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Immune and endocrine aspects of the testis and its relation to male infertility

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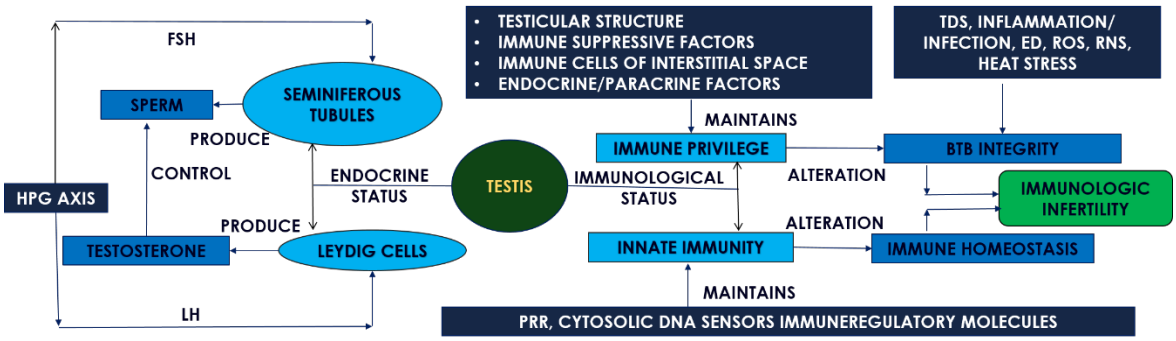
Review/Article

ABSTRACT



Spermatogenesis and steroidogenesis are the two important functions of the testis which are controlled by the hypothalamo-pituitary-gonadal axis of the body. Presence of blood-testis barrier (BTB) in the seminiferous tubules as well as the immune components of the interstitial space also maintains remarkable immune privileged microenvironment in the testis. This helps the sperm autoantigens to escape from the immune attack. The testis also has its own innate immune defensive mechanism to combat against male reproductive tract infection. There are many local immune modulators which maintain the immune privilege and regulate the innate immune mechanism of the testis. Factors like Infection and inflammatory conditions, endocrine disruptors, heat stress, Reactive Oxygen Species, Reactive Nitrogen Species may have impact on the BTB integrity and may finally lead to immunologic infertility. An insight into biomolecules associated with spermatozoal immune mechanism may generate inputs to develop diagnostic tools and modulate fertility.

Keywords: Testis, Immune- privilege, Blood-testis barrier, Immune defensive mechanism, Immunologic infertility

INTRODUCTION

The testes in the male reproductive system synthesize two essential products - sperms - those maintain the health of the male reproductive system and testosterone - needed for the development and maintenance of normal testis function.[1] This feature of the testis characterizes it as endocrine in nature. The synthesis of both the sperm and sex hormone is further regulated by endocrine hormones secreted by hypothalamus and pituitary, as well as locally within the testis. Optimal spermatogenesis requires the action of both testosterone (via androgen receptors) and FSH.[2]

Mammalian spermatogenesis starts from undifferentiated spermatogonia, which then undergo reduction division and enormous transformations as well as changes in genetic makeup, gene expression profile, change in cell surface proteins[3] finally forming spermatozoa. Functional spermatozoa are produced in the seminiferous tubules and finally mature within the epididymis.[4] Only a small number of spermatogonia develop within the testes from fetal to pre-pubertal period; however, once puberty is reached, active spermatogenesis begins,[5] long after the establishment and maturation of the immune system including central immune tolerance to self-antigens. The exclusive sperm membrane proteins developed during maturation of the sperm are considered antigenic and immunogenic for the body and to avoid autoimmune attack on sperm, the testes adopt a unique immune environment for preservation of the reproductive capacity of men.[6]

In particular, the testis is considered to be a remarkable immune privileged organ. The epididymis also maintains immune privileged microenvironment for protecting sperm from an immune attack during maturation and storage.[7] In males, the blood-testis barrier (BTB) and biomolecules in the semen provide an immuno- tolerant microenvironment for spermatozoa.[8] BTB is created by several types of junctions[9] and the Sertoli cells significantly contribute to the formation of this barrier. BTB prevents the passage of circulating substances from entering the inner part of the seminiferous tubules and thus actively exclude immune cells and other factors from entering the seminiferous

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tubules and being exposed to developing germ cells.[2] In addition to their role in BTB formation, Sertoli cells secrete various immunosuppressive factors and thus play significant role in immunosuppression.[10,11]

The defense mechanism of the testis has two aspects: one is to protect the autoantigens from detrimental immune responses and secondly to counteract infections by the attack of microbial pathogens. The immune privileged environment of the testis practice immune tolerance to both auto and alloantigens without evoking immune rejection. Various microorganisms infect the male reproductive system via ascending genital tracts and hematogenous dissemination, which may lead to inflammation and impaired male fertility.[7] To combat against invading pathogens a local response by the testicular cells is observed where immune privilege is overcome by adopting effective antimicrobial innate immune responses.[9] Various cytokines, including IL-1α and TNF- α are secreted by the male germ cells, suggesting the role of male germ cells in regulating the immune response[12,13]. Male germ cells also express Fas ligand (FasL)[14] and FasL-induced apoptosis of Fas-bearing lymphocytes has important contribution in immune suppression.[15] Besides, spermatogenesis, the other important function of the testis - steroidogenesis occur in the in the Leydig cells of the interstitial space. Although Sertoli cells and the germ cells play role in immune responsiveness and maintenance of the immune privilege status, the interstitial spaces also have the immune privilege microenvironment.[16] Various immune components present in the interstitial cells like macrophages, dendritic cells, mast cells, lymphocytes etc. are known to contribute to maintenance of the immune privilege environment. In addition, androgens are produced by the Leydig cells and it exhibit antiviral ability in response to viral infection.[17,18] Researchers have reported that the coordinated action of systemic immune tolerance, the local physical structure and active local immunosuppression maintains the immune privileged environment of the testis.[16]

It is evident that there are many local immune modulators including macrophages, dendritic cells, natural killer cells, mast cells, and T- lymphocyte[19,20,21] which help spermatozoa to escape immune attack. A dense network of dendritic cells[22] and active transforming growth factor β (TGFB) present in the epididymis[23] provide immune tolerance for the auto antigenic spermatozoa. Immune components are also added by the accessory sex glands and efferent ducts during ejaculation.[24] These secretory molecules regulate sperm function[25] and subsequent fertilization events in the female reproductive tract.[26,27]

Testicular innate immunity is particularly critical when systemic immunity is reduced. Pattern recognition receptors (PRRs) have been reported to initiate testicular innate immune responses[16] and subsequently counteract the invading microbes.[28] Several subfamilies of PRRs (Toll like receptors-TLR, RIG I like receptor -RLR, NOD like receptors- NLR) have been identified.[29] These receptors play important roles in the defense against microbial infection and initiate inflammatory response in the male reproductive system.

In humans, different T cell subsets (regulatory T cells, helper T cells, cytotoxic T cells, γδ T cells, and natural killer T cells) have been reported to be involved in the maintenance of immune tolerance and pathogenic immune responses in testicular infection and inflammation. T lymphocytes are the central regulatory molecules in controlling immune response and function either in a contact-dependent manner or by secreting soluble mediators. T lymphocytes maintain immune homeostasis and are involved in the pathogenesis of male infertility.[30] Biomolecules like cytokines maintain pathophysiological functions in the testis and seminal plasma in a coordinated way.[31] During infection, the concentration of pro-inflammatory cytokines increases. Under pathological condition the immune privilege environment is altered due to upregulation Fas system and integrin ligands of the Sertoli cells in response to cytokines.[32] Inflammatory cytokines like IL6, IL17, and IFNA along with NK cells and T- cells are observed in individuals with chronic male reproductive tract infection[33] and this suggests that cytokines are essential during inflammation and infections for successful maintenance of fertility Under physiological and pathological conditions antimicrobial molecules like defensins, cathepsin, and serpine1 present in semen are known to regulate sperm motility and the innate immune response during infection. Defensins offer resistance to viral and bacterial infection and prevent premature hyperactivation of spermatozoa.[34] Defensins are also essential for sperm maturation and protecting spermatozoa from immune attack in the female reproductive tract[4]. Presence of antisperm antibodies (ASA) are also known to be associated in case of infertility and generally cause impairment of various aspects of spermatogenesis including sperm function and sperm-egg binding.[8]

In this review, discussion on the immune-endocrine features of the testis, function of various immuoregulatory molecules in maintaining immune homeostasis during normal and pathological conditions has been done. Though the other parts of the male reproductive system also have their own innate immune mechanism, but those parts are not discussed in this review.

