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Inflammation and Ageing: Probable role in Male infertility

Silpi Acharyya

Taki Govt. College, Taki, Hasnabad, West Bengal, India

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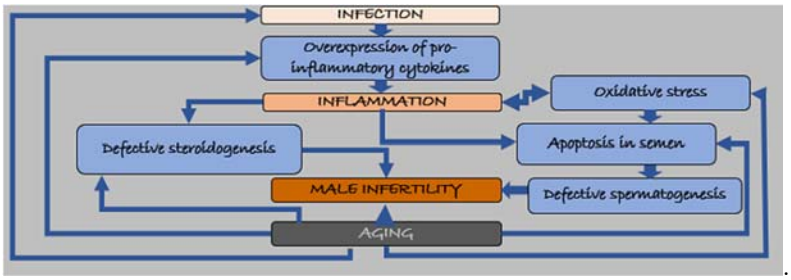
ABSTRACT



A close association between male reproductive tract infection and inflammation with male infertility has been observed in close prominence. Male reproductive tract infection may arise due to various reasons, leading to inflammation with release of inflammatory mediators. Interactions among the mediators with other regulators affect sperm function and in addition, oxidative stress may develop owing to altered spermatogenesis and steroidogenesis. An increased inflammatory condition is observed on advancement of age in males and the incidence of inflammation in male reproductive tract may vary greatly among elderly men. Ageing is a pro-inflammatory condition which give rise to mitochondrial damage, oxidative stress, immunosenescence, endocrinosenescence, finally leading to impairment of the normal sperm function. The study about impact of inflammation on ageing and further its impact on male health draws great significance due to its role as a predictor of pathogenesis in relation to male infertility as well as on overall adverse male health outcome.

Keywords: Ageing, Inflammation, Cytokines, Oxidative stress, Infertility

INTRODUCTION

The association of male reproductive tract infection and inflammation with male infertility have been a prominent phenomonon for evaluation. In addition, the inflammation in the male reproductive tract has also closely been linked to ageing, an another important factor that play role in infertility.[1] On advancement of age, the male reproductive system undergoes retrogressive changes from reproductively active to inactive state and results in decrement of various vital processes including reproductive performance of an individual. It has been reported that the reproductive capabilities of both the sexes decline with age[2,3] but unlike women, men do not undergo a rapid change in their fertility status rather they experience a gradual andropause.[4]

Ageing or senescence is an enormously complex multifactorial and irreversible process[5] and is defined as a "persistent decline in the age-specific fitness components of an organism due to deterioration of vital physiological processes including reproductive performances".[6] It is characterized by a mild chronic proinflammatory stage.[1] Ageing leads to various processes like mitochondrial damage, oxidative stress, immunosenescence, endocrinosenescence, epigenetic modification as well as some age related diseases which are also known to be interconnected with inflammatory responses[7] and ultimately impair normal sperm function. Clinical reports have suggested decline in different semen parameters like semen volume, sperm motility, sperm morphology etc on ageing[4,8] whereas the sperm concentration may not be affected in the similar way.[9]

Inflammation is the "process of responding to injury and tissue damage". Studies regarding male infertility have revealed presence of acute and chronic inflammation in the male

*Corresponding Author: Dr. Silpi Acharyya, Assistant Professor, Department of Zoology, Taki Govt. College, Taki, W.B.
Tel: +919830687508
Email: shilpiacharyya@gmail.com

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genitourinary tract which often are asymptomatic. The inflammatory reactions are immensely related with oxidative stress which in return is harmful to the sperm as it causes damage in the sperm DNA and finally cause apoptosis.[10] Currently a new term "inflammageing" has been introduced to describe the upregulation of inflammation with age.[11] Inflammation in the male reproductive tract are generally caused due to various infectious agents (bacteria, virus, other pathogens) but there may be a broad range of non-infectious processes which may also lead to inflammation. Inflammatory processes are commonly observed in the male reproductive tract on advancement of age. Infection in the reproductive tract causes the innate immune system to become active and release cytokines and other inflammatory mediators which are known to have significant roles in subsequent events.[10] Cytokines are "a family of more than 200 low molecular weight proteins (between 5 and 20 kDa) produced in response to invasion of pathogens or severe injury which play a major role in the inflammatory response by regulating its nature, intensity and duration, and by modulating the communication between the different cells of the immune system.[12] During inflammation, the level of these cytokines and other inflammatory mediators become high and become harmful for sperm production leading to male infertility.[10]

This article reviews the contribution of inflammation to male infertility. It also correlates upregulation of inflammatory processes with ageing and their combined role in male reproductive impairment.

SOURCES OF INFLAMMATION IN MALE REPRODUCTIVE TRACT

There may be several sources which cause inflammation in the male reproductive tract. These include epididymitis, prostatitis, urethritis, and vasitis, (inflammation of the epididymis, prostate, urethra, and vas deferens respectively),[1] ejaculatory duct obstruction, testicular torsion, varicocele etc.[10] Orchitis is an inflammatory reaction of the testes which generally occur as a consequence of previous history of infection like viral mumps or any other bacterial or viral infection.[1] Orchitis and epididymitis can coexist when inflammation in the epididymis spread to the adjacent testis.[13] Inflammation of the extra-testicular duct and male accessory gland may result due to bacterial or viral infection whereas noninfectious causes like dietary factors, trauma, medication, urinary reflux, hormonal changes may also cause inflammation in extra-testicular duct and male accessory gland.[13,14,15,16] It is also evident that there may be incidences of inflammation in the male genital tract without any identifiable reason[17,18,19]. Occurrence of any one of these events in the male genital tract make the immune system respond to the pathogens[10] and the damage in the tissue due to such response may accumulate in the system slowly and gradually; sometimes without showing any symptoms for years and finally causing tissue deterioration.[20] The damage in the cell and tissue are caused due to the release of some enzymes and toxic substances contained in the phagocytic cells. If the inflammation in the male reproductive tract is left untreated, it may cause deleterious effect to system finally causing male infertility.[21,22]

Epididymitis: It is a condition where inflammation occur in the epididymis, a structure which joins the testis to the vas deferens which may result in further complications like scrotal swelling and pain, penile discharge and blood in urine.[10] Older persons with epididymitis often show pyuria and it is frequently caused by enlargement of the prostate gland, which increases the risk of bladder infections.[23] Sexually transmitted infections mainly due to gonorrhea, Chlamydia, *Escherichia coli* was reported to be the main cause of epididymitis in older men whereas other contributing infectious agents may be bacteria like mycobacteria and ureaplasma.[10]

