

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

**4,800**

Open access books available

**122,000**

International authors and editors

**135M**

Downloads

Our authors are among the

**154**

Countries delivered to

**TOP 1%**

most cited scientists

**12.2%**

Contributors from top 500 universities



**WEB OF SCIENCE™**

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.

For more information visit [www.intechopen.com](http://www.intechopen.com)



# Innate Immune Defense in the Male Reproductive System and Male Fertility

*Fei Wang, Ran Chen and Daishu Han*

## Abstract

To protect the male germ cells from adverse immune reaction, the male reproductive system adopts special immune environment such as immunoprivileged status. The male genital organs can be infected by various microorganisms via hematogenous dissemination and ascending genitourinary tracts. To overcome the immunoprivileged status, the male genital organs also adopt their own innate defense against microbial infection. The tissue-specific cells in the male reproductive system are well equipped with innate immune machineries, including pattern recognition receptors (PRRs) and their negatively regulatory system. PRR-initiated immune responses must be tightly regulated by the negative regulatory system for the maintenance of immune homeostasis. The immune homeostasis can be disrupted by unrestrictive innate immune response, which may lead to inflammatory conditions in the male genital tracts, an important etiological factor contributing to male infertility. This chapter describes the current understanding of the innate immune responses in the male reproductive system and their effects on male fertility.

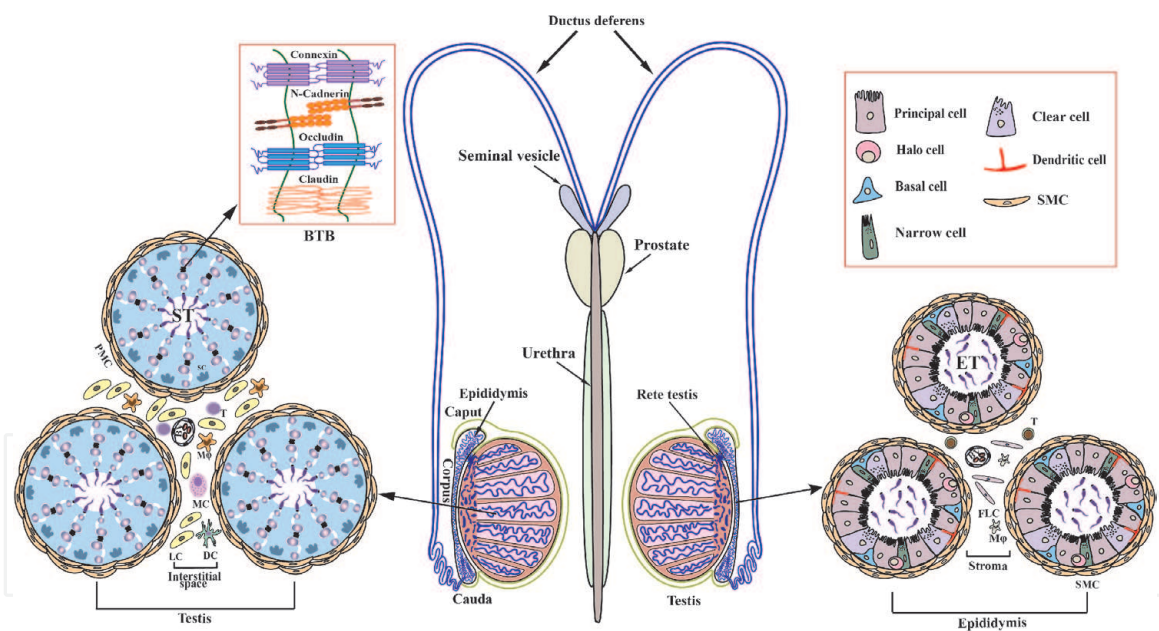
**Keywords:** innate immunity, testis, male fertility, pattern recognition receptor, Tyro3/Axl/Mer receptor tyrosine kinase, orchitis

## 1. Introduction

Innate immunity is the first line of the body defense against microbial infections. The innate immune responses initiated by pattern recognition receptors (PRRs) play critical roles in building the innate immunity and regulating adaptive immunity [1, 2]. PRR-initiated innate immune responses can lead to acute inflammatory conditions, essential for counteracting invading microbes, which must be tightly restricted by the negative regulatory system for maintaining the immune homeostasis. Unrestricted innate immune responses may result in chronic inflammation that can be harmful to the host self [3].

PRRs not only initiate innate immune responses in the immune cells, but also in the nonimmune epithelial cells of various tissues. In particular, the tissue-specific epithelial cells of organs, such as the intestine, lung, and urogenital tracts, which are frequently infected by microorganisms, abundantly express PRRs, and the PRR-initiated innate immune responses play important roles in the tissue defense against microbial infections. The production of functional sperm is necessary for normal fertility, which requires the close cooperation of a whole reproductive

system that is composed of several major organs, including the testes, epididymis, seminal vesicle, and the prostate (**Figure 1**). Sperm cells are produced in the testes, and then mature and are stored in the epididymis. Before ejection, sperm cells are mixed with seminal plasma that is mainly produced by the prostate and seminal vesicle. Sperm is produced post-puberty after the establishment of central immune tolerance. Therefore, sperm production generates immunogenic autoantigens. To avoid detrimental autoimmune responses under physiological conditions, the male reproductive system adopts a unique immune environment. In particular, the testis is a remarkable immunoprivileged organ. The epididymis also has immunoprivileged properties for protecting sperm during maturation and storage from an immune attack. However, the male reproductive system can be infected by various organisms via ascending genital tracts and hematogenous dissemination, which frequently lead to inflammation of the system and impairment of male fertility. The defined inflammation in the male reproductive system includes urethritis, prostatitis, seminal vesiculitis, epididymitis, and orchitis. Ascending bacterial infections represent frequent etiological factors of inflammation in the male reproductive system [4]. Epididymitis and orchitis are predominantly caused by hematogenous dissemination of viruses. Moreover, noninfectious epididymitis and orchitis are also frequently observed. In this regard, male germ damage can be a sterile cause of epididymitis. While all of the inflammatory conditions may perturb male fertility, the inflammatory male infertility is mostly caused by epididymitis and orchitis [5].



**Figure 1.**

*Schematic of the male reproductive system. The male reproductive system is composed of various organs (middle panel), including genital glands, the testes and epididymides; accessory genital glands, the prostates and seminal vesicles; and genital tracts, the ductus deferens and urethra. Both the testis and epididymis belong to the immunoprivileged organs for protecting male germ cells from adverse immune reaction. The testis histologically contains the seminiferous tubule (ST) and interstitial space (left panel). The ST is surrounded by peritubular myoid cells (PMC) and lined by the seminiferous epithelium that is composed of columnar Sertoli cells (SC) embracing different stages of germ cells. The blood-testis barrier (BTB) is formed between adjacent two SC. In the interstitial spaces of the testis, there are mainly Leydig cells (LC), and various immune cells, including macrophages (Mφ), T lymphocytes (T), mast cells (MC), and dendritic cells (DC). Blood vessels (BV) are located in the interstitial spaces. The epididymis can be divided into the caput, corpus, and cauda epididymides. The caput epididymis is connected with the testis via the rete testis. The epididymis is composed of the coiled epididymal tubules (ET) and stroma among the ET (right panel). The ET is surrounded by smooth muscle cells (SMC) and lined by a pseudostratified epithelium that is composed of principal cells, halo cells, basal cells, narrow cells, clear cells, and DC. The stroma areas contain BV, Mφ, T, and certain fibroblast-like cells (FLC). The corpus epididymis is located between the caput and cauda segments, and the cauda epididymis is connected with the ductus deferens following by the urethra.*

Infectious and inflammatory conditions in the male genital tracts represent the most frequent etiological factors contributing to male infertility, which range to 15% in developed countries and 30% in developing countries [6]. In contrast to acute infectious inflammation, which displays evident disease phenotypes, non-infectious chronic inflammatory conditions frequently occur in the male reproductive system and have mild or no visible phenotypes. Therefore, the rates of inflammatory male infertility can be underestimated. The complex inflammatory conditions in the male reproductive system are attributable to its special composition and immune environment. The specificities of the male reproductive system include the following aspects: (1) the male reproductive system is composed of multiple distinct organs, including the testis, epididymis, prostate, and seminal vesicle; (2) this open system can be frequently infected by sexually transmitted pathogens and pathogenic bacteria; (3) the immunogenic sperm is produced, stored, and transported in the male reproductive system; and (4) tissue-specific cells of the male reproductive system abundantly express PRRs that can be activated by ligands from microbial pathogens and endogenous germ cells. Therefore, PRR-initiated innate immune responses in the tissue-specific cells can play important roles in the defense against microbial infection and the initiation of the inflammatory response in the male reproductive system, thereby being involved in health and disease.

## **2. Immune environment in the healthy and diseased testis**

The mammalian testis possesses a unique immune function for protecting the development of immunogenic germ cells from detrimental immune responses and local defenses against microbial infection. The disruption of testicular immune homeostasis may lead to orchitis, one of the etiological factors contributing to male infertility. Notably, the studies on the mechanisms underlying testicular immune regulation and innate defense against microbial infections are mostly carried out using murine models. How these models inform humans remains to be clarified.

### **2.1 Testicular immune privilege**

The testis shares the remarkable status of immune privilege with the eyes, brain, and uterus [7]. The main goal of immune privilege in the testis is to prevent adverse immune responses against male germ cells. The first round of male germ cell development is only completed after puberty, a long time after the establishment of immune self-tolerance, which occurs during fetal and immediately after birth. Therefore, a majority of male germ cells, particularly the late stages of germ cells that are generated during puberty, are stranger to the immune system and can actively induce immune responses elsewhere extra the testis [8]. These immunogenic male germ cells do not induce immune responses in the testis under physiological conditions due to its immunoprivileged environment.

The histological structure and the local immunosuppressive milieu cooperatively produce the testicular immunoprivileged environment.

#### *2.1.1 Testicular structure favoring immune privilege*

The mammalian testis is a complex organ with a highly organized histological structure, many different cell types, and a highly efficient immunosuppressive milieu. The testis is histologically composed of two distinct regions: the seminiferous tubules and the interstitial spaces (**Figure 1**). The seminiferous tubules are surrounded by peritubular myoid cells (PMC). Within the seminiferous tubule, the

columnar Sertoli cells encompassing different stages of male germ cells, including spermatogonia, primary spermatocytes, secondary spermatocytes, round spermatids, and elongated spermatids, form the seminiferous epithelium where male germ cells develop to form sperm. The seminiferous epithelium provides a special micro-environment for germ cell development. Notably, the blood-testis barrier (BTB) is formed by various cellular junctions between adjacent Sertoli cells near the basal side of the seminiferous epithelium. The seminiferous epithelium is separated into two compartments, namely basal and adluminal compartments, by the BTB.

