitial space play a role in this process? Are these germ-cell specific antigens presented by APC outside of the seminiferous tubule? These questions are very intriguing, experimentally challenging and would require to generate new animal and cell-based models to be experimentally answered. On the other hand, answers to these questions can be paradigm-changing, providing a new insight how Sertoli cells in collaboration with immune system establish and maintain the testicles as immune privilege sites.

While studying the data accumulated in publicly available literature, I got fascinated by the Sertoli cell, its complexity, multifunctionality, tremendous importance for successful spermatogenesis and importantly, for their immunomodulatory capabilities, which seems to be still poorly defined. It is of my highest amazement and excitement that Sertoli cell can possess so many crucial biological functions which are tightly and cooperatively regulated. For this reason, during my continuing studies, I would like to investigate the immunological role of Sertoli cell and its interaction with immune cells.

7. List of References

(*=review, **=book chapter)

*Agarwal, A., Mulgund, A., Hamada, A., & Chyatte, M. R. (2015). A unique view on male infertility around the globe. *Reproductive Biology and Endocrinology : RB&E*, *13*, 37. https://doi.org/10.1186/s12958-015-0032-1

*Bhushan, S., & Meinhardt, A. (2017). The macrophages in testis function. *Journal of Reproductive Immunology*, *119*, 107–112. https://doi.org/10.1016/j.jri.2016.06.008

*Bhushan, S., Theas, M. S., Guazzone, V. A., Jacobo, P., Wang, M., Fijak, M., Meinhardt, A., & Lustig, L. (2020). Immune cell subtypes and their function in the testis. *Frontiers in Immunology*, *11*, 583304. https://doi.org/10.3389/fimmu.2020.583304

Breucker, H., Schäfer, E., & Holstein, A. F. (1985). Morphogenesis and fate of the residual body in human spermiogenesis. *Cell and Tissue Research*, *240*(2), 303–309. https://doi.org/10.1007/BF00222339

Bustos-Obregon, E. (1976). Ultrastructure and Function of the Lamina Propria of Mammalian Seminiferous Tubules. *Andrologia*, *8*(3), 179–185. https://doi.org/10.1111/j.1439-0272.1976.tb02132.x

Chemes, H. (1986). The phagocytic function of Sertoli cells: A morphological, biochemical, and endocrinological study of lysosomes and acid phosphatase localization in the rat testis. *Endocrinology*, *119*(4), 1673–1681. https://doi.org/10.1210/endo-119-4-1673

*Cheng, C. Y., & Mruk, D. D. (2009). An intracellular trafficking pathway in the seminiferous epithelium regulating spermatogenesis: A biochemical and molecular perspective. *Critical Reviews in Biochemistry and Molecular Biology*, *44*(5), 245–263. https://doi.org/10.1080/10409230903061207

*Cheng, C. Y., & Mruk, D. D. (2010). A local autocrine axis in the testes that regulates spermatogenesis. *Nature Reviews. Endocrinology*, *6*(7), 380–395. https://doi.org/10.1038/nrendo.2010.71

*Cheng, C. Y., & Mruk, D. D. (2012). The Blood-testis barrier and its implications for male contraception. *Pharmacological Reviews*, *64*(1), 16–64. https://doi.org/10.1124/pr.110.002790

Chung, N. P., Mruk, D., Mo, M. Y., Lee, W. M., & Cheng, C. Y. (2001). A 22-amino acid synthetic peptide corresponding to the second extracellular loop of rat occludin perturbs the blood-testis barrier and disrupts spermatogenesis reversibly in vivo. *Biology of Reproduction*, *65*(5), 1340–1351. https://doi.org/10.1095/biolreprod65.5.1340

Clermont, Y. (1958). Contractile elements in the limiting membrane of the seminiferous tubules of the rat. *Experimental Cell Research*, *15*(2), 438–440. https://doi.org/10.1016/0014-4827(58)90052-1

Cudicini, C., Kercret, H., Touzalin, A. M., Ballet, F., & Jégou, B. (1997). Vectorial production of interleukin 1 and interleukin 6 by rat Sertoli cells cultured in a dual culture compartment system. *Endocrinology*, *138*(7), 2863–2870. https://doi.org/10.1210/endo.138.7.5289

*de Kretser, D. M., Loveland, K. L., Meinhardt, A., Simorangkir, D., & Wreford, N. (1998). Spermatogenesis. *Human Reproduction (Oxford, England)*, *13 Suppl 1*, 1–8. https://doi.org/10.1093/humrep/13.suppl_1.1

Decrouy, X., Gasc, J.-M., Pointis, G., & Segretain, D. (2004). Functional characterization of Cx43 based gap junctions during spermatogenesis. *Journal of Cellular Physiology*, *200*(1), 146–154. https://doi.org/10.1002/jcp.10473

DeFalco, T., Potter, S. J., Williams, A. V., Waller, B., Kan, M. J., & Capel, B. (2015). Macrophages contribute to the spermatogonial niche in the adult testis. *Cell Reports*, *12*(7), 1107–1119. https://doi.org/10.1016/j.celrep.2015.07.015

