Impact of inflammation on male fertility

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1. ABSTRACT

The male uro-genital tract is susceptible to gram-negative bacterial infections that produce a state of inflammation, particularly in the testis and epididymis. Development of germline stem cells into motile spermatozoa takes place in these organs and thus any impairment therein has a direct effect on male fertility. A number of factors are known to impair male fertility including environmental and chemical factors, lifestyle, and infections. The last is a little-known and poorly understood cause of male sub-/ infertility. The presence of the pro-inflammatory cytokines like tumor necrosis factor-alpha (TNF- alpha), interleukin-1alpha (IL-1alpha) and interleukin-1beta (IL-1beta) in the male uro-genital tract following bacterial infections suggests that such infections could have cytokine-mediated anti-fertility effects. Furthermore, inflammation has been associated with elevated levels of reactive oxygen species and oxidative stress both of which affect male fertility. The present article summarizes the effects of inflammation on the testis, epididymis and spermatozoa. We review the correlations between inflammation and oxidative stress vis-à-vis spermatogenesis and discuss the implications of infections on male fertility/ infertility and assisted reproductive technologies for the male.

2. INTRODUCTION

At the start of spermatogenesis, spermatogonia, or immature germ cells divide mitotically to produce diploid cells termed spermatocytes. Spermatocytes undergo two mitotic divisions as they migrate from the base of Sertoli cells to the top or apical end. The meiotic division involves pairs of chromosomes coming together and exchanging DNA to form secondary spermatocytes. Subsequently, the two chromatids separate and form haploid cells called spermatids. These spermatids are then released from the testes into the epididymis where they begin to undergo capacitation and acquire motility (1, 2). Spermatids, now called spermatozoa are thus motile and possess the requirements for their trajectory in the female reproductive tract (3-6). The last leg of capacitation occurs in the cervix where spermatozoa attain hyperactivity and which is the site of acrosomal reaction. The acrosomal reaction enables the spermatozoon to penetrate the zona pellucida, fuse with the oolema and cause fertilization. Thus, male fertility is directly dependent on the uninterrupted completion of spermatogenesis.

Infertility in males is fundamentally due to disruption in the process of spermatogenesis resulting in no or incompetent spermatozoa (7, 8). Different spermatogenic

factors or "symptoms" are considered to lead to infertility such as decreased sperm motility, low sperm count, damage to sperm DNA, and/ or poor semen quality. Seminal quality can be compromised due to a number of factors like high levels of reactive oxygen species (ROS) and oxidative stress (7, 9, 10). Oxidative stress has been known to affect spermatogenesis in the testis, epididymis and at a seminal level (11, 12). In the past few years, new evidence suggests that oxidative stress in the testis can be linked to inflammatory conditions therein (13).

Inflammation is the body's defense against infection or injury where there is an increase in local blood flow, micro-vascular permeability and recruitment of leukocytes to the site of infection. Inflammatory response can be either acute or chronic depending upon the severity and time taken for response. Briefly, inflammatory response starts with hemodynamic changes (like vasodilatations and increased vascular permeability) that facilitate the emigration of circulating leukocytes into the infection site. Leukocytes phagocytose or kill microbes and digest tissue debris and thus protect the host. Cytokines such as interleukins (IL) and tumor necrosis factors (TNFs) are involved in signal transduction during states of inflammation. Inflammation is caused by a number of factors chief among which are infections by gram-negative bacteria. Lipopolysaccharide (LPS), present in the cell wall of gram-negative bacteria is considered to be the endotoxic element that causes inflammation (14). Since the male is susceptible to a number of uro-genital tract infections that cause a state of inflammation in the reproductive tract, it is relevant to know the potential effects of inflammation on the male reproductive system. The present article summarizes the effects of inflammation on the testis, epididymis and spermatozoa, specifically mediated by a few pro-inflammatory cytokines TNF-alpha, IL-1alpha and IL-1beta. We review the correlations between inflammation and oxidative stress vis-à-vis spermatogenesis. We highlight some important markers of inflammation in the semen and discuss the implications of infections on male fertility/ infertility and assisted reproductive technologies for the male.