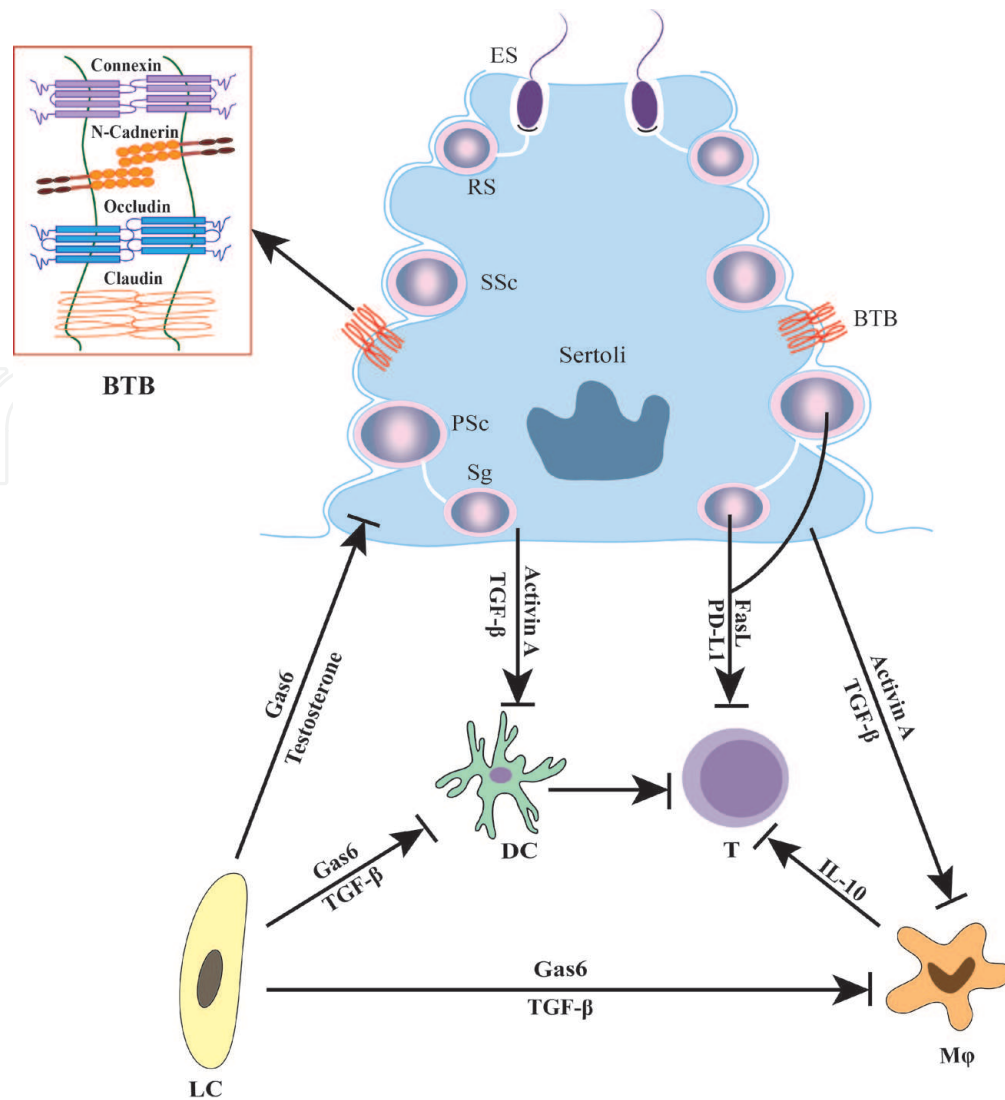
While the interstitial spaces constitute a minor region in the testis, there are many cell types in this region. Leydig cells represent a majority of interstitial cells and produce testosterone. Moreover, immune components, including blood vessels and various immune cells, are found in the interstitial spaces. Macrophages are major immune cells. Several other immune cells, including T lymphocytes, mast cells, and dendritic cells have been found in the testicular interstitial spaces. By contrast, B lymphocytes have yet to be observed in these spaces under physiological conditions. The testicular immune privilege confers two properties: the testis tolerates a large number of immunogenic male germ cells and allo-xenografts without immune rejection or that can survive for a prolonged time. The BTB can isolate most germ cells within the adluminal compartments behind the BTB from the immune components in the interstitial spaces. Therefore, the BTB plays a critical role in maintaining the immunoprivileged status in the adluminal compartments. However, the BTB cannot be fully responsible for the immune privilege of the whole testis because the germ cells outside the BTB, including preleptotene spermatocytes and spermatogonia, also produce immunogenic substances [9]. Moreover, the interstitial spaces themselves are also immunoprivileged because the grafts in the interstitial spaces can survive for an extended period. Therefore, other mechanisms must be involved in the maintenance of the testicular immune privilege.

### *2.1.2 Immunosuppressive milieu*

The testis is a highly organized histological structure composed of a great diversity of cell types. In addition to various stages of developing male germ cells, there are many types of somatic cells in the testis. PMC and Sertoli cells in the seminiferous epithelium and Leydig cells in the interstitial spaces are essential for the testicular functions. Most types of immune cells can be found in the interstitial spaces under physiological conditions. The testicular cells secrete a large spectrum of endocrine and paracrine immunoinhibitory factors, which form a dense network that cooperatively contributes to the immunoprivileged environment in the testis (**Figure 2**).

Leydig cells represent major tissue-specific cell types that produce androgen, mainly the testosterone that is essential for male germ cell development and the functions of many target organs extra the testis. Testosterone levels in the testis are 10-fold higher than in the peripheral circulation. Substantial evidence supports its inhibitory effects on the autoimmune response, which contributes to the difference in autoimmune diseases between males and females. Testosterone is also involved in the maintenance of the testicular immunoprivileged environment because it inhibits the induction of experimental autoimmune orchitis [10]. Testosterone should not directly act on immune cells because they do not have its receptor. In fact, only Sertoli cells express androgen receptors. Therefore, testosterone contributes to the immune privilege via regulating Sertoli cell functions. Accordingly, conditional knockout of the androgen receptor in Sertoli cells impairs the BTB and leads to autoimmune orchitis [11].

Testicular cells secrete a large panel of paracrine immunosuppressive factors that inhibit immune responses. In addition to the endocrine function, Leydig



**Figure 2.** *Immunosuppressive environment in the testis. The systemic immunity in the testis is suppressed by tissue structure and paracrine immunoinhibitory factors. The blood-testis barrier (BTB) sequesters the late stage of germ cells from immune cells in the interstitial spaces. Sertoli cells secrete activin A and transforming growth factor  $\beta$  (TGF- $\beta$ ), and these two factors inhibit immune responses of dendritic cells (DC) and macrophages (M $\phi$ ). Leydig cells produce Gas6, Testosterone, which inhibit immune properties of DC, M $\phi$ , and Sertoli cells. Male germ cells produce soluble Fas ligand (FasL) and programmed death ligand 1 (PD-L1). Both FasL and PD-L1 induce apoptosis of T lymphocytes. M $\phi$  exhibit immunosuppressive properties by producing anti-inflammatory cytokine IL-10 to inhibit effective T cell response. DC in the testis predominantly belong to immature subtype and inhibit effector T cells. Sg, spermatogonia; PSc, primary spermatocytes; SSc, secondary spermatocytes; RS, round spermatid; and ES, elongated spermatids.*

cells produce growth arrest-specific factor 6 (Gas6). Gas6 inhibits innate immune responses through the activation its receptors Tyro3, Axl, and Mer (TAM) receptor tyrosine kinases not only in immune cells [12], but also in Leydig and Sertoli cells [13]. While testicular macrophages represent considerable populations of interstitial cells, they predominantly exhibit immunosuppressive properties by producing anti-inflammatory factor IL-10 and lacking the innate immune machinery [14]. Therefore, testicular macrophages favor immune privilege, rather than inflammatory conditions in the testis. The seminiferous tubular cells also produce multiple immunoinhibitory molecules contributing to the immunoprivileged status. Most notably, besides the formation of the BTB, Sertoli cells produce various anti-inflammatory factors, such as activin A and TGF- $\beta$ , that inhibit the activation of immune cells. The paracrine secretions of Sertoli cells make these cells feasible to prevent immune rejection of grafts after co-transplantation [15]. A main function of the immune privilege is the protection of male germ cells from immune surveillance.

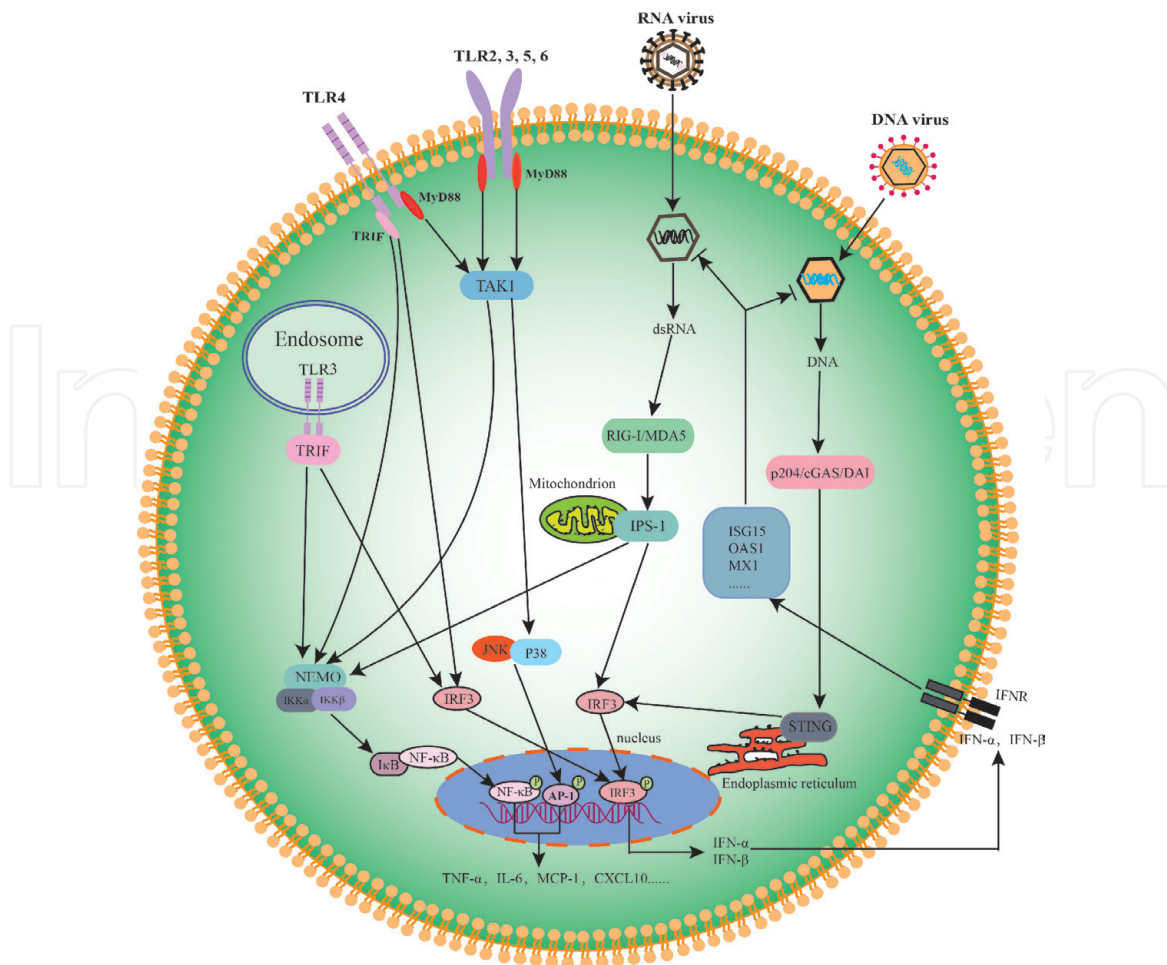
The germ cells also have the ability to inhibit the activation of immune cells. Fas ligand (FasL) and PD-L1 are two major immune checkpoints that act through the induction of T cell apoptosis and inhibition of T cell activation, respectively. Male germ cells abundantly express both membrane-bound and soluble FasL and PD-L1. PD-L1 has been confirmed to contribute to the testicular immunoprivileged state [16]. The role of FasL in maintaining the testicular immune privilege in the interstitial spaces is under debate [17, 18]. Since FasL is predominantly found in germ cells behind the BTB [18], it should be involved in the cleanup of immunity in the adluminal compartment of the seminiferous tubules. This speculation or other roles for FasL have yet to be demonstrated.