*Diekman, A. B., Norton, E. J., Westbrook, V. A., Klotz, K. L., Naaby-Hansen, S., & Herr, J. C. (2000). Anti-sperm antibodies from infertile patients and their cognate sperm antigens: A review. Identity between SAGA-1, the H6-3C4 antigen, and CD52. *American Journal of Reproductive Immunology (New York, N.Y.: 1989)*, *43*(3), 134–143. https://doi.org/10.1111/j.8755-8920.2000.430302.x

Drumond, A. L., Meistrich, M. L., & Chiarini-Garcia, H. (2011). Spermatogonial morphology and kinetics during testis development in mice: A high-resolution light microscopy approach. *Reproduction*, *142*(1), 145–155. https://doi.org/10.1530/REP-10-0431

Dym, M., & Fawcett, D. W. (1970). The blood-testis barrier in the rat and the physiological compartmentation of the seminiferous epithelium. *Biology of Reproduction*, *3*(3), 308–326. https://doi.org/10.1093/biolreprod/3.3.308

*Fijak, M., & Meinhardt, A. (2006). The testis in immune privilege. *Immunological Reviews*, *213*(1), 66–81. https://doi.org/10.1111/j.1600-065X.2006.00438.x

Flickinger, C., & Fawcett, D. W. (1967). The junctional specializations of sertoli cells in the seminiferous epithelium. *The Anatomical Record*, *158*(2), 207–221. https://doi.org/10.1002/ar.1091580210

*França, L. R., Hess, R. A., Dufour, J. M., Hofmann, M. C., & Griswold, M. D. (2016). The Sertoli cell: One hundred fifty years of beauty and plasticity. *Andrology*, *4*(2), 189–212. https://doi.org/10.1111/andr.12165

Gao, J., Wang, X., Wang, Y., Han, F., Cai, W., Zhao, B., Li, Y., Han, S., Wu, X., & Hu, D. (2016). Murine Sertoli cells promote the development of tolerogenic dendritic cells: A pivotal role of galectin-1. *Immunology*, *148*(3), 253–265. https://doi.org/10.1111/imm.12598

Gao, Y., Mruk, D. D., Lui, W.-Y., Lee, W. M., & Cheng, C. Y. (2016). F5-peptide induces aspermatogenesis by disrupting organization of actin- and microtubule-based cytoskeletons in the testis. *Oncotarget*, *7*(39), 64203–64220. https://doi.org/10.18632/oncotarget.11887

**Geyer, C. B. (2017). Setting the stage: The first round of spermatogenesis. In J. M. Oatley & M. D. Griswold (Eds.), *The Biology of Mammalian Spermatogonia* (pp. 39–63). Springer. https://doi.org/10.1007/978-1-4939-7505-1_3

Gliki, G., Ebnet, K., Aurrand-Lions, M., Imhof, B. A., & Adams, R. H. (2004). Spermatid differentiation requires the assembly of a cell polarity complex downstream of junctional adhesion molecule-C. *Nature*, *431*(7006), 320–324. https://doi.org/10.1038/nature02877

*González-Mariscal, L., Betanzos, A., & Avila-Flores, A. (2000). MAGUK proteins: Structure and role in the tight junction. *Seminars in Cell & Developmental Biology*, *11*(4), 315–324. https://doi.org/10.1006/scdb.2000.0178

Gow, A., Southwood, C. M., Li, J. S., Pariali, M., Riordan, G. P., Brodie, S. E., Danias, J., Bronstein, J. M., Kachar, B., & Lazzarini, R. A. (1999). CNS myelin and sertoli cell tight junction

strands are absent in Osp/claudin-11 null mice. *Cell*, *99*(6), 649–659. https://doi.org/10.1016/s0092-8674(00)81553-6

*Griswold, M. D. (2016). Spermatogenesis: The commitment to meiosis. *Physiological Reviews*, *96*(1), 1–17. https://doi.org/10.1152/physrev.00013.2015

*Griswold, M. D., Bishop, P. D., Kim, K.-H., Ping, R., Siiteri, J. E., & Morales, C. (1989). Function of vitamin A in normal and synchronized seminiferous tubulesa. *Annals of the New York Academy of Sciences*, *564*(1), 154–172. https://doi.org/10.1111/j.1749-6632.1989.tb25895.x

*Gu, X., Li, S.-Y., & DeFalco, T. (2022). Immune and vascular contributions to organogenesis of the testis and ovary. *The FEBS Journal*, *289*(9), 2386–2408. https://doi.org/10.1111/febs.15848

Guttman, J. A., Takai, Y., & Vogl, A. W. (2004). Evidence that tubulobulbar complexes in the seminiferous epithelium are involved with internalization of adhesion junctions. *Biology of Reproduction*, *71*(2), 548–559. https://doi.org/10.1095/biolreprod.104.028803

Hadley, M. A., & Dym, M. (1987). Immunocytochemistry of extracellular matrix in the lamina propria of the rat testis: Electron microscopic localization. *Biology of Reproduction*, *37*(5), 1283–1289. https://doi.org/10.1095/biolreprod37.5.1283

*Hedger, M. P. (1997). Testicular leukocytes: What are they doing? *Reviews of Reproduction*, *2*(1), 38–47. https://doi.org/10.1530/ror.0.0020038

*Heinrich, A., & DeFalco, T. (2020). Essential roles of interstitial cells in testicular development and function. *Andrology*, *8*(4), 903–914. https://doi.org/10.1111/andr.12703

**Hess, R. A., & Franca. (2005). Structure of Sertoli Cell. In M. K. Skinner & M. Griswold (Eds.), *Sertoli cell Biology* (pp. 19–40). Elsevier.