TESTICULAR ARCHITECTURE FROM THE ENDOCRINE POINT OF VIEW

The two most important structural part of the testis are the seminiferous tubules and the interstitial space containing Leydig cells. The venue for spermatogenesis is the seminiferous tubules,[4] whereas steroidogenesis take place in the Leydig cells of the interstitial space. The seminiferous tubules comprise the seminiferous epithelium and somatic Sertoli cells. Various stages of developing male germ cells are also observed in the tubules. Peritubular myoid cells reside on a layer of basement membrane surrounding the seminiferous epithelium. Between the tubules is the interstitial space that contains the steroidogenic Leydig cells along with blood and lymphatic vessels, immune cells including macrophages and lymphocytes.[2]

The spermatogenesis starts in the fetal testis and the ability of Leydig cells to produce testosterone under the influence of LH determines the success of spermatogenesis**.** Fetal Leydig cells appear during gestational weeks 7-8 in humans and the

production of testosterone starts under the stimulation of placental human chorionic gonadotropin (hCG). At puberty, under the influence of LH, mesenchymal cells divide and differentiate to form the adult population of Leydig cells.[2] The release of male sex hormone is controlled by the *hypothalamo-pituitary-gonadal axis* which facilitate the formation and maturation of the spermatozoa. This axis is composed of three endocrine organs hypothalamus, anterior pituitary, and the testes. The secretion of gonadotropin-releasing hormone (GnRH) by the hypothalamus initiates control of male reproductive function. The GnRH, in turn, stimulates the anterior pituitary gland to secrete luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH primarily stimulates the testicular secretion of testosterone, while FSH mainly stimulates spermatogenesis.[1] The testes, in turn, feedback on the hypothalamus and the pituitary via testosterone and inhibin secretion, in a negative feedback loop to limit GnRH and gonadotropin production. Sertoli cells (SC) and peritubular myoid cells secrete factors necessary for Leydig cell development and steroidogenesis.[6] The development of the male germ cells is dependent on the structural and nutritional support of the Sertoli cells residing on basement membrane at the peripheral part of the seminiferous tubules. As germ cells lack both androgen and FSH receptors, these hormones act directly on the Sertoli cell surface receptor for controlling spermatogenesis. Sertoli cells also form intercellular tight, occluding and adhesion junctions at their base which prevent the diffusion of substances from the interstitium into the tubules. Thus a *'blood-testis-barrier'* is formed which restricts the diffusion of substances from the interstitium and blood vessels, and thus allows the Sertoli cell to determine the immune privilege microenvironment above the junctions. This barrier effectively divides the seminiferous epithelium into two compartments, the basal compartment where substances from outside the tubule can freely access, and the adluminal compartment, where meiosis and the differentiation of spermatids take place.[1] The formation of BTB creates an **immune privileged environment** in the seminiferous tubule of the testis.

TESTIS AS AN IMMUNE PRIVILEGED ORGAN

Immune privilege implies a special immunological status found in several mammalian tissues, where allografts and xenografts have long survival rates.[35] The testis represents a distinct immune privileged site[16] and this micro-environment in the testis prevent adverse immune responses against male germ cells[36]. An individual acquires the ability to tolerate self-antigen during the development of the immune system.[16] Immune self-tolerance is established during fetal and neonatal stage whereas a majority of male germ cells, particularly the late stages of germ cells are generated during puberty long after the establishment of immune self-tolerance. Male germ cells produced thus are recognized as foreign molecules by the immune system. These autoantigens induce strong autoimmune responses, whereas the intricate structure of the BTB formed protects these auto-antigenic germ cells from the systemic immune attack.[8] The permeability of the BTB is 50–100 times tighter than peripheral endothelial cells[37]. This barrier is also required for the completion of germ cell meiosis and progression into spermiogenesis. Various factors like endocrine hormones [follicle- stimulating hormone (FSH), luteinizing hormone (LH), and androgens) as well as paracrine factors (TGFβ superfamily and retinoid signals)[38] are known to control the integrity of BTB.

The sequestration of autoantigens from the immune system by the blood–testis barrier (BTB) is believed to be critical for testicular immune privilege. Other structures which are benefited from immune privilege are spermatogonia and pre-leptotene spermatocytes, that localize outside the BTB.[16] These observations suggest that multiple mechanisms are involved in the maintenance of testicular immune privilege.

Factors maintaining the immune privilege state of the testis:

Regulation of the immune privileged state in the testis is coordinated by multiple mechanisms and factors which include the testicular structure, immune suppressive milieu and systemic immune tolerance.[39,40]

Testicular structure: The testis consists of a variety of cells and has a unique physical structure. Both the seminiferous tubules and the interstitial space of the testis play important role in spermatogenesis and steroidogenesis respectively. The testicular architecture, the distribution of various cells in the tubules and formation of BTB is already discussed in the previous section of this review.

In addition to their role in BTB formation, SCs have inherent immunosuppressive properties.

Immune suppressive milieu: SCs suppress immune responses by secreting various immune suppressive factors.[10,11] During maturation of spermatozoa, the cytoplasmic compartments of the sperm form residual bodies which are shed before maturation. Most of the developing germ cells undergo apoptosis and the phagocytic removal of the apoptotic germ cells and residual bodies is of great importance in maintaining testicular homeostasis and normal spermatogenesis.[41] Damaged germ cells induce inflammatory responses in the testis[42] and thus removal of apoptotic germ cells and residual bodies in time is important in avoiding autoimmune response. Secretion of various cytokines, including IL-1α and TNF-α by the male germ cells suggests the probable role of the germ cells in regulating the immune response.[13,14] Fas ligand (FasL)[15] and programmed death ligand 1 (PD-L1)[36] are abundantly expressed in male germ cells. An important mechanism for suppression of immune responses is FasL-induced apoptosis of Fas-bearing lymphocytes[15]. However, the contribution of the FasL expressed germ cells in maintaining testicular immune privilege remains to be elucidated. Programmed death receptor-1/programmed death ligand-1 (PD-1/PD-L1) is another T-cell tolerance system. Inhibition of T cell activation take place through PD-1[43] and PD-L1 is constitutively expressed in the testis. It is suggested that the PD-1/PD-L1 system is also involved in the maintenance of testicular immune privilege[44] as they are known to be involved in the survival of islet allografts.

Immune factors of interstitial space

Besides the seminiferous tubules, the interstitial spaces also maintain immune privileged microenvironments.[16] Leydig cells

represent major cell types in the interstitial space and besides, a great variety of immune cells including macrophages, dendritic cells, lymphocytes and mast cells are observed along with.