## **2.2 Innate immunity in the testis**

While the testis is a remarkable immunoprivileged organ, it is not a sterile site. In fact, the testis can be infected by microorganisms from hematogenous dissemination and ascending genital tracts. To overcome the absence of the systemic immune components, the testis adapts its own innate immune defense against microbial infections [19]. Testicular cells express a wide panel of PRRs, which initiate innate immune responses to produce a large number of immunoregulatory factors, including pro-inflammatory cytokines, chemokines, and interferons (IFNs). These factors may activate immune cells to counteract microbial infections or directly restrict microbial replication in the infected cells. PRR-initiated innate immune responses must be negatively regulated because a high level of the immunoregulatory factors for a prolonged period would be harmful to the tissues. Disruption of the innate immune homeostasis may result in orchitis and impair testicular functions. Hereby, we discuss innate immunity in the testis, the cell-to-cell innate immune responses, and their negative regulation in major testicular cells.

### *2.2.1 Innate immune response signaling pathways*

The mechanisms of general innate immune responses are addressed in other chapters. This chapter briefly summarizes PRR-initiated signaling pathways that have been identified in testicular cells (**Figure 3**). Several subfamilies of PRRs, including Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), and cytosolic DNA sensors have been investigated [19]. TLRs, now an established subfamily, were the first PRRs to be identified, which started the field of innate immune receptors and would establish their importance in the initiation of innate immune responses. TLRs exclusively initiate the myeloid differentiation protein 88 (MyD88)-dependent pathways, with the exception of TLR3 and TLR4. TLR3 initiates the Toll/IL-1R-domain-containing adaptor-inducing IFN- $\beta$  (TRIF)-dependent pathway, whereas TLR4 activation triggers both MyD88- and TRIF-dependent pathways [20]. The MyD88 pathway predominantly activates nuclear factor kappa B (NF- $\kappa$ B), thereby inducing the expression of pro-inflammatory cytokines and chemokines. The TRIF-dependent pathway activates NF- $\kappa$ B and IFN regulatory factor 3 (IRF3), thus leading to the induction of type 1 IFNs (IFN- $\alpha$  and IFN- $\beta$ ) and pro-inflammatory cytokines. These cytokines promote the recruitment and activation of leukocytes, and the expression of IFN-inducible antiviral proteins, thereby counteracting invading microbial pathogens. Moreover, TLR signaling facilitates the maturation of antigen-presenting cells, thereby directing adaptive immunity. RLRs include two functional members, retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated protein 5 (MDA5) [21]. RIG-I and MDA5 are cytosolic RNA sensors that recognize cytosolic double-stranded RNA (dsRNA). RIG-I and MDA5 activation triggers signaling through an adaptor IFN- $\beta$  promoter



**Figure 3.** Schematic of PRR signaling pathways in testicular cells. Expression and function of PRRs in most testicular cells have been revealed. PRR signaling pathways have been intensively investigated in mouse Leydig and Sertoli cells. Various TLRs initiate innate immune responses in Leydig and Sertoli cells using adaptor MyD88 except for TLR3 and TLR4. TLR3 initiates TRIF-dependent pathway, whereas TLR4 triggers innate immune responses through both MyD88- and TRIF-dependent pathways. RNA sensors RIG-I and MDA5 initiate innate antiviral responses using adaptor IPS-1 localized in mitochondria. DNA sensors p204, cGAS, and DAI trigger antiviral response using adaptor STING localized in endoplasmic reticulum. MyD88-, TRIF-, and IPS-1-dependent pathways induce the expression of pro-inflammatory cytokines TNF- $\alpha$ , IL-6 and chemokines MCP-1 and CXCL10 through NF- $\kappa$ B activation, and also induce the expression of IFN- $\alpha$  and IFN- $\beta$  through IRF3 activation. STING-dependent signaling pathway predominantly induces IFN- $\alpha$  and IFN- $\beta$  expression. The upregulation of pro-inflammatory cytokines and chemokines facilitate inflammatory conditions in the testis, whereas IFN- $\alpha$  and IFN- $\beta$  are important for the testicular antiviral defense through the induction of antiviral proteins ISG15, OAS1, and Mx1 in autocrine and paracrine manners.

stimulator-1 (IPS-1) that is localized in the mitochondria [22]. The cytosolic DNA sensor initiates the signaling pathway requiring the stimulator of IFN gene (STING) as an adaptor localized in the endoplasmic reticulum [23]. IPS-1-dependent signaling leading to the activation of IRF3 and NF- $\kappa$ B induces the expression of type 1 IFNs and pro-inflammatory cytokines, whereas the STING-dependent signaling pathway predominantly induces type 1 IFN production through the activation of IRF3. Testicular cells adopt cell-specific PRR-initiated innate immune responses.

### 2.2.2 Innate immunity testicular macrophages

Under physiological conditions, testicular macrophages represent approximately 20% of total interstitial cells and 80% of immune cells in the testis. It was believed that testicular cells are the front line of innate testicular defense against microbial infections from hematogenous dissemination. However, the response of testicular macrophages to microbial antigen challenges is relatively weak compared to their



counterparts in other tissues [24]. By contrast, testicular macrophages predominantly produce anti-inflammatory factor IL-10 after challenge with pathogens. Testicular macrophages show disabled innate immune signaling. This phenotype of testicular macrophages favors the immunoprivileged environment of the testis, but weakens its ability to fight invading microbes.

### *2.2.3 Innate antiviral defense of Leydig cells*

Leydig cells represent more than 75% of testicular interstitial cells. The main function of Leydig cells is the production of androgens, mostly testosterone, which are essential for normal spermatogenesis and also function in multiple organs extra the testis. Increasing evidence shows that Leydig cells play important roles in regulating the testicular immunity and the innate defense against viral infection. The antiviral responses of testicular cells were demonstrated more than two decades ago [25, 26]. Rat Leydig cells, as well as other testicular cells express IFNs and antiviral proteins after challenge with viral antigens. Notably, rat and mouse Leydig cells exhibit stronger antiviral ability than human Leydig cells [27]. This observation may explain the reason why viruses from a broad spectrum infect the human testis and impair testicular functions, whereas a natural viral impairment of the testis has not been found in mice. Moreover, the experimental induction of testicular dysfunction using viruses that frequently impair the human testis has not been successful in wild-type mice. Recent studies reveal that mouse Leydig cells express various PRRs that recognize viruses and initiate innate antiviral responses.

Among PRRs, TLRs among PRRs were first examined in mouse Leydig cells. These cells abundantly express TLR3 and TLR4, and their respective ligands trigger innate immune responses [28]. The TLR3-initiated innate immune response in Leydig cells activates NF- $\kappa$ B and IRF3, thereby inducing the expression of pro-inflammatory cytokines, including TNF- $\alpha$  and IL-6, as well as IFN- $\alpha$  and IFN- $\beta$ . The activation of both TLR3 and TLR4 in Leydig cells suppresses the synthesis of testosterone. Diminished testosterone production should be caused by the TLR-induced high level of TNF- $\alpha$  and IL-6 because these cytokines inhibit testosterone synthesis [29, 30]. Notably, TLR-initiated innate immune signaling pathways in Leydig cells are negatively regulated by TAM receptors. TAM signaling is an important negative regulatory system of immunity [12]. TAM receptors and their ligand Gas6 are abundantly expressed in the mouse testis [13]. TAM receptors are expressed in Leydig and Sertoli cells, whereas Gas6 is only expressed in Leydig cells. TAM receptors knockout mice develop autoimmune orchitis, suggesting that the Gas6/TAM signaling is essential for the testicular immunoprivileged environment in mice [31]. The roles of the Gas6/TAM signaling in maintaining testicular immune privilege can be attributed to different mechanisms: (1) Gas6 facilitates the phagocytic removal of apoptotic male germ cells through the activation of TAM receptors, which prevents the release of immunogenic male germ cell antigens [32]; (2) TAM receptors favor central immune tolerance to germ cell autoantigens because Axl and Mer knockout mice are susceptible to male germ antigen-induced autoimmune orchitis [33]; and (3) the inhibition of innate immune responses by the Gas6/TAM signaling in testicular cells, such as Leydig cells, contributes to immune privilege in the testis. Therefore, the interplay between the innate immune responses and their negative regulation is important in the testicular defense against microbial infection and the maintenance of the immunoprivileged status.

In addition to TLRs, mouse Leydig cells constitutively express functional RLRs, including RIG-I and MDA5 [34]. Both RIG-I and MDA5 initiate innate antiviral responses through IPS-1 signaling pathway in Leydig cells after challenge with their ligand dsRNA. IPS-1 signaling activates NF- $\kappa$ B and IRF3 in Leydig cells, thereby

inducing the pro-inflammatory factors TNF- $\alpha$  and IL-6 as well as IFN- $\alpha$  and IFN- $\beta$ . The IFNs subsequently induce the expression of several antiviral proteins, including 2'5'-oligoadenylate synthetase (OAS1), MxGTPase1 (Mx1), and IFN-stimulating gene 15 (ISG15), which leads to the degradation of viral RNA, inhibition of viral gene transcription, and amplification of antiviral signaling [35]. RIG-I- and MDA5-initiated IPS-1 signaling in Leydig cells suppresses testosterone synthesis, which may result in the impairment of testicular functions. Therefore, RIG-I/MDA5-initiated innate immune responses are likely to be the mechanism by which RNA viruses, including mumps virus (MuV), human immunodeficiency virus-1, and Zika viruses, frequently induce dysfunction of the testis [36]. In particular, MuV infection frequently induces orchitis, which results in male infertility. MuV can infect most testicular cells and induce immune responses through the activation of RIG-I/MDA5 in Leydig and Sertoli cells [37]. While MuV induces the expression of IFNs and antiviral proteins for antiviral responses, it also upregulates pro-inflammatory factors and chemokines. These cytokines may facilitate inflammatory conditions and the pathogenesis in the testis by inhibiting testosterone synthesis, inducing germ cell apoptosis, and impairing the BTB integrity [38, 39]. RIG-I/MDA5-initiated innate immune responses in testicular cells are a double-edged sword that both counteracts viral infection and impairs the testicular functions.