**Hess, R. A., & Vogl, A. W. (2015). Sertoli cell anatomy and cytoskeleton. In M. D. Griswold (Ed.), *Sertoli Cell Biology (Second Edition)* (pp. 1–55). Academic Press. https://doi.org/10.1016/B978-0-12-417047-6.00001-6

Hogarth, C. A., Arnold, S., Kent, T., Mitchell, D., Isoherranen, N., & Griswold, M. D. (2015). Processive pulses of retinoic acid propel asynchronous and continuous murine sperm production. *Biology of Reproduction*, *92*(2), 37. https://doi.org/10.1095/biolreprod.114.126326

Holdcraft, R. W., & Braun, R. E. (2004). Androgen receptor function is required in Sertoli cells for the terminal differentiation of haploid spermatids. *Development*, *131*(2), 459–467. https://doi.org/10.1242/dev.00957

**Holthöfer, B., Windoffer, R., Troyanovsky, S., & Leube, R. E. (2007). Structure and function of desmosomes. In *International Review of Cytology* (Vol. 264, pp. 65–163). Academic Press. https://doi.org/10.1016/S0074-7696(07)64003-0

Ikenouchi, J., Furuse, M., Furuse, K., Sasaki, H., Tsukita, S., & Tsukita, S. (2005). Tricellulin constitutes a novel barrier at tricellular contacts of epithelial cells. *The Journal of Cell Biology*, *171*(6), 939–945. https://doi.org/10.1083/jcb.200510043

Jutte, N. H. P. M., Jansen, R., Grootegoed, J. A., Rommerts, F. F. G., Clausen, O. P. F., & Molen, H. J. van der. (1982). Regulation of survival of rat pachytene spermatocytes by lactate supply from Sertoli cells. *Reproduction*, *65*(2), 431–438. https://doi.org/10.1530/jrf.0.0650431

*Kent, T., & Griswold, M. D. (2014). Checking the pulse of vitamin A metabolism and signaling during mammalian spermatogenesis. *Journal of Developmental Biology*, *2*(1), Article 1. https://doi.org/10.3390/jdb2010034

*Koopman, P. (2001). Gonad development: Signals for sex. *Current Biology*, *11*(12), R481–R483. https://doi.org/10.1016/S0960-9822(01)00287-1

Koopman, P., Münsterberg, A., Capel, B., Vivian, N., & Lovell-Badge, R. (1990). Expression of a candidate sex-determining gene during mouse testis differentiation. *Nature*, *348*(6300), 450–452. https://doi.org/10.1038/348450a0

*Kopera, I. A., Bilinska, B., Cheng, C. Y., & Mruk, D. D. (2010). Sertoli–germ cell junctions in the testis: A review of recent data. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1546), 1593–1605. https://doi.org/10.1098/rstb.2009.0251

Kulibin, A. Yu., & Malolina, E. A. (2020). Formation of the rete testis during mouse embryonic development. *Developmental Dynamics*, *249*(12), 1486–1499. https://doi.org/10.1002/dvdy.242

Leblond, C. P., & Clermont, Y. (1952). Definition of the stages of the cycle of the seminiferous epithelium in the rat. *Annals of the New York Academy of Sciences*, *55*(4), 548–573. https://doi.org/10.1111/j.1749-6632.1952.tb26576.x

Lee, N. P. Y., Mruk, D., Lee, W. M., & Cheng, C. Y. (2003). Is the cadherin/catenin complex a functional unit of cell-cell actin-based adherens junctions in the rat testis? *Biology of Reproduction*, *68*(2), 489–508. https://doi.org/10.1095/biolreprod.102.005793

Lemaire, L., Senftleben, A., & Heinlein, U. A. (1992). Characterization by enriched polyclonal antibodies of developmentally regulated and cell type specific mouse testis antigens. *Life Sciences*, *51*(6), 439–448. https://doi.org/10.1016/0024-3205(92)90412-i

*Li, H., Liu, S., Wu, S., Li, L., Ge, R., & Cheng, C. Y. (2020). Bioactive fragments of laminin and collagen chains: Lesson from the testis. *Reproduction (Cambridge, England)*, *159*(3), R111–R123. https://doi.org/10.1530/REP-19-0288

Li, M. W. M., Mruk, D. D., Lee, W. M., & Cheng, C. Y. (2009). Connexin 43 and plakophilin-2 as a protein complex that regulates blood-testis barrier dynamics. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(25), 10213–10218. https://doi.org/10.1073/pnas.0901700106

*Li, N., Tang, E. I., & Cheng, C. Y. (2016). Regulation of blood-testis barrier by actin binding proteins and protein kinases. *Reproduction (Cambridge, England)*, *151*(3), R29-41. https://doi.org/10.1530/REP-15-0463