Among the immune cells, **macrophages** are a major population of antigen presenting cells which regulate the development and function (steroidogenesis) of the Leydig cells[45]. In comparison to macrophages of other tissues, testicular macrophages exhibit relatively low inflammatory responses and high immunosuppressive properties[16] and this can be correlated with the testicular immune privilege. Testicular interstitial M2 macrophages maintain the testicular immune privilege state by increasing the expression of CD163 and IL10 and lowering the expression of TNFA.[46] In contrast circulating macrophages significantly infiltrate the testis in orchitis and are detrimental to spermatogenesis.[47,48] It has been reported that high macrophage numbers in the testis of patients with aspermatogenesis and infertility indicating a negative correlation between circulating macrophages and spermatogenesis.[49]

Dendritic cells (DCs) represent a minor population of the interstitial cells in the normal testis and are the most powerful antigen-presenting cells. DCs are involved in testicular autoimmune response as they are reported to increase in Experimental autoimmune orchitis (EAO).[50] They minimize the autoimmune response by tolerating T cells to auto-antigens under physiological conditions.[16] DCs in the testes predominantly are immature in phenotype and inhibit the action of the effector T cells[1]. Sertoli cells secrete activin A and transforming growth factor β (TGF-β), which inhibit immune responses of dendritic cells (DC) and macrophages.[1]

Lymphocytes are abundant in the interstitial spaces of testis and the most testicular lymphocytes are T cells, among which CD8+ cells are more predominant and CD4+ cells are rare. B cells are absent in the normal testis.[16] The number of lymphocytes is known to increase in case of EAO and infertile patients with sperm autoimmunity.[51,52] This suggests involvement of lymphocytes in testicular pathogenesis under inflammatory conditions. Different T cell subsets (regulatory T cells, helper T cells, cytotoxic T cells, γδ T cells, and natural killer T cells) are known to be involved in the maintenance of immune tolerance and pathogenic immune responses in testicular infection and inflammation. In vitro studies have revealed the fact that there might be some possible interactions of the T cells with Sertoli cells and Leydig cells.[6] Among different subtypes, T regs are known to contribute to testicular immune privilege. They are powerful immunosuppressive cells that promote peripheral immune tolerance and control the autoimmune response to sperm antigens in vasectomy models.[53] The role of natural killer cells in testis is still not reported.

Mast cells are among the most significant immune cell populations in the testis. Their role in maintaining immune privilege is not very clear but they are known to have regulatory mechanism during inflammatory condition of the testis.[16] In addition to the inflammatory regulation, mast cells are essential intermediaries for regulatory T-cell tolerance.[54]

Leydig cells are the major tissue-specific cell types present in the interstitial space that produce androgen, mainly the testosterone. This male sex hormone is essential for germ cell development. There are evidences that testosterone have inhibitory effects on the autoimmune response in both males and females but their mode of action may be different in both the sexes. Testosterone also contributes to the maintenance of immune privileged environment of the testis[55,56] by acting on the Sertoli cells as SCs are the only cells having androgen receptors[55] and thus do not directly act on immune cells. Leydig cells also regulate immune responses by affecting testicular macrophage and lymphocyte numbers.[16]

Peritubular myoid cells are able to communicate with the interstitial cells as they are located outside the BTB. These cells help in transport of the spermatozoa from the testis to the epididymis[57] by utilizing their contractile ability. Peritubular myoid cells also regulate the testicular immune environment by secreting numerous pro-inflammatory and anti-inflammatory cytokines, under physiological and inflammatory conditions.[40,58] Table 1 summarize various immune suppressive molecules found in the testis.

Table I: Various immune suppressive molecules of testis.

Part of the testis	Cell type	Molecules secreted	Reference
Seminiferous tubule	Male germ cells	IL1α, TNF α, Fas L, PD-L-1	12,13,14,36
	Sertoli cells	Fas L, Activin, IGF-1, epidermal growth factor, TGFβ	14, 36, 59
Interstitial space	Peritubular myoid cells	IGF I, TGF-β, IL- 10	59
	Leydig cells	IL-10, Fas L, Activin, IGF-1, epidermal growth factor, TGFβ, Endorphin α and β, GAS 6	36, 59
	Testicular macrophages	Lyso-glyceryl phosphocholine, IL- 10, IL- 13, IL- 35	59
	T lymphocytes (T reg)	IL- 10, TGF- β, TNF- α	59,60,61

Coordinated action of endocrine and paracrine factors

It is evident that testicular immune privilege is maintained by the coordinated action of both endocrine and paracrine factors. Leydig cells suppress both systemic and testicular immune responses to autoantigens by the secretion of androgens. Numerous paracrine cytokines, including various anti-inflammatory factors, are also known to contribute to the maintenance of testicular immune privilege.

Luteinizing hormone (LH) regulates the synthesis of androgens by the Leydig cells. And these androgens in turn regulate immune response by acting on the Sertoli cells. LH antagonists reduce the levels of T regs and increase the levels of NK cells in men[62]. In addition to the endocrine function, Growth arrest-specific factor 6 (Gas6) are produced by the Leydig cells which inhibits innate immune responses through the activation its receptors Tyro3, Axl, and Mer (TAM) receptor tyrosine kinases not only in immune cells[63], but also in Leydig and Sertoli cells.[7]

TESTICULAR INNATE IMMUNITY

Immune system is one of the crucial body systems that guard the body against infections[64]. Innate immune response of the body acts as the first line of defense against microbial infections. Although the testis is a remarkable immune privileged organ, it can be exposed to pathogens derived from blood or through genitourinary tract[36]. The testis adapts its own innate immune defense mechanism against invading microbial infections[7] and to attain this testis overpower this immune privileged environment by inducing a *local innate immune response*[65].

Innate immune response is initiated in the testis with the expression of various pattern recognition receptors (PRR) and produce a large number of immunoregulatory factors, including pro-inflammatory cytokines, chemokines, and interferons (IFNs). These factors either activate immune cells to counteract microbial infections or directly restrict microbial replication in the infected cells. A high level of the immunoregulatory factors for a prolonged period are harmful to the tissues and thus PRR-initiated innate immune responses must be negatively regulated.[36]

PRRs are a superfamily of receptors and are activated by conserved molecular structures of microbial pathogens, termed pathogen-associated molecule patterns (PAMPs). The adaptive immune response induced by PRR activation helps in counteracting microbial infection. Endogenous autoantigens termed damage-associated molecular patterns (DAMPs) are released from damaged tissues and necrotic cells and stimulate inflammation[66]. There are several subfamilies of PRR[67]. These are toll like receptors (TLR), Retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) and NOD-like receptor (NLR). The best studied PRRs are TLRs[68] and 13 TLR members are identified in the mammals. Two functional members, namely, melanoma differentiation-associated protein 5 (MDA5) and RIG-I are present in RLRs and NLRs are known to contain a large number of cytoplasmic PRRs that recognize a broad spectrum of PAMPs and DAMPs[16].

Toll like receptors (TLRs): These are the first PRRs to be identified are the TLRs[33/36] which initiate the innate immune response in SCs by inducing immune -regulatory cytokines, including TNF-a, IL-1, IL-6, MCP-1 and type 1 IFNs[16]. TLRs are located on the innate immune cell surfaces which identify and activate various pathogenic determinants of bacterial cell wall [69]. The TLRs after activation initiate immune responses by recruitment of potent innate immune cells to the site of infection and proinflammatory mediators including the cytokines and chemokines are released from the activated immune cells as well as from the infected cells.[70]

TLRs exclusively initiate the myeloid differentiation protein 88 (MyD88)-dependent pathways, with the exception of TLR3 and TLR4. The Toll/IL-1R-domain-containing adaptor-inducing IFN-β (TRIF)-dependent pathway is initiated by TLR3, and TLR4 activation triggers both MyD88- and TRIF-dependent pathways[71]. Nuclear factor kappa β (NF-κβ) is activated by the MyD88 pathway and induces the expression of pro-inflammatory cytokines and chemokines. The TRIF-dependent pathway activates NF-κB and IFN regulatory factor 3 (IRF3), thus leading to the induction of type 1 IFNs (IFN-α and IFN-β) and pro-inflammatory cytokines. As a result, leukocytes are activated and recruited for the expression of IFN-inducible antiviral proteins, thereby counteracting invading microbial pathogens. Antigen presenting cells (APC) are also matured by TLR signaling facilities and thus adaptive immune response is observed[33/36]. TLR-initiated innate immune responses in Sertoli and Leydig cells are negatively regulated by Gas6/ProS-TAM signaling[72,73]. Different stages of germ cells also express TLRs[74]. Scientific reports reveal the expression of TLR3 in spermatogonia and spermatocytes and TLR11 in spermatids[75,76]. The immune cells of the interstitial space remain separated by the BTB from the cellular components of the adluminal compartments of the seminiferous tubules and thus the role of SCs and germ cells in controlling infection is of great significance.