Mouse Leydig cells constitutively express the cytosolic DNA sensors p204 and STING [40]. The p204/STING signaling can be triggered in Leydig cells after challenge with viral DNA, and induce the expression of IFN- $\alpha$  and IFN- $\beta$ , as well as antiviral proteins. By contrast, viral DNA induces relatively low levels of pro-inflammatory factors in Leydig cells. Accordingly, the viral DNA sensor-initiated innate immune response in Leydig cells does not inhibit testosterone synthesis and rarely impairs male fertility. Therefore, the DNA sensor/STING signaling seems to be an ideal pathway for preventing viral infection in the testis and protecting the testicular function. The differences in antiviral responses and testicular dysfunction after DNA and RNA viral infections are worthy of further investigation.

#### *2.2.4 TLR-initiated innate immune response in Sertoli cells*

TLRs have been well characterized in the murine testis. Functional TLR2 and TLR4 were first demonstrated in mouse Sertoli cells, which opened the study of PRR-initiated innate immune responses in the testis [41]. The expression and function of TLRs in mouse Sertoli cells were subsequently examined in more detail [42–44]. Sertoli cells abundantly express multiple TLRs, including TLR2, TLR3, TLR4, TLR5, and TLR6, and their respective ligands activate TLRs and initiate the innate immune responses. TLR-initiated innate immune responses in Sertoli cells induce the production of TNF- $\alpha$ , IL-6, MCP-1, and IFNs. These cytokines trigger an inflammatory reaction in the testis and facilitate the innate defense against microbial infectious within the seminiferous tubules.

TLR2 and TLR4 in mouse Sertoli cells can be activated by damaged male germ cells, and induce the expression of pro-inflammatory factors and chemokines [45]. These cytokines may impair the BTB integrity, thereby inducing autoimmune orchitis. Male germ cells express high levels of endogenous TLR ligands, such as the high-mobility group box 1 (HMGB1) and several heat shock proteins (HSPs). HMGB1 and HSPs can be released from apoptotic and necrotic male germ cells and subsequently can induce an innate immune response in Sertoli cells, thereby leading to testicular inflammation and dysfunction. Accordingly, extensive apoptosis and necrosis of male germ cells under some pathological conditions, such as physical trauma and cryptorchidism, correlate with autoimmune orchitis [46, 47]. Most male germ cells go to apoptosis before finally developing into sperm. The apoptotic

germ cells must be timely removed before necrosis via phagocytosis by Sertoli cells, which is essential for the normal production of sperm [48]. The removal of apoptotic germ cells by phagocytosis contributes to the healthy testicular functions in several ways. One is the removal of autoantigens released from necrotic germ cells. The defective phagocytosis of apoptotic germ cells by Sertoli cells results in autoimmune orchitis [49]. TLR-imitated innate immune responses by endogenous ligands from damaged germ cells are involved in the development of autoimmune orchitis.

The TLR-initiated innate immune response is also negatively regulated by the Gas6/TAM system [50]. TAM receptor knockout mice increase the activation of TLR3 and TLR4 in Sertoli cells after challenge with TLR ligands, thereby overexpressing pro-inflammatory cytokines and type 1 IFNs. By contrast, Gas6 suppresses the TLR-mediated cytokine expression. The inhibition of TLR activation by the Gas6/TAM system is attributable to the induction of suppressor of cytokine signaling 1 and 3 (SOCS1 and SOCS3) proteins because both SOCS1 and SOCS3 can inhibit TLR signaling [51]. Sertoli cells express most of the major TLRs and can be infected by viruses through hematogenous dissemination as well as bacteria via ascending genital tracts. A broad spectrum of microbes may activate multiple TLRs in Sertoli cells to produce high levels of pro-inflammatory cytokines that can perturb the testicular functions. The negative regulation of TLR signaling by TAM receptors in Sertoli cells is important to prevent the impairment of testicular function by increased inflammatory cytokines.

The testis is predominantly infected by hematogenous viruses, although certain microorganisms may reach to the testis via ascending genital tracts [52]. Therefore, the innate antiviral response in the testis is critical for the prevention of virus-impaired testicular function. In addition to the aforementioned Leydig cells, Sertoli cells also possess antiviral ability by producing IFNs in response to viral infection [25]. Mouse Sertoli cells abundantly express TLR3, which recognizes viral dsRNA and initiates innate antiviral responses by inducing IFN expression [44]. However, the expression levels of RLRs and DNA sensor are relatively low in Sertoli cells compared to Leydig cells [34, 40]. Most studies on PRR-initiated innate antiviral response are focused on Leydig cells. These observations suggest that the innate antiviral machinery is better equipped in Leydig cells than Sertoli cells and that Leydig cells are capable of a more efficient antiviral response than Sertoli cells. Accordingly, Leydig cells produce relatively high levels of IFNs compared with Sertoli cells in response to MuV infection [37]. Therefore, MuV replicates more efficiently in Sertoli cells than in Leydig cells [38]. Notably, Sertoli cells produce relatively high levels of pro-inflammatory cytokines compared to Leydig cells after MuV infection. These studies indicate that Leydig cells are mainly responsible for the testicular defense against viral infection, whereas Sertoli cells predominantly contribute to orchitis in response to MuV infection. The different contributions of Sertoli and Leydig cells to the testicular defense against microbial infections and inflammation remain to be further dissected. The clarification of this issue may provide preventive and therapeutic strategies for viral orchitis, an etiological factor contributing to male infertility [30].

### *2.2.5 Innate defense of male germ cells*

Male germ cells represent the largest populations of testicular cells throughout the entire adulthood. The germ cells are generally thought to be protected by the histological structure and testicular somatic cells. In fact, these germ cells can be infected by microbial pathogens with the predominance of viruses. Therefore, male germ cells also adopt their defense against viral infection. The innate defenses of male germ cells are stage-dependent based on their histological locations. TLR3

is expressed in spermatogonia and spermatocytes that are located both outside and inside of the BTB [53]. TLR3 can be activated by its ligand and initiate innate antiviral responses in these early stages of male germ cells. The germ cells express IFNs and antiviral proteins through TLR3 signaling upon encounter with invading viruses. TLR3-initiated innate antiviral responses in spermatogonia and spermatocytes would contribute to the seminiferous epithelial defense against viral infection. Besides spermatogonia and spermatocytes, round and elongating spermatids express TLR11 [54], which can be activated by its ligands in these late stages of germ cells that resided in the adluminal compartments behind the BTB. TLR 11 recognizes *Toxoplasma gondii* (*T. gondii*) and uropathogenic *Escherichia coli* (UPEC), two major pathogens that can reach the testis via ascending genital tracts. TLR11 activation induces the production of interleukin 12 (IL12) and IFN- $\gamma$  in addition to pro-inflammatory cytokines and chemokines in spermatids. IFN- $\gamma$  favors the immune defense against *T. gondii* [55]. Therefore, the TLR11-initiated innate immune response in spermatids is involved in the testicular defense against *T. gondii* and UPEC infection via ascending genital tracts in mice. However, the functional TLR11 is absent in human beings, which could be a reason why UPEC and *T. gondii* infect and impair the human testis, whereas natural UPEC and *T. gondii* infections in the murine testis have not been found. It has been demonstrated that TLR11 prevents UPEC infection in mice [56]. In addition to TLR11, MDA5 is also constitutively in spermatids. A viral dsRNA analog induces the expression of IFNs and antiviral proteins through MDA5 in spermatids. The expression levels of pro-inflammatory factors, IFNs, and antiviral proteins in male germ cells are relatively low compared to testicular somatic cells. These studies indicate that male germ cells are also equipped with innate immune machinery. Considering their large numbers, male germ cells should be significantly involved in the testicular defense against microbial infections.

In addition to PRR-initiated immune responses, male germ cells also adopt their own specific defense ability, i.e., autophagy [38]. Autophagy is a conserved intracellular degradation pathway that is tightly controlled by a series of regulatory proteins. By fusion with lysosomes, autophagy can break down dysfunctional organelles and large protein aggregates that cannot be degraded by ubiquitination, which plays various important biological roles under pathophysiological conditions [57]. Autophagy is also an important intracellular innate defense system against invading viruses, bacteria, and protozoa by directly uptaking and degrading microorganisms [58]. Mouse male germ cells are abundantly equipped with autophagic machinery [38]. MuV can be internalized into male germ cells, but fails to replicate in vitro, which is in contrast with what happens in the testicular somatic cells. The presence of an inhibitor of autophagy in culture remarkably increases MuV replication in male germ cells. This observation indicates that autophagy plays a critical role in restricting MuV replication and eliminating invading viruses in male germ cells. The antiviral activity of the autophagic pathway does not up-regulate the expression of pro-inflammatory cytokines that can be harmful for the testis to function at a high level. Therefore, autophagy is an ideal solution for the problem of defending male germ cells against microbial infections. The efficient antiviral defense of male germ cells is particularly important for the prevention of not only viral-caused testicular dysfunction, but also sexual viral transmission. While autophagy is an important mechanism underlying the intracellular antiviral response, certain viruses may escape other antiviral mechanisms by hijacking autophagy [59]. Notably, the Zika virus (ZIKV) can be sexually transmitted in humans and impair testicular function and male fertility in mice after experimental infection [60, 61]. ZIKV is detected in the semen and male germ cells of humans and mice for a prolonged period [62, 63]. Viable ZIKV can be isolated from the

spermatozoa of patients with acute infection [64]. These observations suggest that male germ cells can be a reservoir for ZIKV. Previous studies detected human immunodeficiency virus 1 (HIV-1) and hepatitis virus B, typical sexual transmitted viruses, in spermatozoa [65, 66]. Taken together, spermatozoa can be vectors for sexual transmission of certain viruses. In general, while virus families covering a broad spectrum have been detected in the testis and semen [52], only a few of them have been confirmed in male germ cells. Understanding the mechanisms underlying antiviral responses and viral storage in male germ cells is particularly important because it may provide novel clues into the preventive and therapeutic strategies for virus-impaired male fertility and the sexual transmission of pathogens.

### **3. Innate immunity in the epididymis and epididymitis**

The epididymis is the organ where spermatozoa mature and are stored. The epididymis also adopts special structural and immune environments for the protection of spermatozoa maturation from adverse immune responses.