*Li, N., Wang, T., & Han, D. (2012). Structural, cellular and molecular aspects of immune privilege in the testis. *Frontiers in Immunology*, *3*, 152. https://doi.org/10.3389/fimmu.2012.00152

Lie, P. P. Y., Cheng, C. Y., & Mruk, D. D. (2010). Crosstalk between desmoglein-2/desmocollin-2/Src kinase and coxsackie and adenovirus receptor/ZO-1 protein complexes, regulates blood-testis barrier dynamics. *The International Journal of Biochemistry & Cell Biology*, *42*(6), 975–986. https://doi.org/10.1016/j.biocel.2010.02.010

*Luissint, A.-C., Nusrat, A., & Parkos, C. A. (2014). JAM related proteins in mucosal homeostasis and inflammation. *Seminars in Immunopathology*, *36*(2), 211–226. https://doi.org/10.1007/s00281-014-0421-0

Lyon, K. R. P., Bosseboeuf, E., & Vogl, A. W. (2015). An alternative model of tubulobulbar complex internalization during junction remodeling in the seminiferous epithelium of the rat testis. *Biology of Reproduction*, *93*(1), 12. https://doi.org/10.1095/biolreprod.115.128942

Masuda, S., Oda, Y., Sasaki, H., Ikenouchi, J., Higashi, T., Akashi, M., Nishi, E., & Furuse, M. (2011). LSR defines cell corners for tricellular tight junction formation in epithelial cells. *Journal of Cell Science*, *124*(Pt 4), 548–555. https://doi.org/10.1242/jcs.072058

Meineke, V., Frungieri, M. B., Jessberger, B., Vogt, H.-J., & Mayerhofer, A. (2000). Human testicular mast cells contain tryptase: Increased mast cell number and altered distribution in the testes of infertile men. *Fertility and Sterility*, *74*(2), 239–244. https://doi.org/10.1016/S0015-0282(00)00626-9

*Meinhardt, A., & Hedger, M. P. (2011). Immunological, paracrine and endocrine aspects of testicular immune privilege. *Molecular and Cellular Endocrinology*, *335*(1), 60–68. https://doi.org/10.1016/j.mce.2010.03.022

*Mellor, A. L., & Munn, D. H. (2008). Creating immune privilege: Active local suppression that benefits friends, but protects foes. *Nature Reviews Immunology*, *8*(1), Article 1. https://doi.org/10.1038/nri2233

Meng, J., Holdcraft, R. W., Shima, J. E., Griswold, M. D., & Braun, R. E. (2005). Androgens regulate the permeability of the blood-testis barrier. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(46), 16696–16700. https://doi.org/10.1073/pnas.0506084102

*Meroni, S. B., Galardo, M. N., Rindone, G., Gorga, A., Riera, M. F., & Cigorraga, S. B. (2019). Molecular mechanisms and signaling pathways involved in Sertoli cell proliferation. *Frontiers in Endocrinology*, *10*, 224. https://doi.org/10.3389/fendo.2019.00224

*Mital, P., Hinton, B. T., & Dufour, J. M. (2011). The blood-testis and blood-epididymis barriers are more than just their tight junctions. *Biology of Reproduction*, *84*(5), 851–858. https://doi.org/10.1095/biolreprod.110.087452

*Morrow, C. M. K., Mruk, D., Cheng, C. Y., & Hess, R. A. (2010). Claudin and occludin expression and function in the seminiferous epithelium. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1546), 1679–1696. https://doi.org/10.1098/rstb.2010.0025

*Mossadegh-Keller, N., & Sieweke, M. H. (2018). Testicular macrophages: Guardians of fertility. *Cellular Immunology*, *330*, 120–125. https://doi.org/10.1016/j.cellimm.2018.03.009

*Mruk, D. D., & Cheng, C. Y. (2004a). Cell-cell interactions at the ectoplasmic specialization in the testis. *Trends in Endocrinology and Metabolism: TEM*, *15*(9), 439–447. https://doi.org/10.1016/j.tem.2004.09.009

*Mruk, D. D., & Cheng, C. Y. (2004b). Sertoli-Sertoli and Sertoli-germ cell interactions and their significance in germ cell movement in the seminiferous epithelium during spermatogenesis. *Endocrine Reviews*, *25*(5), 747–806. https://doi.org/10.1210/er.2003-0022

*Mruk, D. D., & Cheng, C. Y. (2015). The mammalian blood-testis barrier: Its biology and regulation. *Endocrine Reviews*, *36*(5), 564–591. https://doi.org/10.1210/er.2014-1101

*Nakata, H. (2019). Morphology of mouse seminiferous tubules. *Anatomical Science International*, *94*(1), 1–10. https://doi.org/10.1007/s12565-018-0455-9

**Nef, S., Stévant, I., & Greenfield, A. (2019). Characterizing the bipotential mammalian gonad. In B. Capel (Ed.), *Current Topics in Developmental Biology* (Vol. 134, pp. 167–194). Academic Press. https://doi.org/10.1016/bs.ctdb.2019.01.002

Nel-Themaat, L., Jang, C.-W., Stewart, M. D., Akiyama, H., Viger, R. S., & Behringer, R. R. (2011). Sertoli cell behaviors in developing testis cords and postnatal seminiferous tubules of the mouse. *Biology of Reproduction*, *84*(2), 342–350. https://doi.org/10.1095/biolreprod.110.086900