(RIG-I)-like receptors (RLRs): RLRs initiate antiviral immune response by recognizing ds RNA of different viruses during their replication[77]. The two main components of RLRs-RIG-I and MDA5 are involved in the recognition of viral ds RNA and thereby initiate innate antiviral responses through IPS-1 signaling pathway in Leydig cells. Leydig cells express both RIG I and MDA5 whereas MDA 5 is expressed in the spermatids[78]. IPS-1 signaling activates NF-κβ and IRF3 in Leydig cells, thereby induce the secretion of the pro-inflammatory factors, TNF-α and IL-6 as well as IFN-α and IFN-β. Several antiviral proteins, including 2′5′-oligoadenylate synthetase (OAS1), MxGTPase1 (Mx1), and IFN-stimulating gene 15 (ISG15) are expressed due to stimulation of IFNs and these proteins degrade the viral RNA, inhibits transcription of the viral gene as well as amplification of antiviral signaling[79]. Testosterone synthesis is also suppressed in Leydig cells due to RIG-I- and MDA5-initiated IPS-1 signaling and this may result in the derangement of testicular functions. RIG-I/MDA5- initiated innate immune responses are generally observed during infection of various RNA viruses, like mumps virus (MuV), human immunodeficiency virus-1, and Zika viruses which are responsible for dysfunction of the testis.[80]

NOD like receptors: NLRs are characterized by a common NOD motif[81]. There are more than twenty NLR members identified in humans, and these NLRs recognize a broad spectrum of PAMPs and DAMPs. The mode of action of different NLRs vary in the defense against pathogens. NOD1 and NOD2, induce inflammatory cytokine expression[81] whereas other NLRs are involved in the processing and activation of inflammatory cytokines, including IL-1b and IL-18 which are activators of

inflammasomes[82]. But the function of the inflammasomes is not very clear. The presence of NOD1 and NOD2 mRNAs were detected in some testicular cells, including SCs and germ cells[74], but their functions in these cells have not been examined.

Recently **cytosolic DNA sensors** with antiviral properties have been determined[83]. Mouse Leydig cells constitutively express the cytosolic DNA sensors p204 and STING[84]. Leydig cells on exposure with viral DNA triggers this p204/STING signaling pathway, ultimately leading to the expression of IFN-α and IFN-β, as well as antiviral proteins. Viral DNA induces relatively low levels of pro-inflammatory cytokines and the viral DNA sensor-initiated innate immune response in Leydig cells does not inhibit testosterone synthesis; thereby rarely impairs male fertility[36]. Thus, the DNA sensor/STING signaling may be an ideal pathway for preventing viral infection in the testis.

Role of leukocytes in testicular immunity

Leukocytes are an important factor in immune system and have essential role in immune surveillance and phagocytosis of pathogens including defective spermatozoa. In approximately 20-30% of infertile men, the number of seminal leukocytes is increased as a result of genital tract infection, inflammatory responses, cellular defence mechanism.[85,86] Among diverse types of leukocytes granulocytes originate predominantly from the prostate and seminal vesicles, other types of white blood cells derive predominantly from the epididymis and rete testis[87,88]. Poly morpho nuclear (PMN) granulocytes and macrophages kill foreign material and cells by secreting hydrogen peroxide and superoxide, and pathogens are destroyed through phagocytosis. PMNs further initiate phagocytosis in infections/inflammations and are strong producers of ROS along with macrophages. Leukocytes also secrete both pro and anti-inflammatory cytokines which are further divided into chemokines, interferons, lymphokines, tumor necrosis factors and interleukin - molecules that mediate contact between leukocytes and other immune-reactive cells, like macrophages, etc.[89]

Macrophages are located at the tissue entry site and release proteases, chemotactic neutrophils and endothelial cells responsible for ROS signaling at the inner side of the blood vessels following activation. In addition, each type of leukocyte produces large amounts of ROS to combat infections from invading pathogens by stimulating G6PDH activity, producing high NADPH levels. NADPH oxidase further eliminates NADPH electron to convert oxygen to superoxide anion.[90]

In the immunological and inflammatory mechanism, cytokines play an important role in host response. Interleukins (IL) work by modulating leukocytes to create an inflammatory response, and by decreasing inflammatory cells[90]. ROS produced due to infection activates CXCL, CXCL8, IL-6 and IL-8 cytokines[90,91]. IL-8 produced by macrophage has a negative effect on the fertilizing ability of spermatozoa[90,92]. If the tissue gets damaged due to infection it stimulates IL-1 development in the surrounding environment[90,93]. PMN neutrophils and macrophages in turn secrete IL-6, which interacts with B-lymphocytes that become antibody-producing cells, which can further interfere with sperm function[90,94]. T-cells generate IL-2 in response to mitogen alloantigen antigen and proliferation of T cells initiate the inflammatory response. In addition, numerous studies have shown a correlation between decreased sperm function and elevated levels of IL-6, IL-8 and tumor necrosis factor in seminal plasma, both leading to lipid peroxidation in the sperm cells.[90, 95-97]

Disruption of Testicular Immune Privilege and Male Infertility

The immune privilege condition of the testes is necessary for normal spermatogenesis by preventing immune attacks to gamete specific antigens and paternal major histocompatibility complex (MHC) antigens, However, various factors like infection and inflammations may break the immune tolerance and represent a significant cause of male infertility.[6] If due to some reason the testicular immune privilege is disturbed, immune responses against the TGC autoantigens should be induced. The characteristics of testicular autoimmunity include the detection of inflammatory cell infiltration into the testis, disturbed spermatogenesis, testicular antigens-specific T-cell response, the specific serum autoantibodies, and binding of the autoantibodies and complements in the testis.[5]

Alteration in BTB integrity

The intricate structure of the BTB protects the developing germ cells against the new sperm membrane specific antigens that appear during puberty. Due to the presence of this barrier, the germ cells may conveniently complete their meiotic cycle and advance towards spermiogenesis. Various endocrine and paracrine factors like follicle-stimulating hormone (FSH), luteinizing hormone (LH), androgens, TGFB superfamily and retinoid signaling secretions[38] are known to regulate BTB integrity. The integrity of the BTB can also be affected by a great variety of factors including clinical conditions, endocrine disruptors, inflammation etc. and thus various immune regulatory molecules exhibit immune response. For instance, testicular dysgenesis syndrome (TDS) inclusive of cryptorchidism, hypospadias, and testicular germ cell cancer affects the BTB integrity[98]. These syndromes may develop due to abnormal fetal development of Sertoli and Leydig cells likely due to the genetic interaction of multiple factors including endocrine-disrupting chemicals, lifestyles and obesity[8]. Peptidyl-prolyl cis/trans isomerase (Pin1), Fas ligand (FASLG) system, and toll-like receptors (TLRs) are the examples of various interactors that affect BTB integrity. Pin 1[99] by its reduced expression affects cadherin-catenin multifunctional complex[37] that are essential for maintaining the integrity of the BTB. Scientific reports have revealed the role of TLR10 in the development of spermatogenesis[100] but the immune regulatory functions of TLR in testis and spermatozoa needs more exploration.[39,101]

Biomolecules and male reproductive function

Seminal plasma contains many immune regulatory biomolecules that reacts during inflammation, but their physiological role is not very clear. An insight into biomolecules associated with spermatozoal immune tolerance may generate inputs to the factors regulating fertility.

i. Cytokines: Cytokines are cellular messengers that play key roles in many biological conditions such as immune defense and reproduction.[86,102] The tissue macrophages along with leukocytes are major source of proinflammatory cytokines[103]. Inflammatory cytokines are key immune-regulators in male genital tract infections and modulation of the HPG axis regulation over testicular functions may result in infertility. Proinflammatory cytokines, TNF- α, interleukin (IL)-1α and IL- 1β present in testes, epididymis and spermatozoa, have immunoregulatory roles in the male reproductive tract[4]. A large amount of the immunoregulatory cytokine like IL-6, driven by IL-1, produced by Sertoli cell and germ cells are involved in the development of these cell[104]. Testicular germ cells also produce TNF which has a dual role as a signaling molecule, to regulate Sertoli cell function, in response to toxic insults, as well as its receptor-mediated functions[105]. Testicular development is also regulated by a number of the transforming growth factor β (TGFβ) superfamily members which is critical for sperm development[106]. Colony-stimulating factor-1 (CSF1) and macrophage migration inhibitory factor(MIF) are known to be involved in macrophage and Leydig cell development within the testis. During viral infections, Interferons (IFN-α, β, and γ) are produced by numerous testicular cells[103].