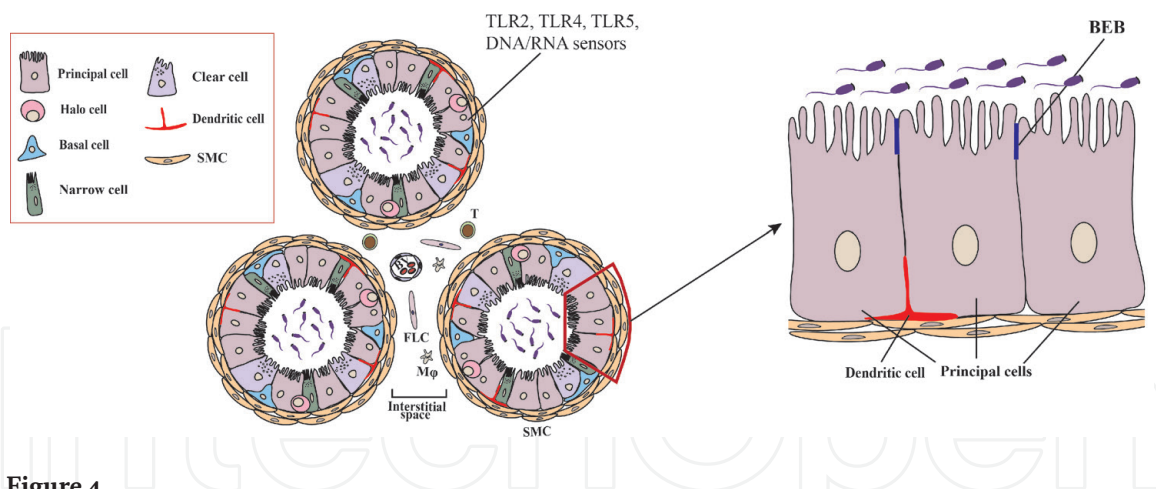
#### **3.1 Histological structure of the epididymis**

The epididymis is composed of a highly coiled tubule of several meters in length and the stroma, which can be divided into three segments, namely the caput, corpus, and cauda epididymis. The caput epididymis connects to the rete testis via the efferent ducts and receives sperm from the testis. The sperm passes through the caput segment and is stored in the corpus and cauda epididymis before ejaculation, which is an essential step for sperm maturation and motility. The epididymal tubule is formed by a pseudostratified epithelium consisting of various cell types, including peritubular myoid cells that surround the tubular epithelium, a majority of principal cells, as well as minor narrow cells, clear cells, and basal cells. The epididymal stroma comprises connective tissue containing certain fibroblasts and blood vessels. The three segments of the epididymis are distinct in morphology and cell compositions. The peritubular myoid cell layer increases, whereas the epithelium decreases, in thickness from the caput to the cauda segments. Various immune cells can be found and are differently distributed in the three segments.

#### **3.2 Epididymal immune environment**

##### *3.2.1 Distribution of immune cells in the epididymis*

The mammalian epididymis is also considered to be immunoprivileged due to its tolerance to sperm, which undergoes maturation and is stored in this organ. However, the immune privilege in the epididymis is not as typical as in the testis. While the blood-epididymis barrier (BEB) is formed between epithelial principal cells near the lumen of the epididymal tubule, it is not as effective as the BTB because certain leukocytes may pass through BEB [67]. Moreover, various leukocytes with immunoregulative properties reside in the epididymis (**Figure 4**). Dendritic cells (DCs) and macrophages are among the major leukocyte populations and show distinct distribution in different segments of the epididymis with abundance in the caput segment and in the basal region of the epididymal epithelium [68]. The protrusions of DCs may reach the lumen via gaps between principal cells. By contrast, DCs are much fewer in the cauda epididymis. A dense network of DCs in the caput epididymis is believed to regulate immune tolerance to antigenic sperm.



**Figure 4.** Schematic of immune environment in the epididymis. Epididymal immune environment comprises local innate immune defense (left panels) and immune privilege (right panel). Dendritic cells and macrophages ( $M\phi$ ) are major leukocytes. Dendritic cells are resided in the basal region of the epididymal tubule and their protrusions can reach the lumen side.  $M\phi$  are predominantly distributed in the stroma. Minor T lymphocytes ( $T$ ) can be also focused in the stroma areas. Various PRRs, including TLR2, TLR4, TLR5, DNA, and RNA sensors are expressed in principal cells, and the principal cells also produce defensins to counteract microbial infections. The immunoprivileged status of the epididymis is mainly maintained by the blood-epididymis barrier (BEB).

Interestingly, the DCs distribution and the fact that inflammation is more frequent in the cauda than caput segments seem to be two related observations. However, while the distribution of DCs and macrophages in the epididymis has been intensively examined, their functions under pathophysiological conditions are mostly speculated and remain to be elucidated [69]. In addition to DCs and macrophages in the basal region of the epididymal tubules, a minor T lymphocyte subset and mast cells can be found in the stroma [70]. The function of immune cells in the stroma remains unknown.

### 3.2.2 Innate immune response in epididymal epithelial cells (EECs)

The epididymis can be frequently infected by ascending microbes, which may result in epididymitis, the most important etiological factor of male infertility. Since major leukocytes in the epididymis predominantly adopt properties contributing to tolerance to sperm, the majority of tissue-specific cells should be responsible for the defense against microbial infections and inflammatory conditions in the epididymis. EECs express a large number of defensins that have potent activities against microbial infection and are important for sperm function [71]. Much like testicular cells, EECs also express a broad spectrum of functional PRRs. The activation of PRRs in EECs initiates the innate immune response, thereby producing pro-inflammatory cytokines, chemokines, and IFNs. These cytokines should play roles in the epididymal defense against invading microbes by impairing microbial survival and the development of inflammatory conditions by recruiting leukocytes from circulation. TLR2 and TLR4 are expressed in the rat EECs [72]. Challenges of EECs with *Staphylococcus aureus* in vitro induce the expression of nitric oxide synthase and production of nitric oxide and TNF- $\alpha$ , which is attributable to the activation of NF- $\kappa$ B and MAPKs. Using gene knockout mice, substantial evidence was gathered showing that TLR4 and TLR5 cooperatively initiate innate immune responses in EECs after infection with UPEC [73]. UPEC induces the expression of TNF- $\alpha$ , IL-6, MCP-1 in EECs, and the TLR4- and TLR5-mediated NF- $\kappa$ B activation. Moreover, UPEC also induces the expression of type 1 IFNs in mouse EECs through the activation of IRF3 in vitro and in vivo, which should be involved in the innate defense against UPEC and inflammatory responses in the epididymis.

In addition to PRRs that recognize bacteria, mouse EECs are also well equipped with PRRs that initiate innate antiviral responses [74]. EECs express TLR3, RIG-I, and cytosolic DNA sensors. The synthetic analogs of viral RNA and DNA induce the expression of type 1 IFNs through the IPS-1 and STING signaling pathways. The IFNs subsequently induce expression of antiviral proteins OAS1, ISG15, and Mx1 in an autocrine manner. Notably, viral RNA induces IFN expression and a remarkable upregulation of TNF- $\alpha$  and MCP-1 in EECs. However, viral DNA induces the high levels of IFNs and antiviral proteins, but moderately upregulates TNF- $\alpha$  and MCP-1. These observations indicate that viral RNA significantly induces both antiviral and inflammatory responses, whereas viral DNA predominantly induces innate antiviral responses in EECs. These phenotypes are also observed in testicular cells. Considering that the high level of TNF- $\alpha$  and MCP-1 may impair sperm survival and favor inflammatory conditions, the IPS-1 and STING antiviral signaling pathways should differentially impact pathophysiological conditions in the testis and epididymis, thus affecting male fertility. This speculation is supported by the fact that RNA viruses such as MuV and HIV-1 frequently cause inflammation in the testis and epididymis, thereby impairing male fertility. By contrast, DNA viral orchitis and epididymitis are not evident. Mechanisms underlying different effects of RNA and DNA viruses on male fertility are interesting issues that are worthy of further examination, which may provide novel clues to develop preventive and therapeutic strategies for virus-impaired male fertility.

### 3.3 Epididymitis

Epididymitis is the most common male genital tract inflammation with scrotal swelling and pain or asymptomatic depending on acute or chronic phenotypes [4]. While acute epididymitis is mostly caused by bacterial infections via the ascending genital tracts, chronic epididymitis is mainly the result of noninfectious stimuli that are associated with various risk factors, including physical trauma, vasectomy, testicular cancer, post systemic or genital tract infection, and adverse effects of certain medications. UPEC, *C. trachomatis*, and *N. gonorrhoeae* are common pathogens responsible for acute infectious epididymitis. The mechanisms underlying the acute epididymitis of rodent models after experimental bacterial infection have been intensively investigated [6]. Epididymitis can be induced by injection of clinically relevant bacteria into the local or mimicking retrograde routes. Similar to observations in clinical patients, the experimental epididymitis in small animals displays reddening, swelling, and enlargement of the scrotum. Massive infiltrations of leukocytes with the predominance of lymphocytes and neutrophils are observed in the experimental epididymitis. These models are valuable to investigate the duration of infection, the mechanisms of disease, and the efficiency of treatments [75].

While antibiotic therapy is effective for acute epididymitis, the treatment of chronic epididymitis has been less successful [76]. Mechanisms underlying chronic epididymitis remain largely unknown, and their further understanding is essential for developing an effective therapy. Although it has been known that chronic epididymitis is associated with various noninfectious risk factors, the pathogenic antigens resulting in noninfectious epididymitis are less understood. Recently, two studies using mouse models showed that damaged germ cells due to different causes induced noninfectious epididymitis characterized by massive macrophage infiltration [77]. Whether the male germ cell damage is a common etiological factor contributing to infectious epididymitis associated with multiple risk factors is the most urgent issue worthy of clarification. Most male germ cells are produced during puberty, when central immune tolerance has been already established. Therefore, male germ cells express a large number of new autoantigens that can induce innate

immune responses. These antigens are not released out and do not induce an immune response in the male reproductive tracts under physiological conditions. However, male germ cell damages produce endogenous TLR agonists that can induce the expression of pro-inflammatory cytokines and chemokines through the activation of TLR2 and TLR4 in Sertoli cells. Both TLR2 and TLR4 are also expressed in mouse EECs, and damaged male germ cells induce the innate immune response in EECs. Whether damaged male germ cells induce epididymitis in human beings should urgently be clarified, as it would be helpful for the development of novel diagnostic and therapeutic approaches for noninfectious epididymitis. In humans, this hypothesis is based on several phenotypes of clinical observations: (1) triggers of the noninfectious epididymitis have not been identified; (2) all known risk factors may damage male germ cells; (3) most cases of noninfectious epididymitis are unilateral and male germ cells might be damaged; and (4) chronic epididymitis is mostly observed in the cauda segment where damaged male germ cells may be stored. Further studies using human samples are required to confirm this hypothesis. In this regard, it is a priority to identify specific germ cell antigens that can induce innate immune responses and inflammation in the epididymis. These antigens in the semen may be used as a diagnostic marker to distinguish the noninfectious and infectious epididymitis, thereby choosing a suitable therapeutic approach. Epididymitis is considered to significantly impair male fertility and may be the most important single factor contributing to male infertility. Epididymitis may impair male fertility through different ways. The inflammatory conditions in the epididymis can impair sperm parameters. Moreover, acute epididymitis may spread to the testis and result in “epididymo-orchitis,” and the orchitis can perturb sperm production. Acute epididymitis can be easily found because patients timely visit outpatient due to the typical symptoms. By contrast, most cases of chronic epididymitis are asymptomatic and are mainly diagnosed in outpatient visitors for infertility, suggesting that chronic epididymitis is closely associated with male infertility. The chronic epididymitis may damage the epididymal tubule and result in tissue fibrosis, thereby leading to the obstruction of the excurrent tubules. It is believed that a great portion of obstructive azoospermia and oligozoospermia, important etiological factors of male infertility, can be caused by acute and chronic epididymitis [78]. Due to lack of common diagnostic and therapeutic standards for chronic epididymitis, the effect of epididymitis on male fertility seems to be underestimated. In particular, chronic asymptomatic epididymitis would be one of the etiological factors of idiopathic male infertility, representing about 50% of total male infertility. Therefore, understanding of mechanisms underlying chronic epididymitis and its impact on male fertility can provide clues for the prevention and therapy of male infertility caused by inflammation in male genital tracts.