Benign Prostatic Hyperplasia (BPH): This is a chronic inflammatory condition of the prostate gland and is the most common urological disorder among elderly men. The inflammation due to BPH may occur as a result of both infectious and noninfectious processes and the latter may be due to some autoimmune response against self-antigens like PSA or prostate specific antigen. These self-antigens are developed in aged men due to hormonal changes or impairment in cellular tolerance.[24] Chronic inflammation in prostate gland due to ageing may have a chance of developing prostatic cancer in aged men.[1]

Urethritis: This is a condition where infection in the urethra or urinary bladder take place which may move to the epididymis too[10]. Urethritis in men is mainly caused due to gonorrhoea and/or Chlamydia and is abundant in younger men whereas nonspecific urethritis has been reported in older men.[19] Calcification is observed in bulbourethral gland of aged men due to ductal obstruction[25] and apoptosis is also reported in the glandular epithelial cells.[26] A decrease in the ratio of acid/neutral glycoprotein in the glandular secretion of senescent men is evident.[27]

Vasitis: This is an inflammatory condition of vas deferens and is of rare occurrence. *Vasitis nodosa* has been reported to be associated with vasectomy independent of the patient's age[28] whereas *tuberculosis vasitis* is found in both young and adult men.[29]

Testicular torsion: In this condition, the testis remain twisted within the scrotum due to some abnormality in the supportive tissues and is characterized by extreme swelling. Due to such torsion, the blood vessels are pinched off, causing disruption in blood circulation to testis and finally causing testicular damage.[10]

Varicocele: In this condition, enlargement of internal spermatic veins take place. The spermatic veins drain blood from the testicles to the abdomen (back to the heart) and in varicocele, the one way valves in the spermatic veins are damaged causing backflow of blood from abdomen to the scrotum. This creates a very unfavorable situation for spermatogenesis to take place.[10]

Obstruction of the ejaculatory duct: Infection in the ejaculatory duct may cause inflammation as scars are produced causing obstruction in it. It is evident that obstruction in the ejaculatory duct can be congenital[30] and, in older men

ejaculatory ducts are mainly blocked due to both chronic prostatitis and BPH.[31]

Orchitis/ Epididymo-orchitis: Orchitis or epididymo-orchitis may occur due to predominantly viral infection specially infections due to haematogenous dissemination of the pathogen.[32,33] Orchitis generally occur secondary to an infection like viral mumps infection and is prevalent in prepubertal male. Besides mumps virus infection, infection due to other viruses like Coxsackie virus types, Epstein-Barr, influenza, varicella, human immunodeficiency viruses may also cause orichitis.[34] Bacterial orchitis is reported to occur in sexually active men as well as in aged men with urinary infection or BPH.[1] Studies have also shown the existence of focal mononuclear orchitis in the testis of aged men.[35] Autoimmune orchitis is another variety of orchitis where chronic testicular inflammation is observed. It is characterized by degeneration and apoptosis of germ cells with an increased number of macrophages, dendritic cells and subsets of T cell population which causes secretion of proinflammatory cytokines.[36,37,38] Moreover, orchitis may occur due to noninfectious sterile reasons like administration of drug (Amiodarone) and heavy metal (mercury) toxicity.[34]

Inflammation can be initiated in the male genital tract as a result of any one of the above mentioned processes. Though the intention of the inflammatory process is to limit damage and restore normal function of the system but the inflammatory response itself may often cause harm to the system.

INFLAMMATORY MEDIATORS AND THEIR ROLE IN MALE INFERTILITY

An inflammatory mediator is known to act on blood vessels or cells to promote an inflammatory response. It is evident that cytokines play a major role in the inflammatory response by modulating the communication between the different cells of the immune system. Besides from immune cells, cytokines are also produced by fibroblasts, endothelial cells, adipocytes and cells of other tissues, such as the testis, ovary etc. Cytokines act by binding on specific receptors located on the cell membrane and their action is pleiotropic which means the same cytokine can have different effects on different cells.[39] On the basis of their way of action, they can be classified into pro-inflammatory cytokines, inflammatory cytokines, anti-inflammatory and cytokines that act as growth factors.[12]

Inflammation is the process of responding to injury and tissue damage and is characterized by an interplay between pro and anti-inflammatory cytokines. In inflammatory process, leukocytes and plasma molecules are released to the site of infection.

Immune privileged environment in testis:

Testis is known to be an ***immune-privileged*** organ[34] and such a condition of the testis is required for better regulation of spermatogenesis by protecting the immunogenic germ cells from destruction. The two main key products of the testis are sperm and testosterone which are synthesized in two separate structurally distinct compartments, the seminiferous tubules and the interstitial space of the testis respectively. Sertoli cells (SC) are the structural platform of the seminiferous tubules within which all stages of spermatogenesis occur. Immune cells, mainly a subset of macrophages and a few scattered mast cells in the human testis are found in close proximity to the tubule perimeter. Leydig cells (LC), which reside in the interstitial space, in close assembly to the immune cells, are the producers of testosterone. Sertoli cells are activated by the follicle stimulating hormone (FSH) whereas the leydig cells are stimulated by luteinizing hormone (LH). Both LH and FSH are produced by the anterior pituitary and thus pituitary has its key role for normal testis function. The least mature spermatogonial stem cells (SSC) and their differentiated progeny are located at the peripheral region of the seminiferous tubule with progressively more mature germ cell types found moving toward the tubule lumen. Tight junctions between adjacent Sertoli cells[40] which separate post-meiotic germ cells (spermatids) from the immune cells present in peri- and inter-tubular (interstitial) spaces and thus prevent immune cell recognition of these “developmentally late” reproductive cells as foreign.[41]

The blood-testis barrier plays a key role in sustaining the immune privileged environment of the testis and is constituted by the tight junctions present between adjacent sertoli cells. Pre-leptotene and leptotene spermatocytes residing in seminiferous epithelium need to pass through the blood-testis barrier at stage VIII of spermatogenesis for the maturation of the spermatozoa.[42] The dynamics of the blood testis barrier is regulated by testosterone and specific cytokines, which promote or disrupt the assembly of the blood-testis barrier.[43] Therefore, cytokines play a pivotal role in gap junctional communication significantly affecting spermatogenesis by restructuring of junctions, both at the SC - SC level and at the SC - germ cell interface in the seminiferous epithelium.[44] Another study has shown that an increase in pro-inflammatory cytokines leads to a deregulation of Cldn11 in SC, which, in turn, causes restructuring of the blood - testis barrier.[45]

Major cytokines in testis:

During infection, the testis is exposed to pathogens derived from blood or through genitourinary tract and to protect itself against all these pathogens, testis overpower this immune privileged environment by inducing a ***local innate immune response***. In the testis, innate immunity is mediated by the leukocytes present in the interstitial space where macrophages along with significant number of lymphocytes and monocytes are the main leukocyte population present. Monocytes promote the inflammatory process by secreting inflammatory cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α), by producing reactive oxygen species (ROS) or extracting chemokines that act as leukotactic agents. During tissue damage, TNF–α, controls movement of leukocytes into tissues and helps in the development of inflammation as well as in the activation of other leukocytes. TNF–α acts by inducing adhesion molecules and chemokines on the endothelium and thus the microbial systems of phagocytes is activated. TNF–α is also known to induce apoptosis. IL–1 shares functions with

Table I: Cytokines and their role in male reproductive function

Name of cytokine	Function
TNF-α	• Regulates spermatogenesis and inhibits steroidogenesis[54]. • Negatively correlated with sperm concentration, motility and morphology[59] • Known to increase in case of unexplained infertility[55] • Induces adhesion molecules and chemokines on the endothelium and activates the microbial system of the phagocytes[46]
IL-1α	• Regulate spermatogenesis by restructuring the blood testis barrier[12,41]. • Introduces apoptosis by chemoattraction of the leukocytes to the site of inflammation and generation of neutrophils and monocytes[60]
IL-1β	• Introduces apoptosis by chemoattraction of the leukocytes to the site of inflammation and generation of neutrophils and monocytes[60]
IL-1	• Regulates spermatogenesis.
IL-2	• Regulates spermatogenesis and is increased in case of dyspermia such as oligozoospermia, asthenozoospermia and teratozoospermia[55] • Negative correlation with testosterone's production by LC and amplifies testosterone's negative feedback on LH production by pituitary[12]
IL-4	• Increased in unexplained infertility[12]
IL-6	• Negative association with spermiogram parameters in case of silent infertility[12]
IL-8	• Increase in case of dyspermia and unexplained infertility[55] • Negative association with spermiogram parameters in case of silent infertility[12]
IL-10	• Regulates spermatogenesis[12]
IL-17	• In positive correlation with the concentration of TNF-α, IL-6 and IL-8[58] • In negative correlation with sperm motility[12].
IL-18	• Negative correlation with semen parameters like sperm concentration and motility • Associated with impaired spermatogenesis[57]
IL-21	• In positive correlation with production of auto-antibody during infertility[12]
IL-23	• Increase in case of orchitis
IFN-γ	• Regulates the function of Sertoli and Leydig cells[41] • Increased in unexplained infertility cases[12]

TNF–α.[46] It has also been reported that cytokines like IL-1, IL-6, TNF-α and TGF-β are also produced by the Leydig cells and sertoli cells of the testis in response to the stimulation of gonadotropins.[47] Both IL1 and IL6 are able to regulate Sertoli cell and spermatogenic cell development.[48] Spermatogenic cells in turn produce TNF-α. Several members of the TGFβ superfamily has a significant role in normal testis development and regulation of fertility by providing developmentally regulated and cell-specific signals. TGFβ1–3, are involved in many aspects of inflammation and immunoregulation.[49] Interferons (IFN; α, β, and γ) are produced by many testicular cells during viral infections. They also regulate Sertoli cell and Leydig cell function as well.[41] MCP-1/CCL2 are first chemokine identified in the testis, which regulates the large testicular macrophage population.[50] Pro-inflammatory cytokines thus act locally as they are produced by the locally activated cells in the testis. They are also produced physiologically by different cell populations in the male gonads and are known to be involved in the normal functioning of the organ.[51] Thus the cytokines are considered to be the natural component of seminal plasma[52] and thus there is a scope for the mutual interaction of macrophages with the leydig cells and sertoli cells of testis inducing steroidogenesis. This highlights the potential of macrophages for either inflammatory or homeostatic functions, depending on the environmental influences to which they are subjected.[53]

Cytokines in regulating various sperm functions:

In a recent study, Loveland and his colleagues have described the role of cytokines in regulation of spermatogenesis[41]. Spermatogenesis is an intricate procedure that requires perfect cell integration between the cells of the testicular microenvironment. Various cytokines like IL-1, IL-1α, IL-2, IL-6, IL-10, TNF-α are known to have significant role in regulation of spermatogenesis, among which IL-1α is known to regulate spermatogenesis by restructuring the blood testis barrier[41]. Spermatogenesis can be regulated by autocrine/ paracrine and endocrine mechanism. Inflammatory cytokines, such as IL-1, IL-2, IL-6, IL-10 and TNF-α, produced by Sertoli cells, leukocytes and germ cells are associated with autocrine/paracrine regulatory mechanism and the endocrine mechanism involves the gonadotropins- LH and FSH. Both autocrine and endocrine mechanisms, regulate each other, controlling the process of spermatogenesis.[54] The cytokine concentration in testis at different conditions affecting spermatogenesis are known to remain quite stable. Any alteration in this stable environment can be the cause of infertility.[12]

Various cytokines are known to be involved in regulating different reproductive functions (Table I). The concentration of serum IL-2, IL-4, IL-6, IL-8, IL-21, TNF-α and IFN-β concentrations are known to increase in case of unexplained infertility and on the other hand in dyspermia such as oligozoospermia, asthenozoospermia and teratozoospermia[55] the concentration of IL-2, IL-6 and IL-8 are increased. IL-21 is reported to be in positive correlation with production of auto-antibody during infertility. IL-2 has negative correlation with the production of testosterone by the Leydig cells of the testis and amplifies negative feedback of testosterone on the production of LH by the pituitary gland. IL-6 and IL-8 have negative association with spermiogram parameters in case of silent infertility.[12]

Various cytokines are also known to have regulatory roles in controlling semen parameters. IL-18 has negative correlation with semen parameters like sperm concentration and motility whereas sperm motility decreases on increase of IL-17.[12] IL-18

is a pro-inflammatory cytokine involved in the innate immunity.[56] IL-18 is found in testicular tissue and elevated IL-18 concentrations have been associated with impaired spermatogenesis, suggesting its involvement in this process[57]. High concentration of IL-17 has been found to be associated with an elevated concentration of TNF-α, IL-6 and IL-8.[58] TNF-α is known to be negatively correlated with sperm concentration, motility and morphology[59] and also controls the movement of leukocytes in the site of tissue damage and play a significant role in the production of inflammation. TNF-α induces adhesion molecules and chemokines on the endothelium and activates the microbial system of the phagocytes.[46] It inhibits steroidogenesis in the Leydig cells and thus has a negative correlation with the serum testosterone concentration.[12] Apoptosis in the semen is induced by the proliferation of T cells, Natural killer cells and differentiation of the beta cells. Apoptosis is also introduced by the IL-1α and IL-1β cells due to chemoattraction of the leukocytes to the site of inflammation and generation of neutrophils and monocytes.[60]