Cytokines have significant role in the maintenance of testicular as well as seminal physiology. TGFB, C- XC motif chemokine ligand 12 (CXCL12), monocyte chemotactic protein 1 and IL1, IL5, IL7, IL13, and IL17 are highly expressed in fertile individuals[107]. These cytokines together regulate the T- cell activity, chemotactic cytokine ligand 3, interferon α (IFNA), and granulocyte colony- stimulating factor (CSF2). The majority of testicular cytokines include bone morphogenic protein (BMP2)[8]. CXCL12 and C- X- C chemokine receptor type 4 (CXCR4) have significant role in Sertoli cell and Leydig cell development and function. The production and differentiation of sperm cells involve BMP2A and TGFB ligand[108]; whereas other molecules like the CXCL12, CXCR4 chemokine ligand and receptor-signaling complex are necessary for migration and colonization of primordial germ cells[109] as well as the maintenance of spermatogonial stem cells[110]. Scientists have reported that the testicular immune privilege environment is maintained by the increased expression of CD163 and IL10 and reduced expression of TNFA by the interstitial M2 macrophages[46]. During testicular infection, cytokines work in a coordinated manner in the testis as well as seminal plasma[31] and an increase in PTGE might stimulate cell- mediated response to spermatozoa. The overall cytokine production is increased during testicular infection[8]. Semen samples with poor fertility status show increased IL6 and IL8 concentration in the seminal plasma and this may be indicative of prostate gland infection as well as leucocytospermia-indicator of infection or inflammation in the male reproductive tract. IL8 is a proinflammatory cytotoxic chemokine and it activates neutrophilic phagocytosis, which might have a key role in combating male genital tract infection[111]. An inverse correlation of IL8 with sperm motility and semen volume has been reported in ejaculatory duct and seminal vesicle dysfunction.[112] In chronic testicular infection, inflammatory cytokines like IL6, IL17, and IFNA along with NK cells and T-cells have been observed[33] and this indicates the role of these inflammatory cytokines in maintaining fertility.

ii. Adipokines: These are hormones produced by the white adipose tissue[65] and have a significant role in the lipid and glucose metabolism, in inflammation[113,114] and in the regulation of the spermatogenesis[115]. Adipokines regulate male gonadal functions through the function of hypothalamo-pituitary gonadal axis.[65]

Leptin, a widely studied adipokine was shown to regulate reproductive functions by synchronizing the hypothalamus-pituitary-gonadal (HPG) axis at both the central and peripheral levels. Leptin facilitates GnRH secretion by acting through neuropeptides in the hypothalamic zona incerta. It releases nitric oxide (NO) which induce GnRH release from GnRH neurons by activating guanylate cyclase and cyclooxygenase[116-118]. Furthermore, leptin stimulates the release of LH and FSH via the NO synthase activation in the gonadotropic cells [116,119]. Increased concentrations of leptin inhibit hCG in a dose dependent manner and thus affect testosterone production by Leydig cells.[65]

Adiponectin is a protein secreted by the adipose tissue which through a variety of signaling pathways, downregulates the expression of TNF-α, IL-6 and IL-18 genes, thus protecting the system from the harmful effect of proinflammatory cytokines by suppressing NFkB action[120]. Adipokines are thought to be a link between metabolic syndrome (MS) and infertility[94]. Men with MS exhibit lower adiponectinemia. BMI, infertility time, and adiponectin serum/SP ratio are independently associated with MS[121].

Resistin is a protein secreted by the adipose tissue and is expressed in interstitial LC and SC of testis. They are expressed under control of the gonadotropins and is expressed at its peak at stages II - IV of the seminiferous epithelial cycle[119,122]. Furthermore, resistin shows a correlation with IL-6, TN-α, elastase and seminal quality. The seminal concentrations of resistin are found to be significantly higher in cases of leucocytospermia[123] and this suggest that resistin could be considered as a marker of inflammation.

Chemerin, also known as tazarotene-induced gene 2 (TIG2) or retinoic acid receptor responder 2 (RARRES2), is synthesized as an inactive precursor, prochemerin, which upon proteolytic cleavage is converted to its active form during inflammation[124]. Chemerin and its receptors CMKLR1 and GPR1 are located in human and rat testes [125] but to a lesser extent in the spermatogonia and spermatocytes and is a novel regulator of gonadal steroidogenesis.[123]

Visfatin, secreted by visceral adipose tissue is present in various tissues, including the testis, Leydig cells, spermatocytes, and spermatozoa[126]. Increased Visfatin concentrations is connected with increased concentration of serum testosterone, increased body and testis weight, and is negatively correlated with blood glucose concentration. Seminal plasma blood level ratio of visfatin indicates local production in the male genital tract, however its role in spermatogenesis is not yet reported in humans.[123]

Vaspin is one of the most recently discovered adipokine which acts as an insulin sensitizer with anti-inflammatory effects[102]. Vaspin is expressed in epididymal, retroperitoneal, and mesenteric adipose tissue and is related to the metabolic state[115].

Progranulin is an adipokine known to increase in cases of obesity or metabolic syndrome and could contribute to the inflammatory mechanisms.

Ghrelin is an endocrine, paracrine and autocrine regulator in human testis is also a multifunctional peptide hormone that affects several biological functions including production of proinflammatory cytokines, and reproduction in many species[127]. Ghrelin is also present in the human testis and particularly in Leydig and Sertoli cells but not in germ cells[128] and has an indirect effect on spermatogenesis.[129]

iii. Defensins: Defensins are biomolecules those offer resistance to viral and bacterial infection and prevent premature hyper activation of spermatozoa[34]. α and β defensins, human neutrophil peptide1–3, human defensin 5, 6, and human β defensin- 1 (HBD1) are observed in human spermatozoa and testes. There is evidence that human spermatozoa on incubation with defensin improve sperm motility as well as pregnancy rate[130]. During capacitation, binding of spermatozoa to the epithelia of oviduct through glycosylation is conducted by DEFB126[34]. DEFB126 also protects the sperm during its transit through female reproductive tract.[131]

iv. Antisperm antibodies: There are reports that naturally occurring antisperm antibodies (ASA) exist in many species, including humans[132]. Circulating ASAs and their role in affecting fertility was reported since long[4] and various reasons have been identified for formation of ASA in males. These include damage of BTB due to local inflammation[133], tumors[8], toxicants[134,135], testicular sperm extraction procedures[136], decrease in both cellular and humoral immunomodulatory factors in seminal plasma[137] and antigenic cross- reaction between microorganisms and spermatozoa[138]. Other reasons may include mechanical obstructions in the excurrent duct system due to inflammatory and infectious agents[139], varicocele[140,141], injury to the genital organs[142], occlusion of vas deferens[143], and epididymal inflammation[144]. In case of sexually transmitted disease, ASA may bind to the spermatozoa and induce immune response[145].

ASAs secreted in seminal plasma react with spermatozoal antigens and agglutinate spermatozoa.[8] ASA production associated with infertility indicates impairment of various aspects of spermatogenesis, including sperm function and sperm- egg binding[8]. Various sperm parameters like motility, sperm functional membrane integrity, acrosomal integrity, and the ability to penetrate the cervical mucus are known to be affected due to secretion of ASAs.[79,101,107,146] ASAs have been also associated with acrosomal disorders, DNA instability and can impair fertility in normozoospermic individuals[147]. ASA positive human sperm has revealed 27 novel proteins including T- complex protein 1 subunit θ, arylsulfatase A, and arrestin domain- containing protein- 5. These proteins are known to be involved in spermatozoon–oocyte interaction[148], presence of ASAs against these proteins may result in failure of sperm- zona binding and infertility.