#### **4. Innate immunity and inflammation in male accessory glands**

Healthy male fertility not only requires normal testicular and epididymal functions for sperm development and maturation, but also needs the functions of the male accessory glands, including mainly the prostate and seminal vesicle, which produce majority of seminal plasma essential for sperm function and fertility. As organs downstream of the epididymis, both the prostate and seminal vesicle can be infected before the epididymis by retrograde microorganisms via the ascending urethra. Therefore, infectious prostatitis and seminal vesiculitis are more frequent than the inflammation in the epididymis and testis. While prostatitis and seminal vesiculitis are not considered as important etiological factors of male infertility, these inflammations may impair sperm parameters and cause male subfertility [79–81].



## **4.1 Prostate pathophysiology**

The prostate is the largest accessory sex gland of the male genital tract and plays an essential role in facilitating fertility. The main function of the prostate is to secrete the prostate fluid that contributes up to 30% semen volume. The prostate fluid contains a large number of factors essential for healthy reproduction by protecting sperm during its travel through the female genital tract [82]. The well-known functions of these prostatic factors include ejaculation controlling, semen clotting and liquefaction, sperm activation and capitation, and female genital tract remodeling for fertilization and implantation. The prostate is composed mainly of an epithelial duct and a small volume occupied by a stroma. Epithelial cells are responsible for fluid secretion, and stromal cells are essential for the maintenance of tissue homeostasis in both the physical structure and microenvironment, and they are necessary for the secretory function of epithelial cells. The prostate is the organ with the most prevalent diseases.

Three major pathogenic conditions affect the prostate, namely benign prostatic hyperplasia, prostate cancer, and prostatitis. All three morbidities are associated with the immune response in the prostate. Benign prostatic hyperplasia is extremely prevalent in males over 50 years old and almost 90% of men older than 80 years suffer from the disease [83]. Prostate cancer is the most common cancer in men older than 60 years and is the second most prevalent cause of cancer-related deaths in men, second only to lung cancer in men [84]. Moreover, prostatitis is the most common inflammation of the urogenital tract in men younger than 50 years, in which chronic noninfectious prostatitis without defined etiological factors encompasses more than 90% of cases [79]. All three pathological conditions in the prostate are associated to local inflammation.

## **4.2 Inflammation and the diseased prostate**

Various leukocytes, including lymphocytes, macrophages, and mast cells, have been found in the prostatic stroma. These leukocytes are involved in prostatitis toward allo- and autoantigens [85]. A set of observations suggest the association between chronic prostatitis and benign prostatic hyperplasia, including: (1) leukocyte infiltration in benign prostatic hyperplasia; (2) positive correlation between a history of prostatitis and later development of benign prostatic hyperplasia; (3) non-steroidal anti-inflammatory drugs reduce the clinical symptoms; and (4) inflammation facilitates the progression of benign prostatic hyperplasia. In addition to immune cells, prostatic epithelial and stromal cells can be inducers of prostatitis, because these tissue-specific cells express several functional TLRs, including TLR4, TLR5, TLR7, and TLR9 [86]. The activation of TLRs in prostatic cells induces the expression of pro-inflammatory cytokines and chemokines, and these cytokines subsequently recruit and activate immune cells leading to inflammatory conditions.

The chronic inflammation in the prostate plays a role in cancer development. The association between TLR-initiated innate immune response and prostate cancer has been revealed [87]. The role of TLRs in prostate cancer seems complex and might be a “double-edged sword” in cancer progression. There is evidence showing that the activation of TLR3 in prostate cancer cells inhibits cancer cell growth by different mechanisms. TLR3 activation in the prostate cancer cells *in vivo* induces an innate immune response that increases infiltration of T lymphocytes and NK cells into the tumor, thereby suppressing cancer growth [88]. Moreover, TLR3 signaling induces apoptosis of prostate cancer cells *in vitro* [89]. By contrast, the TLR3 level is associated with the recurrence of prostate cancer in humans [90]. In addition, several other TLRs, including TLR2, TLR4, and TLR9 also facilitate the growth

and invasion of prostate cancer cells. The mechanisms behind the TLR3 signaling inhibition of prostate cancer development and the facilitation of tumor progression by other TLRs remain to be clarified. In this regard, the specific effects of distinct TLR signaling pathways and TLR-mediated cytokine production on cancer cell growth and apoptosis should be focused. The clarification of TLR functions in regulating the prostate cancer development can aid in developing immunotherapy against the prostate tumorigenesis by manipulating the TLR signaling pathways. In fact, several drugs targeting TLR signaling have been used in the clinic for the treatment of cancer patients [91].

### **4.3 Adverse effect of prostatitis on male fertility**

Prostatitis, the most common inflammation among urology outpatients younger than 50 years, is characterized by dysuria, nocturia, pelvic, and perineal pain, and ejaculatory disturbances [92]. Prostatitis comprises two subtypes of acute and chronic inflammation. Acute prostatitis is induced by an acute infection of bacteria, including mostly *E. coli* and sexually transmitted pathogens *N. gonorrhoeae* and *C. trachomatis*. Therefore, acute prostatitis is sensitive to antimicrobial treatment. By contrast, chronic prostatitis is a hallmark in nonbacterial inflammation, which represents more than 90% of all prostatitis cases. The etiology and mechanism underlying chronic prostatitis are poorly understood. Since prostatic secretions significantly contribute to the seminal plasma that plays a role in natural fertilization, inflammation in the prostate may alter the components of seminal plasma and thereby impairs fertility. While seminal plasma is not indispensable for successful reproduction using assisted technology, fertilization, and fetal development are compromised without exposure of spermatozoa and female genital tract to seminal plasma [82]. Acute prostatitis scarcely alters sperm parameters and impairs male fertility. However, chronic prostatitis has a negative effect on sperm parameters and male fertility. Persistently increased levels of inflammatory factors, including TNF- $\alpha$ , IL-6, IL-1 $\beta$ , IFN- $\gamma$ , and reactive oxygen species, in the semen of chronic prostatitis patients are associated with abnormal sperm parameters and reduction male fertility [79]. Since both acute and chronic prostatitis rarely results in the obstruction of genital tracts that may lead to male infertility, the roles of the prostate on health male fertility have been neglected. However, the enthusiasm and interest in this area have been increasing in recent years. The prostate secretes numerous substances that regulate fertility through the protection of sperm function, modulation of immunity in the female tract, and preservation of proper embryo implantation. The consequences of prostatitis on fertility should be considered for the assessment of male infertility.

## **5. Innate immunity and inflammation in the seminal vesicles**

The seminal vesicle is another major male accessory gland besides the prostate and produces approximately 70% of the seminal plasma volume. The production of prostaglandin and promotion of semen coagulation are two major functions of the seminal vesicles.

Prostaglandin affects sperm parameters and sperm-oocyte interaction [93]. Inflammation in the seminal vesicles can alter prostaglandin production and semen coagulation [94], thereby impairing sperm function and fertility. Microbial infections may lead to vesiculitis, which frequently occurs with prostatitis and is then termed prostate-vesiculitis [81]. The vesiculitis is considered as an etiological factor of hemospermia. The immunity in the seminal vesicle and the vesiculitis, as well as their effects on male fertility, is largely neglected and remain unknown. A recent

study examined the expression and function of PRRs in seminal vesicle epithelial cells. TLR3, TLR4, and various viral DNA and RNA sensors are expressed in the epithelial cells. These PRRs can initiate the innate response in the seminal vesicle epithelial cells, thereby expressing pro-inflammatory cytokines and chemokines. PRR-initiated innate immune responses alter prostaglandin synthesis and semen coagulation. These observations provide insights into mechanisms underlying vesiculitis and its potential adverse effect on the functions of the seminal vesicle.

## **6. Conclusions**

Infection and inflammation in the male reproductive system are major etiological factors for male infertility. The male reproductive system possesses a special immune environment to protect the organism from the sperm's antigens and prevent microbial infection. The innate immune responses in the male genital tracts are involved in the regulation of immune environment and their pathophysiology. While the immune regulation in the testis and epididymis has been intensively investigated, immunity in the accessory glands is less understood. Further research in the field will be helpful for understanding mechanisms underlying infectious and inflammatory male infertility or subfertility, which can aid in the development of preventive and therapeutic approaches for the inflammation in the reproductive system.

## **Acknowledgements**

This work was supported by grants from CAMS Initiative for Innovative Medicine (Nos. 2017-I2M-B and R-06, 2017-I2M-3-007) and the State Key Research and Developmental Project of China (Nos. 2016YFA0101001 and 2018YFC1003902).

## **Conflict of interest**

The authors declare no conflict of interest.