Cytokines in regulation of steroidogenesis:

Leydig cells are the main source of testicular steroid hormones that control spermatogenesis and secondary male sexual characteristics. The hypothalamic-pituitary-gonadal axis (HPG axis) in men controls production of testosterone hormones. The axis consists of three essential endocrine glands: the hypothalamus, anterior pituitary, and the testes. The gonadotropin-releasing hormone (GnRH) is secreted from the hypothalamus and reaches the anterior pituitary gland via the hypophyseal portal system. It stimulates the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) into the bloodstream by the gonadotropic cells of pituitary. The production of testosterone by the Leydig cells is induced by LH, whereas FSH induces the Sertoli cells to secrete androgen-binding protein (ABP) and inhibin which play an essential role in initiation and progression of spermatogenesis. In absence of GnRH, the release of FSH is also controlled by the inhibin-activin-follistatin system. Inhibin is secreted from the Sertoli cells and acts as an FSH inhibitor, while activin is secreted by the Sertoli cells and the pituitary gland and stimulates FSH secretion. Follistatin is an activin-binding protein secreted from the gonads and the pituitary gland, and inhibit activin-stimulated FSH release.[61] There are evidences that inflammatory cytokines reduce testosterone production by activating TLR2 receptors on leydig cells.[62] A recent study on young men has revealed elevated levels of pro-inflammatory cytokines, such as TNF-α and chemokines is associated with low concentrations of serum testosterone.[63] Further studies to elucidate the relationship between IL-2, LH and testosterone revealed the use of recombined IL-2 causes decreased production of testosterone by leydig cells. In addition, testosterone's negative feedback on LH secretion at the pituitary is amplified by IL-2. Thus, it seems that testosterone production can be affected by inflammation at many levels of the HPG axis.[64]

EFFECT OF INFLAMMATION IN MALE GENITAL TRACT:

Infections can promote inflammation in the male genital tract and may have two types of effect. In *acute inflammation* blood supply and capillary permeability increase allowing larger serum molecules to enter the site of infection and thereby increasing leukocyte concentration. When acute inflammation fails to eliminate infection, a condition called *chronic inflammation* take place. In this condition, macrophages and other phagocytic cells are activated and trigger the coordinated action of cytokines.[10]

Inflammatory response*:*

Infection in the male genital tract give rise to *inflammatory response* which is characterized by pain, redness, swelling and heat at the site of infection. This leads to an increased local seminal flow (responsible for the heat and redness) due to increase in diameter and a reduction in the velocity of seminal flow specially along the surface of the local seminal vesicles. In normal condition, the leukocytes remain at the centre of the seminal vesicles where the seminal flow is fastest but during inflammation the blood vessels lining the testis are dilated and the leukocytes move from the center to the periphery and interacts with the endothelial cells. In addition, fluid is accumulated due to increase in membrane permeability leading to swelling and pain. The migration of leukocytes out of the seminal vesicles depends on adhesive interactions activated by the release of inflammatory mediators. Thus, infection or physical damage to male genital tract sets in motion the recruitment of phagocytic cells to the site of damage.[10] The direct association between acute or chronic inflammation leads to the development of infertility. The reduced semen quality during the inflammatory process can result from impairment of accessory gland functions, obstruction of sperm transport, and dysregulation of spermatogenesis.[10]

Inflammatory damage and production of oxidative stress:

Oxidative stress (OS) is an imbalance between reactive oxygen species (ROS) and antioxidant scavengers of the cellular system and is considered as an important underlying mechanism associated with ageing, age-related chronic diseases and reproductive decline in males. It is triggered by various pathological conditions including inflammation, ischemia, ageing and any event of male reproductive decline.[65]

Production of Reactive oxygen species: The broad term ROS is used to describe oxygen-derivatives that are highly reactive and contribute to oxidative stress (OS). They have important cellular regulatory roles and are being implicated in numerous pathologies. ROS includes free radicals such as various sub-classes of molecules comprising of unstable oxygen ions such as hydroxide, superoxide etc. Free radicals are basically reactive chemical species containing unpaired valence electron(s) in their outer orbit. These unpaired electrons make free radicals highly reactive towards cellular components and cause damage to them.[66,67] The terms free radicals and reactive oxygen species (ROS) are frequently used interchangeably, however, not all ROS are free radicals.[68] For example, Hydrogen peroxide (H_2O_2), is named among ROS, but is not a free radical.

Endogenous ROS are produced intracellularly via numerous cellular mechanisms, which may vary within different tissues. During oxidative phosphorylation mechanism, ROS is generated by the mitochondria via a series of redox reactions and there is a transfer of electrons through five mitochondrial protein complexes. A small leakage of electrons from protein complex I and complex III results in the formation of superoxide radicals due to partial reductions of the oxygen consumed by the mitochondria. Superoxide radical is then metabolized into H_2O_2 by superoxide dismutase (SOD) 1 and SOD2 in the mitochondria.[69] H_2O_2 may be partially reduced further to produce a hydroxyl radical (OH^-). Moreover, other types of ROS can be generated directly (enzymatically) or indirectly from O_2^- .[70,71] Additionally, superoxide can undergo further metabolism by nitric oxide to produce the highly detrimental oxidant peroxynitrite that denatures proteins and affects mitochondrial membrane integrity.[69] Low and medium concentrations of ROS are involved in cellular defensive mechanism against infectious agents, signal transduction, and response against mitogens, carboxylation, peroxidation, however, high levels of ROS lead to various cellular damage[72,73,74]. Mt DNA is highly vulnerable to oxidative stress than nuclear DNA as because it is devoid of histone protein and there is no DNA repair mechanism in the mitochondrial DNA system[75]. Consequently, on accumulation of mutations in mtDNA, mitochondrial dysfunction take place, which in turn leads to an overproduction of ROS, oxidative damage and decrease in ATP/ADP ratio.[76,77]