Nel-Themaat, L., Vadakkan, T. J., Wang, Y., Dickinson, M. E., Akiyama, H., & Behringer, R. R. (2009). Morphometric analysis of testis cord formation in Sox9-EGFP mice. *Developmental Dynamics: An Official Publication of the American Association of Anatomists*, *238*(5), 1100–1110. https://doi.org/10.1002/dvdy.21954

*Oatley, J. M., & Brinster, R. L. (2012). The germline stem cell niche unit in mammalian testes. *Physiological Reviews*, *92*(2), 577–595. https://doi.org/10.1152/physrev.00025.2011

*O'Donnell, L. (2014). Mechanisms of spermiogenesis and spermiation and how they are disturbed. *Spermatogenesis*, *4*(2), e979623. https://doi.org/10.4161/21565562.2014.979623

*O'Donnell, L., Nicholls, P. K., O'Bryan, M. K., McLachlan, R. I., & Stanton, P. G. (2011). Spermiation. *Spermatogenesis*, *1*(1), 14–35. https://doi.org/10.4161/spmg.1.1.14525

O'Donnell, L., Rebourcet, D., Dagley, L. F., Sgaier, R., Infusini, G., O'Shaughnessy, P. J., Chalmel, F., Fietz, D., Weidner, W., Legrand, J. M. D., Hobbs, R. M., McLachlan, R. I., Webb, A. I., Pilatz, A., Diemer, T., Smith, L. B., & Stanton, P. G. (2021). Sperm proteins and cancer-testis antigens are released by the seminiferous tubules in mice and men. *The FASEB Journal*, *35*(3), e21397. https://doi.org/10.1096/fj.202002484R

*Oliver, E., & Stukenborg, J.-B. (2020). Rebuilding the human testis in vitro. *Andrology*, *8*(4), 825–834. https://doi.org/10.1111/andr.12710

Ozaki-Kuroda, K., Nakanishi, H., Ohta, H., Tanaka, H., Kurihara, H., Mueller, S., Irie, K., Ikeda, W., Sakai, T., Wimmer, E., Nishimune, Y., & Takai, Y. (2002). Nectin couples cell-cell adhesion and the actin scaffold at heterotypic testicular junctions. *Current Biology*, *12*(13), 1145–1150. https://doi.org/10.1016/S0960-9822(02)00922-3

Patriarca, C., Colecchia, M., & Clerici, C. A. (2019). Enrico Sertoli and the supporting cells of the testis "Morphology is function". *Pathologica*, *111*(4), 375–381. https://doi.org/10.32074/1591-951X-32-19

*Pointis, G., Gilleron, J., Carette, D., & Segretain, D. (2010). Physiological and physiopathological aspects of connexins and communicating gap junctions in spermatogenesis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1546), 1607–1620. https://doi.org/10.1098/rstb.2009.0114

*Pointis, G., & Segretain, D. (2005). Role of connexin-based gap junction channels in testis. *Trends in Endocrinology & Metabolism*, *16*(7), 300–306. https://doi.org/10.1016/j.tem.2005.07.001

Practice Committee of the American Society for Reproductive Medicine. (2015). Diagnostic evaluation of the infertile male: A committee opinion. *Fertility and Sterility*, *103*(3), e18-25. https://doi.org/10.1016/j.fertnstert.2014.12.103

Raverdeau, M., Gely-Pernot, A., Féret, B., Dennefeld, C., Benoit, G., Davidson, I., Chambon, P., Mark, M., & Ghyselinck, N. B. (2012). Retinoic acid induces Sertoli cell paracrine signals for spermatogonia differentiation but cell autonomously drives spermatocyte meiosis. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(41), 16582–16587. https://doi.org/10.1073/pnas.1214936109

Riccioli, A., Starace, D., Galli, R., Fuso, A., Scarpa, S., Palombi, F., De Cesaris, P., Ziparo, E., & Filippini, A. (2006). Sertoli cells initiate testicular innate immune responses through TLR activation. *Journal of Immunology (Baltimore, Md.: 1950)*, *177*(10), 7122–7130. https://doi.org/10.4049/jimmunol.177.10.7122

Rival, C., Guazzone, V. A., von Wulffen, W., Hackstein, H., Schneider, E., Lustig, L., Meinhardt, A., & Fijak, M. (2007). Expression of co-stimulatory molecules, chemokine receptors and proinflammatory cytokines in dendritic cells from normal and chronically inflamed rat testis. *Molecular Human Reproduction*, *13*(12), 853–861. https://doi.org/10.1093/molehr/gam067

Rodriguez, I., Ody, C., Araki, K., Garcia, I., & Vassalli, P. (1997). An early and massive wave of germinal cell apoptosis is required for the development of functional spermatogenesis. *The EMBO Journal*, *16*(9), 2262–2270. https://doi.org/10.1093/emboj/16.9.2262

Russell, L. (1977a). Desmosome-like junctions between Sertoli and germ cells in the rat testis. *The American Journal of Anatomy*, *148*(3), 301–312. https://doi.org/10.1002/aja.1001480302