IMMUNE ENDOCRINE FACTORS AND MALE INFERTILITY

The hypothalamic- pituitary gonadal (HPG) axis contributes to maintaining normal reproductive function. The local immunological environment in the reproductive tract is maintained by gonadal steroids and LH and this condition promotes immune tolerance. Androgens downregulate pro-inflammatory cytokines in males. Testosterone is involved in T- cell apoptosis in testes[149], which is important for the maintenance of testicular immune privilege. Androgens exert their immunosuppressive function on testicular leukocytes by regulating the balance between pro- and anti- inflammatory cytokine expression in the Sertoli, Leydig, and peritubular myoid cells[20]. CD106 is also found to be expressed in Leydig cells and the basal parts of the SCs in human testes[150]. inflammation in the testes can destroy the Leydig cell group, and the elimination of $CD8^+$ or $CD8^-$ T cells diminish the testicular inflammation, thus protecting the germ cells[151]. Recently, a case was found with a significantly decreased number of Leydig cells in the testes, along with T lymphocyte infiltration, under infection with COVID-19, and the expression of the virus receptor angiotensin-converting enzyme 2 (ACE2) was proved to be highest in the testes[152,153]. Thus, dysfunction of the immune endocrine factors may be related to male infertility.

Endocrine disruptors and male infertility

Endocrine disrupting chemicals (EDCs) are able to interrupt the closed feedback loops of the hormonal and homeostatic systems[154]. The group of known ED is extremely heterogeneous. These include ubiquitous synthetic substances used as industrial lubricants and solvents, and their by-products, plastics, plasticizers, pesticides, drugs, as well as non-steroidal anti-inflammatory drugs. Natural chemicals such as genistein, a phytoestrogen[155] and heavy metals[156] can also have endocrine-disruptive effects. Most EDs are known to act as imperfect ligands (either agonists or antagonists) for nuclear and membrane receptors (both steroidal and non-steroidal hormones) and interfere with hormone regulated cell signaling pathways and gene expression[157]. Most EDs are supposed to act through several mechanisms, which may have synergistic or antagonistic outcomes.[158] Some EDs are also capable of modifying bioavailability of hormone by disrupting its secretion and transport or altering the enzymatic pathways involved in hormone synthesis and metabolism.[159,160] Recently, many anti-virilizing EDs (e.g., phthalates and BPA) have been found which reduce prostaglandin biosynthesis by acting as cyclooxygenase inhibitors.[161]

Due to the presence of distinctive methylation patterns and epigenetic markers[162] the male germ cells are considered as the most vulnerable cells. It is evident that an association exists between inferior semen quality parameters and increased urinary and serum levels of phthalates,[163] PCB,[164] PBDE[165,166] and BPA.[167] The normal functioning of the germ cells and spermatogenesis supporting cells are hampered by EDCs. The blood–testis barrier is disrupted due to intrauterine exposure of BPA and this may lead to infertility through germ cell loss via immunological activity,[168] higher incidences of *testicular*

dysgenesis syndrome (TDS),[169] which include cryptorchidism, hypospadias and testicular cancer.[170]

The main cellular trait of TDS is impaired Leydig cells function[171,172]. In mild cases, men have low testosterone levels, slightly decreased penile/testicular volumes and poor semen quality, while in the more severe cases there is also hypospadias or cryptorchidism and an increased risk of testicular cancer[173]. Epidemiological data suggest that human developmental exposure to environmental levels of EDC (e.g., phthalates, PCB and pesticides) is indeed connected to an increased risk of TDS features such as hypospadias and cryptorchidism.[174-177]

Hypospadias is a condition in which the urethral meatus is on the ventral side of the penis, affects about 0.4% of males at birth and *Cryptorchidism* is defined as the failure of one or both testicles to descend into the scrotal sac and is the most common congenital abnormality in male children, affecting 2–4% of full-term males.[178] It may lead to infertility and even testicular cancer in adulthood.[179] Developmental exposure to EDC may act on Leydig cells by i) reducing insulin-like factor 3 expression[180] and ii) impairing steroidogenesis (resulting in relative testosterone deficiency) respectively.[181] The fetal testicular androgen production helps in differentiation of the male reproductive system[182] and thus, disruption of androgen activity by EDC during the virilization period (around 8–14 weeks into human fetal development) may cause TDS.[183]

Another cause of male infertility may be occupational pesticide exposure. PCB,[184] phthalates,[185] cypermethrin,[186] dieldrin[187] and EE[188] are known to cause reduced steroidogenesis in Leydig cells and Phthalates[189] are known to be involved in germ cell apoptosis. Arsenic also acts as an endocrine disruptor and alters the function of the hypothalamus- pituitary- testicular axis and subsequently reproductive hormones. Arsenic affects testicular architecture, leading to decreased testicular weight, reduced sperm concentration and function.[89,90/134,135]

ROS, RNS and heat stress in male infertility

Oxygen is crucial for living organisms, but some of its derivatives such as peroxyl (□ROO-) and hydroxyl (□OH) radicals, superoxide (□O_2-) anion, and hydrogen peroxide (H_2O_2) could be harmful to the cells. These derivatives are Reactive Oxygen Species (ROS) which are known to have link with male infertility.[190] Leukocytes and spermatozoa are two main sources of ROS in the male reproductive tract, and these cells produce ROS as part of regulatory roles during inflammation and cellular defense.[56/65] ROS influence spermatozoal DNA damage, impair sperm motility, reduce sperm concentration and damage the sperm membrane, thus decreasing the sperms potential to fuse with the oocyte.[191] Excessive production of reactive oxygen species (ROS), by the leukocytes may induce oxidative stress (OS) and oxidative damage to the spermatozoa[89]. During continuous adenosine triphosphate (ATP) production by the mitochondria in the spermatozoa, ROS may be released as by-products from oxygen (O_2) consumed due to redox reactions occurred.[192,193] Dysfunctional mitochondria are thus the major source of excessive ROS production, which create an unfavorable imbalance in the redox status.

ROS is also known to produce immature spermatozoa and leukocytes (mainly neutrophils and macrophages) in the male genital tract. In approximately 20-30% of infertile men having genital tract infection, the number of seminal leukocytes is elevated resulting in a significantly higher ROS production [194,195]. Bacterial infection triggers defensive mechanism by the host tissue (testis, epididymis, prostate gland and seminal vesicles) via unique or non-specific immunity at their entry sites.[196] During male genital tract infection, PMN granulocytes and macrophages produce excessive ROS and secrete cytokines.[190] Numerous studies have shown a correlation between decreased sperm function and elevated levels of IL-6, IL-8 and tumor necrosis factor in seminal plasma, both leading to lipid peroxidation in the sperm cells[97]. Leukocytes release large amounts of superoxide into phagocytic vesicles during the killing action of pathogens and these ROS in turn cause substantial damage to the healthy tissues including male reproductive system as well as the sperms. ROS destroy the sperm membrane, impair sperm movement, and damage sperm DNA. Oxidative stress also causes change in the structure of the spermatozoal flagella during epididymal maturation, causing impaired motility following ejaculation.[197] According to Depuydt and his colleagues,[198] elevated ROS have been associated with male accessory gland infection, including the urethra, prostate, deferent ducts, seminal vesicles, epididymis, or testes.

Additional ROS in the male reproductive tract is known to be produced from poor lifestyle, smoking, consumption of alcohol, pesticides, exogenous estrogens and heavy metal toxicity, nutritional deficiencies etc.