ROS production in spermatozoa: ROS production in ejaculate is a normal physiological process. In the male reproductive tract and ejaculate, ROS have critical role in regulation of essential reproductive processes, like spermatogenesis, epididymal transport, spermatozoa maturation and post-ejaculation processes such as motility, capacitation and the acrosome reaction.[65] In addition, increased ROS production in the testes and epididymis may take place due to external factors like biological agents, radiation, excess heat, some medications, radiation, exposure to heavy metals, and smoking.[78] Human spermatozoal membrane contains a high quantity of polyunsaturated fatty acids, which are the primary targets of oxidative stress; besides which other targets of oxidative stress include biomolecules, such as structural proteins and nucleic acids.[78,79] Spermatozoa contain poor amount of antioxidant enzymes like superoxide dismutase, glutathione peroxidase, and catalase due to presence of a small amount of cytoplasm, that too concentrated at the centre of sperm head, which makes it unlikely for any antioxidant to protect spermatozoal membrane from head to tail[80] and therefore are vulnerable to oxidative damage. High oxidative stress leads to an increase in lipid peroxidation, DNA damage and apoptosis, which ultimately causes reduced sperm motility and vitality.[81,82] It is also evident that increased ROS levels may be a major cause of idiopathic male infertility.[82]

Inflammatory damage: In normal condition, ROS is generated by the spermatozoa to regulate various reproductive functions and the excess ROS is continually inactivated by the antioxidants present in the seminal plasma. When the effect of ROS overwhelms the defensive action of the antioxidants, then only oxidative stress develops in the male genital tract[83]. ROS promote inflammatory damage to the cells by inducing lipid peroxidation which cause reduction in the membrane fluidity inhibiting fertilization. ROS induced DNA damage involves base modifications, abasic sites, single strand (ss) and double strand (ds) DNA breaks, and DNA protein cross-links[81]. whereas sperm protein damage cause enzyme inhibition, denaturation and protein degradation.[84] During inflammation, the inflammatory mediators promote overproduction of ROS (Reactive Oxygen Species) giving rise to i*nflammatory damage* in the male reproductive tract due to excessive oxidative stress.[85] Increased levels of cytokines like TNF-α, Interleukin1 alpha (IL–1 α) and interleukin1 beta (IL1–β) during inflammation induce apoptosis in semen via proliferation and differentiation of beta cells, proliferation of T cells and natural killer cells. This causes chemoattraction of leucocytes to the site of inflammation and thus generating neutrophils and monocyte, consequently resulting in an increase in ROS of the seminal plasma as well as a product of lipid peroxidation named malondialdehyde (MDA). During infection, when the tissues are damaged the same cytokines that are known for immune modulation for the male gonad appear in large concentrations in semen and participates in inflammation thus causing increased leukocyte population in the semen. The migration of leukocytes out of the seminal vesicles to the site of infection depends on adhesive interactions activated by the release of inflammatory mediators. Neutrophils exposed to TNF–α generates oxygen radicals and nitric oxide, and release their stored granule contents leading to both host defence and local tissue damage.[10]

AGEING AND INFLAMMATION: IMPACT ON MALE INFERTILITY:

Ageing and inflammation are two closely linked processes and due to their close association, the term inflammageing has been introduced currently.[11] Ageing in the male reproductive system includes changes in testicular function, spermatogenesis and erectile function.[86] There are evidences that ageing in men is characterized by an imbalance between the pro and anti-inflammatory cytokines[1] and various changes in the male genital tract due to inflammageing have also been studied since long.

Impact on Testicular architecture:

In course of ageing, dramatic changes occur in the testis, from the neonatal and infantile period to puberty onset, adulthood, and finally senescence. The testicular morphology is known to change on ageing. The mean testicular volume tends to increase between 11 and 30 years of age, remains constant between 30 and 60 years of age, and decreases gradually every year after age 60.[87] Histomorphological studies have revealed that the number of germ cells and Sertoli cells in the testes decrease with aging. During the course of aging, the narrowing of the seminiferous tubules is also reported due to the thickening of the tunica propria of the basal membrane of seminiferous tubuli and simultaneous reduction of the

seminiferous epithelium causing the testis to vascularize.[88] The decrease in the number of Sertoli cells and germ cells on ageing is characterized by decline of seminiferous tubules. During the course of aging, a portion of Sertoli cells are reported to be multinucleated with a high number of mitochondria with tubular cristae, while another portion of sertoli cells have immature nuclei and sparse cytoplasmic organelles. Vacuolization in the cytoplasm of Sertoli cells and accumulation of lipid droplets within them occur due to phagocytosis of abnormal germ cells by the sertoli cells. Testicular fibrosis is also reported due to vascular changes and on progression of fibrosis with age, germinal epithelium separates from the blood supply. This develops in tubular involution and is thought to have an essential role in testicular atrophy on ageing.[89] The number of Leydig cells are also known to decrease with age having underdeveloped endoplasmic reticulum within them.[89]

A chronic testicular inflammation is observed during autoimmune orchitis and this is characterized by degeneration and apoptosis of germ cells, increased population of macrophages, dendritic cells and T cell subsets including Th1, Th17 and T(regs) cells, as well as by the presence of specific anti-sperm antibodies and chemokines. These molecules are known to alter the normal immune-suppressor microenvironment of the testis by the secretion of pro-inflammatory cytokines such as interferon IL-6 and TNFα.[1] Leydig cells show higher incidence of apoptosis during ageing[90]. In addition, the number of testicular macrophages increase in number and change ultrastructurally[91]. Human testicular macrophages express and secrete IL-1β and TNFα.[92] In aged men, increased levels of the circulating proinflammatory cytokines IL-1β, IL-6 and TNFα have been reported[93,94] 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.[12] The close association that exists normally between macrophages and Leydig cells are maintained on advancement of age but the close interdigitation between them is lost[69].The imbalance between the levels of pro and anti-inflammatory cytokine molecules is reported to be one of the fundamental mechanisms of ageing. Thus, inflammation can be correlated with the testicular ageing process. On ageing, hyperactivation of the macrophages take place which upregulates COX2 expression and an increased production of prostaglandins (well-known mediators of inflammation)[95]. It has been established that IL-1β and its receptors are expressed in macrophages and Leydig cells of the human testis, and this cytokine induces COX2 expression and prostaglandins production in both cell types.[96]

Impact on spermatogenesis:

Studies have revealed that changes in the histoarchitecture of the testis on ageing has great contribution to low spermatogenesis. Xu and his colleagues in 2013 have shown that the testicular tissue of aged men shows progressive degenerative spermatogenesis and in their testis spermatogenesis take place in some of the seminiferous tubules while adjacent tubules showed absence of spermatogenesis.[97] Due to the decline in the number of seminiferous tubules in the testis on ageing, there are reduced number of sertoli cells and germ cells in the testis reflecting its adverse effect on spermatogenesis. Kimura and his colleagues in 2003 have reported about higher occurrence of apoptosis in primary spermatocytes of aged men[98] whereas in another report it is said that alterations of spermatogenesis does not seem to significantly compromise fertility in the elderly[99]. It has been reported that high levels of the prionflammatory cytokines TNFα, IL -1α and IL--1ß are very harmful to sperm production[10]. Immunohistochemical studies revealed that COX2 is detected only in testes showing abnormal spermatogenesis but not in normal testes.[100] Tryptase is a serine protease which releases from testicular mast cells causing fibrosis of the peritubular cells also target PAR2-immunoreactive interstitial cells leading to induction of COX2 as well as upregulation of prostaglandin synthesis.[101] Futher, elevated ROS have been described in infertile men with impaired spermatogenesis, at least in the tubular wall.[102]