Russell, L. (1977b). Movement of spermatocytes from the basal to the adluminal compartment of the rat testis. *American Journal of Anatomy*, *148*(3), 313–328. https://doi.org/10.1002/aja.1001480303

Russell, L., & Clermont, Y. (1976). Anchoring device between Sertoli cells and late spermatids in rat seminiferous tubules. *The Anatomical Record*, *185*(3), 259–277. https://doi.org/10.1002/ar.1091850302

Russell, L. D. (1979a). Further observations on tubulobulbar complexes formed by late spermatids and sertoli cells in the rat testis. *The Anatomical Record*, *194*(2), 213–232. https://doi.org/10.1002/ar.1091940204

Russell, L. D. (1979b). Observations on the inter-relationships of Sertoli cells at the level of the blood-testis barrier: Evidence for formation and resorption of Sertoli-Sertoli tubulobulbar complexes during the spermatogenic cycle of the rat. *American Journal of Anatomy*, *155*(2), 259–279. https://doi.org/10.1002/aja.1001550208

Russell, L. D. (1979c). Spermatid-Sertoli tubulobulbar complexes as devices for elimination of cytoplasm from the head region late spermatids of the rat. *The Anatomical Record*, *194*(2), 233–246. https://doi.org/10.1002/ar.1091940205

Russell, L. D., Saxena, N. K., & Turner, T. T. (1989). Cytoskeletal involvement in spermiation and sperm transport. *Tissue and Cell*, *21*(3), 361–379. https://doi.org/10.1016/0040-8166(89)90051-7

Salanova, M., Stefanini, M., De Curtis, I., & Palombi, F. (1995). Integrin receptor α6β1 is localized at specific sites of cell-to-cell contact in rat seminiferous epithelium. *Biology of Reproduction*, *52*(1), 79–87. https://doi.org/10.1095/biolreprod52.1.79

Sang, Y., Ortega, M. T., Blecha, F., Prakash, O., & Melgarejo, T. (2005). Molecular cloning and characterization of three beta-defensins from canine testes. *Infection and Immunity*, *73*(5), 2611–2620. https://doi.org/10.1128/IAI.73.5.2611-2620.2005

*Schuppe, H.-C., Meinhardt, A., Allam, J. P., Bergmann, M., Weidner, W., & Haidl, G. (2008). Chronic orchitis: A neglected cause of male infertility? *Andrologia*, *40*(2), 84–91. https://doi.org/10.1111/j.1439-0272.2008.00837.x

Sekido, R., Bar, I., Narváez, V., Penny, G., & Lovell-Badge, R. (2004). SOX9 is up-regulated by the transient expression of SRY specifically in Sertoli cell precursors. *Developmental Biology*, *274*(2), 271–279. https://doi.org/10.1016/j.ydbio.2004.07.011

*Sharma, A., Minhas, S., Dhillo, W. S., & Jayasena, C. N. (2020). Male infertility due to testicular disorders. *The Journal of Clinical Endocrinology and Metabolism*, *106*(2), e442–e459. https://doi.org/10.1210/clinem/dgaa781

*Sharpe, R. M., McKinnell, C., Kivlin, C., & Fisher, J. S. (2003). Proliferation and functional maturation of Sertoli cells, and their relevance to disorders of testis function in adulthood. *Reproduction*, *125*(6), 769–784. https://doi.org/10.1530/rep.0.1250769

Siu, M. K. Y., Lee, W. M., & Cheng, C. Y. (2003). The interplay of collagen IV, tumor necrosis factor-alpha, gelatinase B (matrix metalloprotease-9), and tissue inhibitor of metalloproteases-1 in the basal lamina regulates Sertoli cell-tight junction dynamics in the rat testis. *Endocrinology*, *144*(1), 371–387. https://doi.org/10.1210/en.2002-220786

Skinner, M. K., Tung, P. S., & Fritz, I. B. (1985). Cooperativity between Sertoli cells and testicular peritubular cells in the production and deposition of extracellular matrix components. *The Journal of Cell Biology*, *100*(6), 1941–1947.

*Slack, J. M. W. (2018). What is a stem cell? *WIREs Developmental Biology*, *7*(5), e323. https://doi.org/10.1002/wdev.323

Smith, B. E., & Braun, R. E. (2012). Germ cell migration across Sertoli cell tight junctions. *Science (New York, N.Y.)*, *338*(6108), 798–802. https://doi.org/10.1126/science.1219969

Snyder, E. M., Small, C., & Griswold, M. D. (2010). Retinoic acid availability drives the asynchronous initiation of spermatogonial differentiation in the mouse. *Biology of Reproduction*, *83*(5), 783–790. https://doi.org/10.1095/biolreprod.110.085811

Solari, A. J., & Fritz, I. B. (1978). The ultrastructure of immature sertoli cells. Maturation-like changes during culture and the maintenance of mitotic potentiality. *Biology of Reproduction*, *18*(3), 329–345. https://doi.org/10.1095/biolreprod18.3.329

Streilein, J. W. (1993). Immune privilege as the result of local tissue barriers and immunosuppressive microenvironments. *Current Opinion in Immunology*, *5*(3), 428–432. https://doi.org/10.1016/0952-7915(93)90064-Y