IntechOpen

IntechOpen


### **Author details**

Fei Wang, Ran Chen and Daishu Han\*  
Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, School  
of Basic Medicine, Peking Union Medical College, Beijing, China

\*Address all correspondence to: [dshan@ibms.pumc.edu.cn](mailto:dshan@ibms.pumc.edu.cn)

### **IntechOpen**

---

© 2019 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

## References

- [1] O'Neill LA, Golenbock D, Bowie AG. The history of toll-like receptors - redefining innate immunity. *Nature Reviews. Immunology*. 2013;**13**:453-460. DOI: 10.1038/nri3446
- [2] Iwasaki A, Medzhitov R. Control of adaptive immunity by the innate immune system. *Nature Immunology*. 2015;**16**:343-353. DOI: 10.1038/ni.3123
- [3] Newton K, Dixit VM. Signaling in innate immunity and inflammation. *Cold Spring Harbor Perspectives in Biology*. 2012;**4**(3):a006049. DOI: 10.1101/cshperspect.a006049
- [4] Haidl G, Haidl F, Allam JP, et al. Therapeutic options in male genital tract inflammation. *Andrologia*. 2019;**51**:e13207. DOI: 10.1111/and.13207
- [5] Trojian TH, Lishnak TS, Heiman D. Epididymitis and orchitis: An overview. *American Family Physician*. 2009;**79**:583-587
- [6] Fijak M, Pilatz A, Hedger MP, et al. Infectious, inflammatory and 'autoimmune' male factor infertility: How do rodent models inform clinical practice? *Human Reproduction Update*. 2018;**24**:416-441. DOI: 10.1093/humupd/dmy009
- [7] Stein-Streilein J, Caspi RR. Immune privilege and the philosophy of immunology. *Frontiers in Immunology*. 2014;**5**:110. DOI: 10.3389/fimmu.2014.00110
- [8] Tung KS, Teuscher C, Meng AL. Autoimmunity to spermatozoa and the testis. *Immunological Reviews*. 1981;**55**:217-255
- [9] Yule TD, Montoya GD, Russell LD, et al. Autoantigenic germ cells exist outside the blood testis barrier. *Journal of Immunology*. 1988;**141**:1161-1167
- [10] Fijak M, Schneider E, Klug J, et al. Testosterone replacement effectively inhibits the development of experimental autoimmune orchitis in rats: Evidence for a direct role of testosterone on regulatory T cell expansion. *Journal of Immunology*. 2011;**186**:5162-5172. DOI: 10.4049/jimmunol.1001958
- [11] Meng J, Greenlee AR, Taub CJ, et al. Sertoli cell-specific deletion of the androgen receptor compromises testicular immune privilege in mice. *Biology of Reproduction*. 2011;**85**:254-260. DOI: 10.1095/biolreprod.110.090621
- [12] Rothlin CV, Carrera-Silva EA, Bosurgi L, et al. TAM receptor signaling in immune homeostasis. *Annual Review of Immunology*. 2015;**33**:355-391. DOI: 10.1146/annurev-immunol-032414-112103
- [13] Wang H, Chen Y, Ge Y, et al. Immunoexpression of tyro 3 family receptors—tyro 3, Axl, and Mer—and their ligand Gas6 in postnatal developing mouse testis. *The Journal of Histochemistry and Cytochemistry*. 2005;**53**:1355-1364. DOI: 10.1369/jhc.5A6637.2005
- [14] Winnall WR, Muir JA, Hedger MP. Rat resident testicular macrophages have an alternatively activated phenotype and constitutively produce interleukin-10 in vitro. *Journal of Leukocyte Biology*. 2011;**90**:133-143. DOI: 10.1189/jlb.1010557
- [15] Suarez-Pinzon W, Korbitt GS, Power R, et al. Testicular sertoli cells protect islet beta-cells from autoimmune destruction in NOD mice by a transforming growth factor-beta1-dependent mechanism. *Diabetes*. 2000;**49**:1810-1818. DOI: 10.2337/diabetes.49.11.1810
- [16] Cheng X, Dai H, Wan N, et al. Interaction of programmed death-1

and programmed death-1 ligand-1 contributes to testicular immune privilege. *Transplantation*. 2009;**87**:1778-1786. DOI: 10.1097/TP.0b013e3181a75633

[17] Bellgrau D, Gold D, Selawry H, et al. A role for CD95 ligand in preventing graft rejection. *Nature*. 1995;**377**:630-632. DOI: 10.1038/377630a0

[18] D'Alessio A, Riccioli A, Lauretti P, et al. Testicular FasL is expressed by sperm cells. *Proceedings of the National Academy of Sciences of the United States of America*. 2001;**98**:3316-3321. DOI: 10.1073/pnas.051566098

[19] Zhao S, Zhu W, Xue S, et al. Testicular defense systems: Immune privilege and innate immunity. *Cellular and Molecular Immunology*. 2014;**11**:428-437. DOI: 10.1038/cmi.2014.38

[20] Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: Update on toll-like receptors. *Nature Immunology*. 2010;**11**:373-384. DOI: 10.1038/ni.1863

[21] Ori D, Murase M, Kawai T. Cytosolic nucleic acid sensors and innate immune regulation. *International Reviews of Immunology*. 2017;**36**:74-88. DOI: 10.1080/08830185.2017.1298749

[22] Loo YM, Gale M Jr. Immune signaling by RIG-I-like receptors. *Immunity*. 2011;**34**:680-692. DOI: 10.1016/j.immuni.2011.05.003

[23] Paludan SR, Bowie AG. Immune sensing of DNA. *Immunity*. 2013;**38**:870-880. DOI: 10.1016/j.immuni.2013.05.004

[24] Bhushan S, Meinhardt A. The macrophages in testis function. *Journal of Reproductive Immunology*. 2017;**119**:107-112. DOI: 10.1016/j.jri.2016.06.008

[25] Dejuq N, Chousterman S, Jegou B. The testicular antiviral defense system: Localization, expression, and regulation of 2'5' oligoadenylate synthetase, double-stranded RNA-activated protein kinase, and mx proteins in the rat seminiferous tubule. *The Journal of Cell Biology*. 1997;**139**:865-873. DOI: 10.1083/jcb.139.4.865

[26] Dejuq N, Dugast I, Ruffault A, et al. Interferon-alpha and -gamma expression in the rat testis. *Endocrinology*. 1995;**136**:4925-4931. DOI: 10.1210/endo.136.11.7588226

[27] Le Tortorec A, Denis H, Satie AP, et al. Antiviral responses of human Leydig cells to mumps virus infection or poly I:C stimulation. *Human Reproduction*. 2008;**23**:2095-2103. DOI: 10.1093/humrep/den207

[28] Shang T, Zhang X, Wang T, et al. Toll-like receptor-initiated testicular innate immune responses in mouse Leydig cells. *Endocrinology*. 2011;**152**:2827-2836. DOI: 10.1210/en.2011-0031

[29] Xiong Y, Hales DB. The role of tumor necrosis factor-alpha in the regulation of mouse Leydig cell steroidogenesis. *Endocrinology*. 1993;**132**:2438-2444. DOI: 10.1210/endo.132.6.8504748

[30] Van der Hoek KH, Woodhouse CM, Brannstrom M, et al. Effects of interleukin (IL)-6 on luteinizing hormone- and IL-1beta-induced ovulation and steroidogenesis in the rat ovary. *Biology of Reproduction*. 1998;**58**:1266-1271

[31] Zhang Y, Li N, Chen Q, et al. Breakdown of immune homeostasis in the testis of mice lacking Tyro3, Axl and Mer receptor tyrosine kinases. *Immunology and Cell Biology*. 2013;**91**:416-426. DOI: 10.1038/icb.2013.22

[32] Xiong W, Chen Y, Wang H, et al. Gas6 and the tyro 3 receptor

tyrosine kinase subfamily regulate the phagocytic function of Sertoli cells. *Reproduction*. 2008;**135**:77-87. DOI: 10.1530/REP-07-0287

[33] Li N, Liu Z, Zhang Y, et al. Mice lacking Axl and Mer tyrosine kinase receptors are susceptible to experimental autoimmune orchitis induction. *Immunology and Cell Biology*. 2015;**93**:311-320. DOI: 10.1038/icb.2014.97

[34] Zhu W, Chen Q, Yan K, et al. RIG-I-like receptors mediate innate antiviral response in mouse testis. *Molecular Endocrinology*. 2013;**27**:1455-1467. DOI: 10.1210/me.2013-1075

[35] Sadler AJ, Williams BR. Interferon-inducible antiviral effectors. *Nature Reviews. Immunology*. 2008;**8**:559-568. DOI: 10.1038/nri2314

[36] Liu W, Han R, Wu H, et al. Viral threat to male fertility. *Andrologia*. 2018;**50**:e13140. DOI: 10.1111/and.13140

[37] Wu H, Shi L, Wang Q, et al. Mumps virus-induced innate immune responses in mouse Sertoli and Leydig cells. *Scientific Reports*. 2016;**6**:19507. DOI: 10.1038/srep19507

[38] Wu H, Zhao X, Wang F, et al. Mouse testicular cell type-specific antiviral response against mumps virus replication. *Frontiers in Immunology*. 2017;**8**:117. DOI: 10.3389/fimmu.2017.00117

[39] Jiang Q, Wang F, Shi L, et al. C-X-C motif chemokine ligand 10 produced by mouse Sertoli cells in response to mumps virus infection induces male germ cell apoptosis. *Cell Death and Disease*. 2017;**8**:e3146. DOI: 10.1038/cddis.2017.560

[40] Zhu W, Liu P, Yu L, et al. p204-initiated innate antiviral response in mouse Leydig cells. *Biology of Reproduction*. 2014;**91**:8. DOI: 10.1095/biolreprod.114.119396

[41] Riccioli A, Starace D, Galli R, et al. Sertoli cells initiate testicular innate immune responses through TLR activation. *Journal of Immunology*. 2006;**177**:7122-7130. DOI: 10.4049/jimmunol.177.10.7122

[42] Palladino MA, Johnson TA, Gupta R, et al. Members of the toll-like receptor family of innate immunity pattern-recognition receptors are abundant in the male rat reproductive tract. *Biology of Reproduction*. 2007;**76**:958-964. DOI: 10.1095/biolreprod.106.059410

[43] Wu H, Wang H, Xiong W, et al. Expression patterns and functions of toll-like receptors in mouse sertoli cells. *Endocrinology*. 2008;**149**:4402-4412. DOI: 10.1210/en.2007-1776

[44] Starace D, Galli R, Paone A, et al. Toll-like receptor 3 activation induces antiviral immune responses in mouse sertoli cells. *Biology of Reproduction*. 2008;**79**:766-775. DOI: 10.1095/biolreprod.108.068619

[45] Zhang X, Wang T, Deng T, et al. Damaged spermatogenic cells induce inflammatory gene expression in mouse Sertoli cells through the activation of toll-like receptors 2 and 4. *Molecular and Cellular Endocrinology*. 2013;**365**:162-173. DOI: 10.1016/j.mce.2012.10.016

[46] Nistal M, Riestra ML, Paniagua R. Focal orchitis in undescended testes: Discussion of pathogenetic mechanisms of tubular atrophy. *Archives of Pathology and Laboratory Medicine*. 2002;**126**:64-69. DOI: 10.1043/0003-9985(2002)126<0064:FOIUT>2.0.CO;2

[47] Goldacre MJ, Wotton CJ, Seagroatt V, et al. Immune-related disease before and after vasectomy: An epidemiological database study. *Human Reproduction*. 2007;**22**:1273-1278. DOI: 10.1093/humrep/dem010