Impact on semen parameters:

Ageing has been reported to cause a change in various semen parameters like daily sperm production, total sperm count, and sperm viability[89]. Daily sperm production is negatively correlated with age in men in general[89]. Men with age of 45 or above show gradual decrease in the semen volume due to functional decline of accessory glands. In addition, sperm morphology is also affected with aging and the percentage of sperms with normal morphology begins to decrease after the age of 40. Recent studies have shown that paternal aging leads to decrease in sperm parameters like sperm volume, sperm motility, and sperm morphology except for sperm concentration.[103] Besides ageing alone, the pathophysiological basis of effects of age on semen parameters may be due to factors associated with age, as for example, vascular diseases, obesity, infections of the accessory reproductive glands or an accumulation of toxic substances. Due to insufficient number of seminal vesicles, semen volume and seminal fructose concentration have been reported to decrease with age, since the seminal vesicle contributes most to ejaculate volume.[104] Altered functions of prostate gland and the epididymis leads to decreased sperm motility as the swimming ability of spermatozoa is acquired during epididymal transit and motility is dependent on dilution into seminal plasma.[105] It is known that Prostate-specific-antigen (PSA) and α-glucosidase are the markers secreted by the prostate and the epididymis respectively and are positively correlated to sperm motility,[106] and the concentration of these markers decrease with age. Age-dependent alterations of the epididymis is assumed to lead to disturbed mitochondrial functioning, as an important part of epididymal sperm maturation is the activation of sperm mitochondria,[78,80,82] which could by itself already be altered via several mechanisms. High levels of certain cytokines in semen are often linked with a decrease in the quality of the semen parameters.[10] Role of cytokines involved in altered semen parameters have already been discussed in the earlier section of the review.

Impact on steroidogenesis:

Ageing in men is known to cause prominent changes in steroidogenesis. As ageing causes decrease in the number of the Leydig cells, serum testosterone levels are also known to decrease with age consequently. The other reasons for the reduced serum testosterone level on ageing are deterioration of testicular perfusion, and disturbance in diurnal rhythm of GnRH and chorionic gonadotropin secretion[89]. ***Primary hypogonadism*** is a condition where testosterone deficiency due to a testicular defect take place, whereas hypogonadism due to gonadotropin deficiency is called ***secondary hypogonadism***. Studies conducted with spermatic vein plasmas and testicular tissues from older men have shown a clear decrease in the levels of testosterone and its precursors (pregnenolone, progesterone, 17 alpha- hydroxyprogesterone, 17 alphahydroxypregnenolone, androstenedione, androstenediol, dehydroepiandrosterone, and dehydroepiandrosterone sulfate).[89]

The progressive reduction in total and free serum concentrations of androgens (testosterone, dihydrotestosterone, 3α -androstanediol, 3α -androstanediol glucuronide) in men with advancement of age is accompanied by an increase in the gonadotropins circulating levels. Testosterone is locally converted to estrogen by the enzyme aromatase in many tissues[61] however, Estrone (E1) and estradiol (E2) have been shown to decrease with aging.[89] Although testosterone is the main source of plasma E2, decline in testosterone levels with aging is poorly reflected in plasma E2 levels. This discrepancy can be explained by increases in aromatase activity and body fat mass observed during aging, as E2 is highly positively correlated with body fat index. The age-dependent decrease in the steroidogenic and spermatogenic activities of the testis have been associated with an increase in the inflammatory status of the tissue.[99,107] Low levels of serum androgens in elderly men is correlated with increased levels of the circulating proinflammatory cytokines.[93,94] IL-1β, TNFα and prostaglandins are known 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 steroidogenesis[108]. TNF- α activates DAX-1 a member of the nuclear receptor family, which acts as a co-repressor of many nuclear receptors and a regulator of steroidogenic genes.[109]

Furthermore, increase in the number of lipid vacuoles in testicular macrophages can be associated with alterations in cholesterol metabolism. 25-hydroxycholesterol produced by testicular macrophages is transferred to Leydig cells and is utilised in the androgen biosynthesis. The loss of macrophage-Leydig cell interdigitations during ageing might contribute to age-related decreased steroidogenesis. Testicular macrophages in aged individual also accumulate lipofuscin granules, which might be related to the increased cytokine production and further contribute to the decline of steroidogenesis during senescence.[110]

Impact on hypothalamic pituitary gonadal axis:

Changes in the hypothalamic-pituitary axis also have an effect on reproductive aging in men. Aging causes a reduction in the secretion of GnRH, which in turn leads to smaller LH and testosterone pulses. LH pulses in older men are more frequent and smaller in comparison to the younger men. The gonadotropin response to exogenous GnRH is impaired due to ageing and this increases LH pulse size, and reduces in vitro LH bioactivity.[89] Aging attenuates the inhibitory effect of free testosterone on GnRH release and also suppresses the feedback by bioavailability and total testosterone concentrations. Although ageing may cause decline in the density of androgen and estrogen receptor the deterioration of negative feedback by testosterone has a simple effect on GnRH release.[111]

Impact on other parts of the male genital tract:

Inflammation of the urogenital system due to identified and nonidentified reason is often correlated with ageing. Enlargement of the prostate gland in older men is one of the most common age-related diseases and is defined as benign prostatic hyperplasia (BPH). Benign prostatic hyperplasia (BPH) is of common occurrence among the aged men.[112] Age-related prostate enlargement is known to be caused by hyperplasia of basal cells and stromal cells, such as muscle and fibroblasts, located in the transitional zone around the urethra. Stromal volume has been observed to increase in patients with symptomatic BPH along with a decrease in apoptosis of stromal cells.[89] Enlarged nuclei, decline in basal epithelial layer and proliferation and abnormal differentiation of secretory cells give rise to a condition called Prostatic intraepithelial neoplasia (PIN). PIN is regarded as pre-invasive stage of prostatic adenocarcinoma.[89] In elderly men with BPH, expression of some inflammatory mediators including chemokines like (CXCL1, CXCL2, CXCL5, CXCL6, CXCL12) and interleukins (IL-11, IL-33) are upregulated[113] and they promote proliferation of both epithelial and fibroblastic/myofibroblastic cell types which is a characteristic feature of BPH in aged men. In this condition, COX2 enzyme (key enzyme for prostaglandin biosynthesis) is overexpressed which may even lead to prostratic cancer in aged men.[114,115] Role of IL-6 in prostate cancer has also been reported.[116] BPH inflammation in elderly men due to noninfectious reason may occur due to autoimmune responses against self-antigens such as the prostate-specific antigen (PSA) and its antigenicity is associated with the impairment of cellular tolerance processes and/or hormonal changes in ageing men.[112] Chronic inflammation and advanced age are the two main risk factors for the development of prostate cancer.