Su, L., Mruk, D. D., Lee, W. M., & Cheng, C. Y. (2010). Differential effects of testosterone and TGF-β3 on endocytic vesicle-mediated protein trafficking events at the blood-testis barrier. *Experimental Cell Research*, *316*(17), 2945–2960. https://doi.org/10.1016/j.yexcr.2010.07.018

Su, L., Mruk, D. D., Lie, P. P. Y., Silvestrini, B., & Cheng, C. Y. (2012). A peptide derived from laminin-γ3 reversibly impairs spermatogenesis in rats. *Nature Communications*, *3*, 1185. https://doi.org/10.1038/ncomms2171

*Svingen, T., & Koopman, P. (2013). Building the mammalian testis: Origins, differentiation, and assembly of the component cell populations. *Genes & Development*, *27*(22), 2409–2426. https://doi.org/10.1101/gad.228080.113

*Sylvester, S. R., & Griswold, M. D. (1994). The testicular iron shuttle: A "nurse" function of the sertoli cells. *Journal of Andrology*, *15*(5), 381–385. https://doi.org/10.1002/j.1939-4640.1994.tb00468.x

Tang, E. I., Lee, W. M., & Cheng, C. Y. (2016). Coordination of actin- and microtubule-based cytoskeletons supports transport of spermatids and residual bodies/phagosomes during spermatogenesis in the rat testis. *Endocrinology*, *157*(4), 1644–1659. https://doi.org/10.1210/en.2015-1962

**The Editors of Encyclopaedia Britannica. (2023). Spermatogenesis | Description & Process. *Encyclopedia Britannica*. https://www.britannica.com/science/spermatogenesis#/media/1/559418/143155

Thoma, M. E., McLain, A. C., Louis, J. F., King, R. B., Trumble, A. C., Sundaram, R., & Buck Louis, G. M. (2013). The prevalence of infertility in the United States as estimated by the current duration approach and a traditional constructed approach. *Fertility and Sterility*, *99*(5), 1324-1331.e1. https://doi.org/10.1016/j.fertnstert.2012.11.037

Toyama, Y. (1976). Actin-like filaments in the Sertoli cell junctional specializations in the swine and mouse testis. *The Anatomical Record*, *186*(4), 477–491. https://doi.org/10.1002/ar.1091860403

Tung, K., Harakal, J., Qiao, H., Rival, C., Li, J. C. H., Paul, A. G. A., Wheeler, K., Pramoonjago, P., Grafer, C. M., Sun, W., Sampson, R. D., Wong, E. W. P., Reddi, P. P., Deshmukh, U. S., Hardy, D. M., Tang, H., Cheng, C. Y., & Goldberg, E. (2017). Egress of sperm autoantigen from seminiferous tubules maintains systemic tolerance. *Journal of Clinical Investigation*, *127*(3), 1046–1060. https://doi.org/10.1172/JCI89927

*Van Itallie, C. M., & Anderson, J. M. (2014). Architecture of tight junctions and principles of molecular composition. *Seminars in Cell & Developmental Biology*, *0*, 157–165. https://doi.org/10.1016/j.semcdb.2014.08.011

*Vogl, A. W. (1989). Distribution and function of organized concentrations of actin filaments in mammalian spermatogenic cells and Sertoli cells. *International Review of Cytology*, *119*, 1–56. https://doi.org/10.1016/s0074-7696(08)60648-8

*Vogl, A. W., Pfeiffer, D. C., Mulholland, D., Kimel, G., & Guttman, J. (2000). Unique and multifunctional adhesion junctions in the testis: Ectoplasmic specializations. *Archives of Histology and Cytology*, *63*(1), 1–15. https://doi.org/10.1679/aohc.63.1

Vogl, A. W., Soucy, L. J., & Foo, V. (1985). Ultrastructure of Sertoli-cell penetrating processes found in germ cells of the golden-mantled ground squirrel (spermophilus lateralis). *The American Journal of Anatomy*, *172*(1), 75–86. https://doi.org/10.1002/aja.1001720106

*Vogl, A. W., Vaid, K. S., & Guttman, J. A. (2008). The Sertoli cell cytoskeleton. *Advances in Experimental Medicine and Biology*, *636*, 186–211. https://doi.org/10.1007/978-0-387-09597-4_11

*Walker, W. H. (2011). Testosterone signaling and the regulation of spermatogenesis. *Spermatogenesis*, *1*(2), 116–120. https://doi.org/10.4161/spmg.1.2.16956

Wang, M., Fijak, M., Hossain, H., Markmann, M., Nüsing, R. M., Lochnit, G., Hartmann, M. F., Wudy, S. A., Zhang, L., Gu, H., Konrad, L., Chakraborty, T., Meinhardt, A., & Bhushan, S. (2017). Characterization of the micro-environment of the testis that shapes the phenotype and function of testicular macrophages. *The Journal of Immunology*, *198*(11), 4327–4340. https://doi.org/10.4049/jimmunol.1700162

*Washburn, R. L., Hibler, T., Kaur, G., & Dufour, J. M. (2022). Sertoli cell immune regulation: A double-edged sword. *Frontiers in Immunology*, *13*, 913502. https://doi.org/10.3389/fimmu.2022.913502

Wiebe, J. P., Kowalik, A., Gallardi, R. L., Egeler, O., & Clubb, B. H. (2000). Glycerol disrupts tight junction-associated actin microfilaments, occludin, and microtubules in Sertoli cells. *Journal of Andrology*, *21*(5), 625–635.