Seminal plasma contains endogenous enzymatic scavengers such as Superoxide dismutase (SOD), catalase and glutathione peroxidase, as well as exogenous non-enzymatic antioxidants such as vitamins C and E, zinc, coenzyme Q10 and ubiquinol[198]. A fine balance between ROS and antioxidants needs to be maintained for normal reproductive function.[191,197]

Nitrosative stress is caused by high levels of reactive nitrogen species (RNS) and peroxynitrite is known to be the most toxic RNS[8]. Peroxynitrite- induced nitrosative stress causes impaired sperm function; however, it does not cause cell death in human spermatozoa.[199,200] An association between high levels of peroxynitrite and decreased sperm quality has been reported in infertile men. Peroxynitrite is also associated with decreased mitochondrial membrane potential, adenosine triphosphate (ATP), and motility. Decreased sperm viability and increased sperm DNA fragmentation may take place due to prolonged exposure to peroxynitrite.[200]

Heat stress suppresses immunity and impacts reproduction mainly by elevating ROS production and subsequently affecting sperm mitochondrial ROS generation, sperm membrane fluidity, and DNA integrity[201]. This may also stimulate the expression of heat shock proteins (HSPs) including hypoxia upregulated 1, HSPC1, HSP86, HSPA5, HSPD1, HSP60, HSP70, and testis-specific chaperon HSPA2 on the sperm surface, impacting the fertilization potential of the individual. During genital tract infection the expression of HSPs are upregulated and finally leads to reproductive failure.[202] The concentration of some of the

cytokines like CXCL5, CXCL16, CXCL8, IL1b, IL10, CSF3, chemokine- motif ligand 3, and TNFA are increased at high ROS levels.[203]

Male reproductive tract infection in infertility

The association of male reproductive tract infection and inflammation with male infertility is being a topic of research since long.[56] Scientific reports reveal that 15% of male infertility is caused by male genital urinary tract infections,[14] affecting different sites of male reproductive system, such as testis, epididymis, and male accessory glands.[15] Both sexually and non-sexually transmitted diseases may impair spermatogenesis at different levels of sperm production, maturation and transport by urogenital infections. Different mechanisms are proposed to explain how infection or inflammation in the reproductive system can reduce male fertility.[18-20] One of the major causes involve induction of testicular oxidative stress (OS) due to overproduction of reactive oxygen species (ROS).[21-23] The inflammatory reactions associated with oxidative stress in return is harmful to the sperm as it causes damage in the sperm DNA and finally cause apoptosis.

Infection in the reproductive tract activates the innate immune system and release cytokines and other inflammatory mediators[204] Cytokines produced in response to invasion of pathogens or severe injury play a major role in the inflammatory response and modulate the communication between the different cells of the immune system.[194] During inflammation, the level of these cytokines and other inflammatory mediators become high and become harmful for sperm production leading to male infertility.[204]

Variety of micro-organisms are responsible for male infertility. They generally colonize in the semen irrespective of their origin of infection (either in the main genital tract or genitourinary tract of the male). Bacterial agents such as genital mycoplasmas (*Ureaplasma urealyticum* and *Mycoplasma hominis)* generally invade the genital tracts.[205] These microorganisms cause *Urethritis, prostatitis* and few instances of orchitis. Ureaplasmas are also reported to cause non chlamydial, non-gonococcal urethritis in males.[206] Elevated concentrations of elastase in granulocyte of the seminal plasma are also indicators of male genital tract infections. According to WHO guideline around 20-30% infertile men have asymptomatic genital inflammations.[207,208] Changes in sperm characteristics and apoptotic markers, semen contamination by excess leukocytes due to inflammation in the male genial tract, generation of reactive oxygen species (ROS) due to infection are considered to be the possible factors that contribute for the infertility among the male population.

Infections caused by Mycoplasmas (*M. genitalium* and *M. hominis)* reduce sperm motility and morphology and can induce sperm DNA damage due to high ROS production induced by inflammation.[209] *Ureaplasma urealyticum* and *Ureaplasma parvum* are found in the urethra and bind directly to sperm following ejaculation.[210,211] This is thought to cause increased DNA damage and loss of membrane integrity through ROS formation. *E. coli* reduces sperm motility and vitality and increases DNA damage.[212] Leukocytes are recruited in *E. coli* infection and thus neutrophils produce RO.[212] Proinflammatory cytokines, such as IL-6, can break cell membranes directly, decreasing sperm motility.[213] Similarly, the *E. coli* reduces sperm membrane integrity by directly binding to their membrane and adding porins to the membrane of the spermatozoa.[214] This causes a significant reduction in viability. In case of symptomatic HIV infections monocytes, macrophages, and leucocytes are recruited in semen.[215] Both hepatitis B and hepatitis C viruses have been shown to cause male infertility. Hepatitis B virus move through the blood–testis barrier and transmit its genome directly into spermatozoa leading to defective spermiogenesis and lower fertilization levels.[216] In men with chronic hepatitis B, high concentrations of IL-18 are found in the seminal plasma which causes natural killer cells to secrete proinflammatory cytokine INF- ɣ[217]. Unlike hepatitis B, the hepatitis C virus, does not pass through the blood–testis barrier and does cause direct oxidative stress to spermatozoa.[215] A systemic elevation of TNF- α and NO are observed in infections with chronic hepatitis C[218] and the consequences are chronic inflammation, and activation of lymphocyte and polymorphonuclear leucocyte take place.[219] Polymorphonuclear leucocytes generate ROS via NOX2, resulting in a loss of the mitochondrial membrane potential in the spermatozoa. This causes further propagation of ROS and OS. Hepatitis C-induced OS causes reduction in sperm motility (without affecting the ejaculatory volume), apoptosis and enhanced DNA damage.[218,219]

Disruption in either the HPG axis, spermatogenesis or transport of sperm in the tract are observed in *Sexually transmitted diseases (STD).* A high titer of seropositive chlamydia antibodies is observed in STD and this is a marker of severe infections finally leading to male infertility.[220]

In case of *acute and chronic bacterial prostatitis*, pathogens can influence sperm directly or indirectly through the activation of cytokines such as IL-6, IL- 8 or TNF- α in the seminal tract. Oxidative stress is produced due to elevated levels of cytokine and this causes a reduction in the testosterone level affecting the spermatozoa[86,221] In testicular infection, increased IL-6, IL- 8, and TNF- α levels may also impact sperm transit during ejaculation. The main function of BTB is to isolate sperm cells from all other body compartments and when a virus crosses this barrier, they may remain active or latent and may subsequently be transported by the male reproductive system, especially in semen.[222] Leukocytes found in the semen are able to carry the virus, contributing to the infection of all other cells in semen. This suggests that the blood-testis barrier cannot protect semen or spermatozoa completely, because when the spermatozoa leave the seminiferous tubules, they are exposed to an infection prone environment by many viruses that may cause infection in the male reproductive system.

Obesity and male infertility

Several studies have investigated the impact of obesity on the semen parameters. The sedentary lifestyle and fat rich diet disrupts male reproductive functions by reducing semen quality as well as by altering the physical and molecular structure of germ cells. Obese men are reported to have reduced sperm concentration, abnormal sperm morphology, altered sperm

motility and compromised sperm chromatin condensation and the reason behind it may be long exposure of germ cells to high aromatase activity. The size of the adipocytes also affects testosterone concentrations.[223] Furthermore, sperm DNA fragmentation in obese males, indicates poor quality of spermatogenesis.

The main contributors to male reproductive problems in obese men include endocrine and inflammatory factors[224,225] Obesity is reported to induce systemic inflammation by activating a TH-1 lymphocyte-dependent chronic inflammatory process.[224] The inflammatory progression gradually affects the functions of the testes, epididymis, and male accessory glands.[226] The proinflammatory mediators, interact with the control units of the HPG axis, thereby affecting the functioning of the testes[127]. Hypogonadotropic hypogonadism and deteriorated semen parameters are associated with impaired steroidogenesis and spermatogenesis, in addition, excessive reactive oxygen species (ROS) may generate and promote testicular Oxidative Stress (OS) causing both hormonal imbalance and direct damage to sperm, including sperm membrane damage, mitochondrial and nuclear DNA fragmentation and adverse epigenetic changes in the sperm DNA.[90,194] Several studies have revealed that obesity is positively correlated with high testicular oxidative stress.[221] This causes DNA damage, deformity and damaged plasma membrane integrity in sperm.[227,228]

Chronic inflammatory conditions are reported to be associated with obesity and Metabolic syndrome (MetS) which lead to increased expression of inflammatory cytokines among both testis and other accessory male sex organs including the ejaculate.[45] It is reported that, C-reactive protein (CRP) may be considered as probable key marker for reproductive tract inflammation caused by obesity and MetS. Obesity reduces the adherence between Sertoli cells and spermatogenic cells, and also reduces the expression of proteins forming tight junctions causing disputed blood-testis barrier among the obese patients.[229]