In case of infection in bulbourethral gland, the glycoprotein profile detected in the glandular secretion is also affected by age. It shows a decrease in the ratio of acid/neutral glycoprotein in senescent men.[27] Infections of the ejaculatory duct can promote inflammation by the formation of scars leading to a blockage and azoospermia. It has been proposed that in older men, ejaculatory ducts are mainly blocked by both chronic prostatitis and BPH.[31] It can be said that ageing and

inflammation are closely interconnected in extratesticular ducts and accessory sex organs, affecting male reproductive function.

Free radical theory of ageing and its effect on the male reproductive function:

Presently no single theory can explain the probable cellular or molecular mechanism of ageing however, De la Fuente and Miquel (2009) have proposed the oxidation-inflammation theory as the main cause of ageing.[117] Ageing is characterized by accumulation of cellular damage (due to genomic factors and/or oxidative stress) over time and is closely associated with damage at DNA and protein level leading to cellular senescence[118]. The free radical theory of ageing states that excessive endogenous and exogenous ROS is detrimental to homeostatic mechanism of the cell.[119] This suggests that excessive oxidative stress (OS) causes an accumulation of macromolecular damage and this is associated with age related functional decline of the organism. As telomeres are unable to maintain their lengths due to end replication problem, cellular senescence is also preprogramed into the genome. In course of ageing, a decline in protective systems against cellular stressors, including ROS take place.[118] Cellular senescence is associated with increased cell size, accumulation of protein aggregates, increased protein damage due to oxidation, giant mitochondria (producing increasing amounts of ROS with age), enlarged nucleus, increased lipofuscin accumulation and reduced proteasomal and lysosomal function. Production of numerous secretory biomarkers including pro-inflammatory cytokines, growth factors, matrix metalloproteinases and ROS causes the genome to be associated with a reduced capacity for DNA repair, resulting in an increased accumulation of genetic mutations and finally leading to cellular senescence. Dysfunctional mitochondria are the major factors for excessive ROS production, they create an unfavorable imbalance in the redox status, and are thus considered a major cause of cellular ageing.[120]

There are two main sources of ROS in the male reproductive tract, namely leukocytes and spermatozoa and these cells produce ROS as part of regulatory roles during inflammation and cellular defense. 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, co-enzyme Q10 and ubiquinol.[121] 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 and these are known to increase on ageing. Pathological conditions such as varicocele and spinal cord injuries are also mediated by OS as well as by the ageing process itself.[122]

Overproduction of ROS has been correlated with reduced sperm concentration and motility, morphological derangements and damage to both cellular and mitochondrial DNA, as well as mitochondrial membrane potential (MMP).[122,123,124] OS also causes change in the structure of the spermatozoal flagella during epididymal maturation, causing impaired motility following ejaculation.[65] High concentrations of H_2O_2 are formed in abnormal spermatozoa which initiate the apoptosis via caspase 3 activation and annexin-V binding (as the sperms are prevented to be exposed to antioxidants such as catalase and melatonin).[105] Thus, a fine balance between ROS and antioxidants needs to be maintained by various enzymatic and non-enzymatic processes.[65,122,] Sperm DNA damage can be evidenced due to increased 8-hydroxy-2'-deoxyguanosine (8OHdG), a known marker of DNA oxidative damage[105] and on ageing there is an increased rate of damage in sperm DNA and decreased DNA repair mechanism.[125] Sperm DNA fragmentation Index (DFI) is also positively correlated with sperm paternal aging. On aging poor quality DNA, lipids, and proteins are formed due to an imbalance between antioxidant defence mechanisms and ROS production.[126] Overproduction of oxidative stress leads to increased spermatozoal DNA damage and apoptosis.[127] Though apoptosis is good for spermatogenesis, it has been reported that ageing causes an imbalance between proliferation of spermatogonia and apoptosis of different germ cell types.[128] Expression of Ki-67, a marker of cell proliferation activity and apoptotic rate were found to be decreased in aged spermatogonia as well as primary spermatocytes.[98] DNA damage repair proteins as well as apoptosis markers, such as cleaved PARP-1 and active caspase-3 are differentially expressed in aged spermatocytes and it is said that overactivation of PARP-1 leads to initiation of apoptosis. Besides, DNA damage, extensive protein oxidation in spermatozoal and seminal plasma proteins associated with ROS has been reported. The markers for OS are DJ-1, PIP and lactotransferrin and PRDX's.[121] In human testis, a study reported that the proinflammatory prostaglandin 15d-PGJ2 generates ROS.[129]

Leukocytospermia is known to be associated with male reproductive tract infection and inflammation, and the associated immune response increases ROS production as well as downregulates the action of endogenous antioxidants in order to induce OS as part of the immune response. OS plays a significant role in a local inflammatory response associated with acute or chronic infections of the reproductive tract.[130,131] Pro-inflammatory cytokines, particularly TNFα, IL1β and IL6, are known to modulate both OS and antioxidant status by increasing production of ROS and OS in seminal fluid.[132] Systemic inflammatory diseases are also associated with poor fertility outcomes. Leukocytospermia is associated with increased superoxide and H_2O_2-positive spermatozoa associated with apoptotic markers caspase 3/7 and glutathione activation.[131] Leukocyte induced OS can also be neutralised with Vitamin C, Vitamin E, Glutathione and Co Enzyme Q10,[65] suggesting a central role of OS in the pathogenesis of male reproductive tract inflammation.

OBESITY: ANOTHER PROBABLE FACTOR FOR MALE INFERTILITY:

Nowadays, obesity is a global health problem and an association between obesity and male infertility has been studied in recent years. Several studies have investigated the impact of obesity on the semen parameters. Our sedentary

lifestyle and fat rich diet fats affects male reproductive functions not only by reducing semen quality but also by altering the physical and molecular structure of germ cells. Reduced sperm concentration, abnormal sperm morphology, abnormal sperm motility and compromised sperm chromatin condensation are observed in semen of obese person and long exposure of germ cells in high aromatase activity may be the reason behind it. It is evident that the size of the adipocytes rather than the aromatase expression, affect testosterone concentrations.[133] Furthermore, sperm DNA fragmentation is commonly observed in obese males, indicating poor quality of spermatogenesis. Increased body mass index (BMI) can also affect spermatogenesis by causing an increase in scrotal temperature. Futhermore, increased fat tissue in scrotum and adjoining area can have adverse effect on the reproductive potential of a man. Obesity may induce systematic oxidative stress[134] which is again considered to be one of the potential reasons for male infertility.