- [48] Fei W, Han D. Sertoli cell phagocytosis: An essential event for spermatogenesis. In: Mukherjee S, editor. *Phagocytes*. Rijeka, Croatia: IntechOpen; 2019
- [49] Pelletier RM, Yoon SR, Akpovi CD, et al. Defects in the regulatory clearance mechanisms favor the breakdown of self-tolerance during spontaneous autoimmune orchitis. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*. 2009;**296**:R743-R762. DOI: 10.1152/ajpregu.90751.2008
- [50] Sun B, Qi N, Shang T, et al. Sertoli cell-initiated testicular innate immune response through toll-like receptor-3 activation is negatively regulated by Tyro3, Axl, and mer receptors. *Endocrinology*. 2010;**151**:2886-2897. DOI: 10.1210/en.2009-1498
- [51] Liew FY, Xu D, Brint EK, et al. Negative regulation of toll-like receptor-mediated immune responses. *Nature Reviews. Immunology*. 2005;**5**:446-458. DOI: 10.1038/nri1630
- [52] Dejuqc N, Jegou B. Viruses in the mammalian male genital tract and their effects on the reproductive system. *Microbiology and Molecular Biology Reviews*. 2001;**65**:208-231. DOI: 10.1128/MMBR.65.2.208-231.2001
- [53] Wang T, Zhang X, Chen Q, et al. Toll-like receptor 3-initiated antiviral responses in mouse male germ cells in vitro. *Biology of Reproduction*. 2012;**86**:106. DOI: 10.1095/biolreprod.111.096719
- [54] Chen Q, Zhu W, Liu Z, et al. Toll-like receptor 11-initiated innate immune response in male mouse germ cells. *Biology of Reproduction*. 2014;**90**:38. DOI: 10.1095/biolreprod.113.114421
- [55] Suzuki Y, Orellana MA, Schreiber RD, et al. Interferon-gamma: The major mediator of resistance against *Toxoplasma gondii*. *Science*. 1988;**240**:516-518
- [56] Zhang D, Zhang G, Hayden MS, et al. A toll-like receptor that prevents infection by uropathogenic bacteria. *Science*. 2004;**303**:1522-1526. DOI: 10.1126/science.1094351
- [57] Choi AM, Ryter SW, Levine B. Autophagy in human health and disease. *The New England Journal of Medicine*. 2013;**368**:651-662. DOI: 10.1056/NEJMra1205406
- [58] Deretic V, Saitoh T, Akira S. Autophagy in infection, inflammation and immunity. *Nature Reviews. Immunology*. 2013;**13**:722-737. DOI: 10.1038/nri3532
- [59] Wileman T. Autophagy as a defence against intracellular pathogens. *Essays in Biochemistry*. 2013;**55**:153-163. DOI: 10.1042/bse0550153
- [60] Musso D, Roche C, Robin E, et al. Potential sexual transmission of Zika virus. *Emerging Infectious Diseases*. 2015;**21**:359-361. DOI: 10.3201/eid2102.141363
- [61] Meinhardt A. Infection: A new threat on the horizon - Zika virus and male fertility. *Nature Reviews. Urology*. 2017;**14**:135-136. DOI: 10.1038/nrurol.2016.265
- [62] Matusali G, Houzet L, Satie AP, et al. Zika virus infects human testicular tissue and germ cells. *The Journal of Clinical Investigation*. 2018;**128**:4697-4710. DOI: 10.1172/JCI121735
- [63] Robinson CL, Chong ACN, Ashbrook AW, et al. Male germ cells support long-term propagation of Zika virus. *Nature Communications*. 2018;**9**:2090. DOI: 10.1038/s41467-018-04444-w
- [64] Joguet G, Mansuy JM, Matusali G, et al. Effect of acute Zika virus infection



on sperm and virus clearance in body fluids: A prospective observational study. *The Lancet Infectious Diseases*. 2017;**17**:1200-1208. DOI: 10.1016/S1473-3099(17)30444-9

[65] Huang JM, Huang TH, Qiu HY, et al. Studies on the integration of hepatitis B virus DNA sequence in human sperm chromosomes. *Asian Journal of Andrology*. 2002;**4**:209-212

[66] Muciaccia B, Corallini S, Vicini E, et al. HIV-1 viral DNA is present in ejaculated abnormal spermatozoa of seropositive subjects. *Human Reproduction*. 2007;**22**:2868-2878. DOI: 10.1093/humrep/dem288

[67] Hedger MP. Immunophysiology and pathology of inflammation in the testis and epididymis. *Journal of Andrology*. 2011;**32**:625-640. DOI: 10.2164/jandrol.111.012989

[68] Shum WW, Smith TB, Cortez-Retamozo V, et al. Epithelial basal cells are distinct from dendritic cells and macrophages in the mouse epididymis. *Biology of Reproduction*. 2014;**90**:90. DOI: 10.1095/biolreprod.113.116681

[69] Da Silva N, Barton CR. Macrophages and dendritic cells in the post-testicular environment. *Cell and Tissue Research*. 2016;**363**:97-104. DOI: 10.1007/s00441-015-2270-0

[70] Flickinger CJ, Bush LA, Howards SS, et al. Distribution of leukocytes in the epithelium and interstitium of four regions of the Lewis rat epididymis. *The Anatomical Record*. 1997;**248**:380-390. DOI: 10.1002/(SICI)1097-0185(199707)248:3<380:AID-AR11>3.0.CO;2-L

[71] Dorin JR, Barratt CL. Importance of beta-defensins in sperm function. *Molecular Human Reproduction*. 2014;**20**:821-826. DOI: 10.1093/molehr/gau050

[72] Zhao YT, Guo JH, Wu ZL, et al. Innate immune responses of epididymal epithelial cells to *Staphylococcus aureus* infection. *Immunology Letters*. 2008;**119**:84-90. DOI: 10.1016/j.imlet.2008.05.002

[73] Cheng L, Chen Q, Zhu W, et al. Toll-like receptors 4 and 5 cooperatively initiate the innate immune responses to uropathogenic *Escherichia coli* infection in mouse epididymal epithelial cells. *Biology of Reproduction*. 2016;**94**:58. DOI: 10.1095/biolreprod.115.136580

[74] Zhu W, Zhao S, Liu Z, et al. Pattern recognition receptor-initiated innate antiviral responses in mouse epididymal epithelial cells. *Journal of Immunology*. 2015;**194**:4825-4835. DOI: 10.4049/jimmunol.1402706

[75] Ludwig M, Johannes S, Bergmann M, et al. Experimental *Escherichia coli* epididymitis in rats: A model to assess the outcome of antibiotic treatment. *BJU International*. 2002;**90**:933-938

[76] Haidl G, Allam JP, Schuppe HC. Chronic epididymitis: Impact on semen parameters and therapeutic options. *Andrologia*. 2008;**40**:92-96. DOI: 10.1111/j.1439-0272.2007.00819.x

[77] Wang F, Liu W, Jiang Q, et al. Lipopolysaccharide-induced testicular dysfunction and epididymitis in mice: A critical role of tumor necrosis factor alpha. *Biology of Reproduction*. 2019;**100**:849-861. DOI: 10.1093/biolre/iy235

[78] Schuppe HC, Pilatz A, Hossain H, et al. Urogenital Infection as a risk factor for male infertility. *Deutsches Ärzteblatt International*. 2017;**114**:339-346. DOI: 10.3238/arztebl.2017.0339

[79] Motrich RD, Salazar FC, Bresler ML, et al. Implications of prostate inflammation on male fertility.

Andrologia. 2018;**50**:e13093. DOI: 10.1111/and.13093

[80] Motrich RD, Maccioni M, Molina R, et al. Reduced semen quality in chronic prostatitis patients that have cellular autoimmune response to prostate antigens. *Human Reproduction*. 2005;**20**:2567-2572. DOI: 10.1093/humrep/dei073

[81] La Vignera S, Vicari E, Condorelli RA, et al. Male accessory gland infection and sperm parameters (review). *International Journal of Andrology*. 2011;**34**:e330-e347. DOI: 10.1111/j.1365-2605.2011.01200.x

[82] Robertson SA, Sharkey DJ. Seminal fluid and fertility in women. *Fertility and Sterility*. 2016;**106**:511-519. DOI: 10.1016/j.fertnstert.2016.07.1101

[83] Fibbi B, Penna G, Morelli A, et al. Chronic inflammation in the pathogenesis of benign prostatic hyperplasia. *International Journal of Andrology*. 2010;**33**:475-488. DOI: 10.1111/j.1365-2605.2009.00972.x

[84] Siegel R, Ma J, Zou Z, et al. Cancer statistics, 2014. *CA: A Cancer Journal for Clinicians*. 2014;**64**:9-29. DOI: 10.3322/caac.21208

[85] Vykhovanets EV, Resnick MI, Marengo SR. The healthy rat prostate contains high levels of natural killer-like cells and unique subsets of CD4+ helper-inducer T cells: Implications for prostatitis. *The Journal of Urology*. 2005;**173**:1004-1010. DOI: 10.1097/01.ju.0000149130.06055.f2

[86] Konig JE, Senge T, Allhoff EP, et al. Analysis of the inflammatory network in benign prostate hyperplasia and prostate cancer. *The Prostate*. 2004;**58**:121-129. DOI: 10.1002/pros.10317

[87] Zhao S, Zhang Y, Zhang Q, et al. Toll-like receptors and prostate cancer.

*Frontiers in Immunology*. 2014;**5**:352. DOI: 10.3389/fimmu.2014.00352

[88] Chin AI, Miyahira AK, Covarrubias A, et al. Toll-like receptor 3-mediated suppression of TRAMP prostate cancer shows the critical role of type I interferons in tumor immune surveillance. *Cancer Research*. 2010;**70**:2595-2603. DOI: 10.1158/0008-5472.CAN-09-1162

[89] Harashima N, Inao T, Imamura R, et al. Roles of the PI3K/Akt pathway and autophagy in TLR3 signaling-induced apoptosis and growth arrest of human prostate cancer cells. *Cancer Immunology, Immunotherapy*. 2012;**61**:667-676. DOI: 10.1007/s00262-011-1132-1

[90] Gonzalez-Reyes S, Fernandez JM, Gonzalez LO, et al. Study of TLR3, TLR4, and TLR9 in prostate carcinomas and their association with biochemical recurrence. *Cancer Immunology, Immunotherapy*. 2011;**60**:217-226. DOI: 10.1007/s00262-010-0931-0

[91] Vacchelli E, Galluzzi L, Eggermont A, et al. Trial watch: FDA-approved toll-like receptor agonists for cancer therapy. *Oncoimmunology*. 2012;**1**:894-907. DOI: 10.4161/onci.20931

[92] Khan FU, Ihsan AU, Khan HU, et al. Comprehensive overview of prostatitis. *Biomedicine and Pharmacotherapy*. 2017;**94**:1064-1076. DOI: 10.1016/j.biopha.2017.08.016

[93] Lee TC, Ho HC. Effects of prostaglandin E2 and vascular endothelial growth factor on sperm might lead to endometriosis-associated infertility. *Fertility and Sterility*. 2011;**95**:360-362. DOI: 10.1016/j.fertnstert.2010.08.040

[94] Liu B, Song Z, Su S, et al. Abnormal expression of Sg I is closely related to seminal vesiculitis. *Urology*. 2016;**88**:227 e229-227 e214. DOI: 10.1016/j.urology.2015.08.031