An altered reproductive hormonal profile is observed in obese males. Both Obesity and MetS reduce the testosterone and progesterone level in male, probably by modifying the steroidogenic pathway.[230] Increased conversion of testosterone into estrogens causes elevated estrogen concentrations associated with high bioavailability of aromatase.[231,232] High estrogen concentrations and low testosterone concentration in obese men is a potential reason for male infertility. A scientific research has revealed that weight reduction leads to an increase in testosterone concentrations, SHBG, anti- Müllerian hormone (AMH) and free androgen index (FAI). Obesity is also known to affect the spermatozoa DNA methylation.[16]

Ageing and male infertility

Ageing and inflammation are two closely linked processes and ageing in the male reproductive system includes changes in testicular function, spermatogenesis and erectile function.[233] An imbalance between the pro and anti-inflammatory cytokines is evident in aged men. Histomorphological studies have revealed that the number of germ cells and Sertoli cells in the testes decrease with ageing. During the course of ageing, the seminiferous tubules are narrowed due to the thickening of the tunica propria of the basal membrane of seminiferous tubuli as well as simultaneous reduction of the seminiferous epithelium. This causes the testis to vascularize,[234] which results in Testicular fibrosis and germinal epithelium separates from the blood supply. This condition of the testis also leads to testicular atrophy.[235] Leydig cells decrease in number with age showing higher incidence of apoptosis.[236] In aged men, increased levels of the circulating proinflammatory cytokines IL-1β, IL-6 and TNFα have been reported [237,238] and concentrations of pro-inflammatory cytokines, such as TNF-α, IFN-α, IL-6, IL-12, IL-17 and IL-23, were observed to increase in orchitis[16]. Hyperactivation of the macrophages during ageing upregulates COX2 expression associated with an increased production of prostaglandins (well-known mediators of inflammation.[239]

Reduction in number of seminiferous tubules, on ageing leads to reduced number of sertoli cells and germ cells in the testis reflecting its adverse effect on spermatogenesis. High levels of the proinflammatory cytokines TNFα, IL -1α and IL--1ß during ageing are very harmful to sperm production[4]. Immuno-histochemical studies revealed that COX2 production is linked with abnormal spermatogenesis.[240] Furthermore, elevated ROS level have been described in infertile men with impaired spermatogenesis.[241]

Ageing changes various semen parameters like daily sperm production, total sperm count, and sperm viability.[235,89] Functional decline of accessory glands shows gradual decrease in the semen volume in men with age of 45 or above. Ageing also affects sperm morphology[242] Due to insufficient number of seminal vesicles, semen volume and seminal fructose concentration have been reported to decrease with age.[243] As the swimming ability of spermatozoa is acquired during epididymal transit and motility is dependent on dilution into seminal plasma, altered functions of prostate gland and the epididymis leads to decreased sperm motility.[244] High levels of certain cytokines in semen are often linked with a decrease in the quality of the semen parameters.[4]

Ageing in men is known to cause prominent changes in steroidogenesis. Due to decrease in Leydig cell number on ageing, serum testosterone levels are also known to decline consequently. IL-1β, TNFα and prostaglandins are reported to be responsible for the decline of testosterone biosynthesis due to ageing. TNF-α acts through the activation of nuclear factor kappa B (NFkB), which, in turn, inhibits the transactivation of orphan nuclear receptors, responsible for the regulation of steroidogenesis108. TNF- α activates DAX-1 a member of the nuclear receptor family, which acts as a corepressor of many nuclear receptors and a regulator of steroidogenic genes.[245]

Changes in the hypothalamic-pituitary axis also have an effect on reproductive ageing in men. Ageing causes a reduction in the secretion of GnRH, which in turn leads to smaller LH and testosterone pulses.

One of the most common age-related disease is benign prostatic hyperplasia (BPH) where the prostate gland is reported to enlarge in older men. Age related prostate enlargement is known to be caused by hyperplasia of basal cells and stromal cells, located in the transitional zone around the urethra. Some

inflammatory mediators including chemokines like (CXCL1, CXCL2, CXCL5, CXCL6, CXCL12) and interleukins (IL-11, IL-33) are expressed and upregulated[246] and these promote proliferation of both epithelial and fibroblastic/myofibroblastic cell types in elderly men with BPH. In this condition, COX2 enzyme (key enzyme for prostaglandin biosynthesis) is overexpressed which may even lead to prostratic cancer in aged men.[247,248] IL-6 is also reported to have significant role in prostate cancer[249]. Infections of the ejaculatory duct can promote inflammation leading to a blockage and azoospermia. In older men, ejaculatory ducts are mainly blocked by both chronic prostatitis and BPH.[250] Thus, ageing and inflammation have significant contribution in extra-testicular ducts and accessory sex organs, affecting male reproductive function.

CONCLUSION

Male infertility is accompanied by many different inflammatory /infectious processes. Each one has a unique damage mechanism and special susceptibility to antibiotics. Oxidative stress may serve as a common pathway by which several factors can cause male infertility. Inflammatory reactions within the male genital tract have a negative impact on sperm quality and consequently, infertility. Furthermore, the process of inflammation generates ROS in addition to inherent oxidative stress generated by sperm cells. This causes toxic effects on human spermatozoa. Among the main sources of seminal ROS, leukocytes are depicted as significant contributors in male infertility. Leucocytospermia is reportedly associated with impairments in sperm maturation and functions but the exact functions of leukocytes in seminal plasma is yet to be defined. An imbalance between pro-oxidative and antioxidative substances in semen leads to metabolic and functional disorders of the male germ cells and may be a primary cause of some types of infertility.

The male reproductive system possesses a special immune microenvironment to protect the organism from the sperm's antigens and prevent microbial infection. Due to its remarkable immunoprivileged status and effective local innate immunity the testis exhibits special defense mechanism. Infection and inflammation in the male reproductive system are two major etiological factors for male infertility. The innate immune responses in the male genital tracts are involved in the regulation of immune environment and their pathophysiology.

Although the testis is regarded as an immunologically privileged organ and resistant to inflammatory responses, it is also highly susceptible to infection as well as autoimmune inflammation. It is evident that disruption of the testicular immunological environment may lead to various reproductive malfunctions due to invasion of pathogens including chronic orchitis. Proper understanding of the mechanisms underlying testicular immune homeostasis has important implications for studying male immunological infertility or subfertility. The causes of immune infertility can be elucidated by understanding of the molecular composition of the sperm- plasma membrane, structural domains and mechanisms that lead to a successful pregnancy. The immuneregulatory mechanism of various biomolecules depends on the balance between physiological and pathological conditions modulated by tolerogenic and pathogenic cells of the reproductive immune system. The innate defense functions of germ cells deserve great attention because of the large number of unique cells within the testis. The function of PRRs and their role in maintaining immune homeostasis are relevant areas for future research. In PRR initiated testicular innate immunity, very little is known about the functions of inflammasomes and cytosolic DNA sensors, and thus need further research. Defense against microbial pathogens is critical for hosts to recover from infection but the inflammatory response of the cytokines may cause damage to the host. The detrimental effects of inflammatory cytokines that are secreted by the testicular cells need to be clarified.

Besides, the increasing global prevalence of obesity together with the concurrent decline in male fertility also makes researchers curious about finding any association between these two factors. Obese men have high adipose tissue deposition, which is considered as toxin depots and sources of several hormones and adipokines. These hormones may influence the HPG regulatory axis as well as directly testicular cells to impair male reproductive functions. It is evident that obese men are likely to have elevated levels of inflammatory markers in their seminal plasma which positively correlate with deteriorated sperm quality.

The effects of ageing on men's fertility are of scientific interest since long and age-related alterations lead to gradual changes in men's androgen levels as well as spermatogenesis. These progressive changes consequently result in decline in both quality and quantity of spermatozoa. Ageing and age-related reproductive decline in males are closely associated with an imbalance between ROS production and the scavenging mechanism of the antioxidants present in the seminal plasma. Various factors like lifestyle, genetic and environmental factors are known to modulate this balance.

Considering treatment, further research in this field is required for understanding mechanisms underlying infectious and inflammatory male infertility or subfertility, which can be helpful in the development of preventive and therapeutic approaches for the inflammation in the reproductive system.

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