An altered reproductive hormonal profile is observed in obese males. They have elevated estrogen concentrations due to increased conversion of testosterone into estrogens associated with high bioavailability of aromatase.[134,135] High estrogen concentrations and low testosterone concentration in obese men is a potential reason for male infertility. Testosterone helps in adhesion of sertoli cells to the developing germ cells; hence, germ cells may undergo phagocytosis due to low intra-testicular androgen concentrations. Obesity is also responsible for hypogonadotropic hypogonadism with lower concentrations of FSH, LH, inhibin B and sex hormone-binding protein (SHBG). They are involved in the regulation of the functions of the sertoli cells and hence regulates spermatogenesis.[134,135,136] A potential risk factor for hypogonadism, sexual dysfunction and, possibly, reduced fertility is sleep apnea which is of frequent occurrence in obese men. Sleep changes affect neuroendocrine function by lowering LH and testosterone concentrations.[134,136] 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.[12]

Adipokines:

These are hormones produced by the white adipose tissue which is considered to be an endocrine organ involved in energy homeostasis. They have a significant role in the lipid and glucose metabolism, in inflammation and in the regulation of the immune system[137,138]. Adipokines regulate male gonads through the function of hypothalamo-pituitary gonadal axis. Various adipokines are:

Leptin: Leptin, a polypeptide of 146 amino acids is predominantly expressed in adipose tissue and it regulates energy expenditure, body weight, fat mass and puberty.[139] 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.[135,139,140] Obese men have increased leptin concentrations which may contribute to male infertility through leptin's resistance or insufficiency at the hypothalamic level. High leptin concentrations lead to low serum testosterone concentrations and abnormal seminal parameters. This occurs due to inhibitory action for the conversion of 17(OH) progesterone into testosterone. Males having hypothalamic hypogonadism have leptin receptor deficiency which results in delayed pubertal development, atrophic testis and impaired spermatogenesis.[134,135] Furthermore, leptin stimulates the release of LH and FSH via the NO synthase activation in the gonadotropic cells.[135,141] Increased concentrations of leptin inhibit hCG in a dose dependent manner and thus affect testosterone production by Leydig cells. Thus leptin regulates male reproductive function by stimulating hypothalamus and pituitary and inhibiting the gonadal function.

Adiponectin: 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 pro-inflammatory cytokines by suppressing NFkB action.[142] However, presently, there is not much evidence for the relationship between adiponectin and reproduction.

Resistin: Resistin is a protein secreted by the adipose tissue and is expressed in interstitial LC and SC of testis. Their expression is under control of the gonadotropins and is expressed at its peak at stages II - IV of the seminiferous epithelial cycle.[141,143] Furthermore, resistin shows a correlation with IL-6, TN-α and seminal quality. Resistin concentrations are increased in cases of leukocytospermia and smoking, suggesting that resistin may play a regulatory role in the inflammation of the male reproduction system.[144]

Visfatin: Visfatin is secreted by visceral adipose tissue. It is present in various tissues, including the testis. Increased Visfatin concentrations causes increased concentration of serum testosterone, increased body and testis weight, and is negatively correlated with blood glucose concentrations[12]

Other adipokines known are *vaspin, chemerin, and progranulin* and their role in regulation of testicular function needs to be explored.

GHLERIN AND ITS ROLE IN MALE INFERTILITY

Ghrelin is a GH-releasing peptic peptide that helps in energy homeostatic mechanism and has effects on male reproductive function. It is found in the leydig cells and its expression in LC is dependent on their various differentiation states. They are found in low levels in immature LC and in increased levels in differentiated, adult-type LC. An inhibitory role of ghrelin in the immature LC proliferation has also been reported and this is known to be associated with changes in expression of stem cell factor (SCF) gene, a key signal in spermatogenesis and regulator of LC development[141]. Furthermore, a positive correlation exists between testosterone and circulating ghrelin concentrations.[12]

CONCLUSION

Male infertility is accompanied by a variety of disorders including hormonal, histological as well as physical problems. It

is often caused due to infection resulting in inflammation having a negative impact on normal sperm function. Multiple genetic and environmental factors accelerate aging in somatic cells, as well as in male reproductive cells. The effects of aging on men's fertility are studied since long and age-related alterations lead to gradual changes in men's androgen levels as well as spermatogenesis. Consequently, these progressive changes 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 and lifestyle, genetic and environmental factors are known to modulate this balance. Although low and medium level ROS are important for normal physiological function including fertilization potential of an individual, overproduction of ROS have roles in all male age-related phenomenon affecting sexual behavior and seminal quality. Several studies have also investigated the impact of obesity on male's status of fertility and the sedentary lifestyle and a fat rich diet affects male reproductive system not only by reducing semen quality, but also by altering the physical and molecular structure of germ cells. Involvement of pro-inflammatory and inflammatory cytokines in male infertility indicates the role of inflammation in regulating the reproductive function in men. Although the involvement of cytokines in male infertility is complex and complicated, strong supportive evidence on the role of leukocytes and cytokines in male reproduction are present. Rigorous living conditions and desire for a better lifestyle often cause postponing of parenthood until older ages. Though it is evident women's age is more important in parenthood as well as reproductive potential than in men, recent studies on reproduction propose that men can maintain their fertility until older ages. Although paternal age does not affect fecundity directly as an independent factor, it may have significant role in combination with the maternal age. Paternal age has a significant influence on the risk of certain diseases and correlates with a number of complications in the offspring. The children of older fathers show tendency of some genetic abnormalities, childhood cancers, and several neuropsychiatric disorders. Keeping this in mind couples should be more conscious about their daily life style and should be more practical while planning for offspring.

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AUTHOR BIOGRAPHY

Silpi Acharyya is an Assistant Professor in Zoology in West Bengal Education Service and is presently posted at Taki Govt. College, Hasnabad, West Bengal, India. She did both her graduation and post-graduation courses in Zoology from University of Calcutta and obtained her Ph.D degree from the same University on the topic entitled "Sperm DNA characteristics and Male Infertility". Her area of specialization is Advanced cytology - Genetics and Biochemistry and has a research interest in the area of Male Reproductive Biology. She has a teaching experience of more than 13 years and has published articles in leading journals.