**Wong, C., & Cheng, C. Y. (2005). The blood-testis barrier: Its biology, regulation, and physiological role in spermatogenesis. In *Current Topics in Developmental Biology* (Vol. 71, pp. 263–296). Academic Press. https://doi.org/10.1016/S0070-2153(05)71008-5

Wong, E. W. P., Mruk, D. D., & Cheng, C. Y. (2008). Biology and regulation of ectoplasmic specialization, an atypical adherens junction type, in the testis. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, *1778*(3), 692–708. https://doi.org/10.1016/j.bbamem.2007.11.006

Xia, W., Wong, E. W. P., Mruk, D. D., & Cheng, C. Y. (2009). TGF-β3 and TNFα perturb blood–testis barrier (BTB) dynamics by accelerating the clathrin-mediated endocytosis of integral membrane proteins: A new concept of BTB regulation during spermatogenesis. *Developmental Biology*, *327*(1), 48–61. https://doi.org/10.1016/j.ydbio.2008.11.028

**Yan Cheng, C., & Mruk, D. D. (2015). Biochemistry of Sertoli cell/germ cell junctions, germ cell transport, and spermiation in the seminiferous epithelium. In M. D. Griswold (Ed.), *Sertoli Cell Biology (Second Edition)* (pp. 333–383). Academic Press. https://doi.org/10.1016/B978-0-12-417047-6.00012-0

Yan, H. H. N., & Cheng, C. Y. (2005). Blood–testis barrier dynamics are regulated by an engagement/disengagement mechanism between tight and adherens junctions via peripheral adaptors. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(33), 11722. https://doi.org/10.1073/pnas.0503855102

Yan, H. H. N., & Cheng, C. Y. (2006). Laminin alpha 3 forms a complex with beta3 and gamma3 chains that serves as the ligand for alpha 6beta1-integrin at the apical ectoplasmic specialization in adult rat testes. *The Journal of Biological Chemistry*, *281*(25), 17286–17303. https://doi.org/10.1074/jbc.M513218200

*Yan, H. H. N., Mruk, D. D., Lee, W. M., & Cheng, C. Y. (2007). Ectoplasmic specialization: A friend or a foe of spermatogenesis? *BioEssays*, *29*(1), 36–48. https://doi.org/10.1002/bies.20513

Yan, H. H. N., Mruk, D. D., Lee, W. M., & Cheng, C. Y. (2008). Blood-testis barrier dynamics are regulated by testosterone and cytokines via their differential effects on the kinetics of protein endocytosis and recycling in Sertoli cells. *The FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology*, *22*(6), 1945–1959. https://doi.org/10.1096/fj.06-070342

Young, J. S., Guttman, J. A., Vaid, K. S., Shahinian, H., & Vogl, A. W. (2009). Cortactin (CTTN), N-WASP (WASL), and Clathrin (CLTC) are present at podosome-like tubulobulbar complexes in the rat testis. *Biology of Reproduction*, *80*(1), 153–161. https://doi.org/10.1095/biolreprod.108.070615

Young, J. S., Takai, Y., Kojic, K. L., & Vogl, A. W. (2012). Internalization of adhesion junction proteins and their association with recycling endosome marker proteins in rat seminiferous epithelium. *Reproduction (Cambridge, England)*, *143*(3), 347–357. https://doi.org/10.1530/REP-11-0317

Yuan, L., Liu, J. G., & Höög, C. (1995). Rapid cDNA sequencing in combination with RNA expression studies in mice identifies a large number of male germ cell-specific sequence tags. *Biology of Reproduction*, *52*(1), 131–138. https://doi.org/10.1095/biolreprod52.1.131

*Zhao, S., Zhu, W., Xue, S., & Han, D. (2014). Testicular defense systems: Immune privilege and innate immunity. *Cellular & Molecular Immunology*, *11*(5), 428–437. https://doi.org/10.1038/cmi.2014.38

Zhou, L., Lopes, J. E., Chong, M. M. W., Ivanov, I. I., Min, R., Victora, G. D., Shen, Y., Du, J., Rubtsov, Y. P., Rudensky, A. Y., Ziegler, S. F., & Littman, D. R. (2008). TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing RORgammat function. *Nature*, *453*(7192), 236–240. https://doi.org/10.1038/nature06878

Zimmermann, C., Stévant, I., Borel, C., Conne, B., Pitetti, J.-L., Calvel, P., Kaessmann, H., Jégou, B., Chalmel, F., & Nef, S. (2015). Research Resource: The Dynamic Transcriptional Profile of Sertoli Cells During the Progression of Spermatogenesis. *Molecular Endocrinology*, *29*(4), 627–642. https://doi.org/10.1210/me.2014-1356

*Zirkin, B. R., & Papadopoulos, V. (2018). Leydig cells: Formation, function, and regulation. *Biology of Reproduction*, *99*(1), 101–111. https://doi.org/10.1093/biolre/ioy059