3. FFECTS OF INFLAMMATION ON MALE REPRODUCTIVE SYSTEM

Inflammation has been known to affect the twin testicular functions of steroidogenesis and spermatogenesis. Marked decreases in the circulating levels of luteinizing hormone and testosterone are detected when there is inflammation (15-17). When bacterial inflammation is induced by injecting LPS, there are decreased testosterone levels due to a reduction in the activity of Steroid Acute Regulatory (StAR) protein, a key regulator of steroidogenesis (18). The co-occurrence of high levels of pro-inflammatory cytokines (like IL-1beta, TNF-alpha) with the inhibition of StAR protein activity suggests cross-talk between these cytokines and StAR protein (18-20). A recent study found that inflammation caused spermatogenic arrest and deceleration in sperm maturational processes (21). Interestingly, inflammation has a cell-specific effect on various germ cells. Spermatocytes and spermatids seem to be primarily affected while spermatogonia are often seen to be unaffected (21). Although a number of studies report inflammation in the epididymis, it is difficult to ascertain whether this is a consequence of testicular effects or if the epididymis can be the primary target of inflammation. In conditions of epididymal inflammation, the count and motility of caudal sperm are decreased and often there is obstructive azoospermia. Inflammation affects the prostate by suppressing the secretion of citric acid and gamma-glutamyl transpeptidase by the gland. Most importantly, in conditions of inflammation, there is infiltration of leucocytes into semen and production of anti-sperm antibodies. Inflammatory conditions increase rigidity of sperm flagellar membrane by decreasing lipid content of the membrane. This decreases sperm motility and causes sperm agglutination and asthenozoospermia (22, 23). Ensuing defects in acrosome reaction render the sperm incapable of penetrating the oolemma. Moreover, the integrity of sperm DNA is damaged leading to an increase in the number of apoptotic sperm (24). Sperm or germ cell apoptosis is regulated by ROS and related to the oxidant/ antioxidant status of the testis (25).

4. INFLAMMATION: A CAUSE OXIDATIVE STRESS

Prior to discussing the correlation between inflammation and oxidative stress, it is necessary to understand the importance of the oxidant/ antioxidant system and its role in spermatogenesis. ROS like hydrogen peroxide (H_2O_2), hydroxyl radical (OH), and superoxide anion (O_2-) are highly reactive oxygen free radicals which contain one of more unpaired electrons (8). Small amounts of ROS are required for capacitation, hyperactivation, and acrosome reaction, and sperm-oocyte fusion (26). However, ROS, in high amounts, can be harmful to sperm as these radicals try to poach hydrogen atoms from polyunsaturated fatty acid of sperm membrane in order to fill their valence (27). ROS take up the hydrogen atoms causing peroxidation of membrane lipids, loss of adenosine triphosphate (ATP), and loss of membrane fluidity and integrity (28-30). The reduction in intracellular ATP compromises axonemal protein phosphorylation leading to sperm immobilization (31). Antioxidants act as defense mechanisms against the pathological effects of ROS production and oxidative stress (32).

To prevent excesses of ROS and maintain a balance, cells contain a number of antioxidants that degrade ROS to form non-radical molecules like water (33). Antioxidants are of two types: (i) enzymatic and (ii) non-enzymatic antioxidants. Three main enzymatic antioxidants include superoxide dismutase (SOD), catalase and glutathione peroxidase (GPX). SOD converts the superoxide anion to H_2O_2. Both catalase and GPX neutralize H_2O_2 into water and oxygen. Three main non-enzymatic antioxidants include glutathione, Vitamin C and Vitamin E. Glutathione and Vitamin C directly neutralize ROS while Vitamin E is required to recycle Vitamin C (34). Spermatozoal antioxidant defense mechanisms are important because spermatozoa contain very little cytoplasm (approximately 20 μm^3), which is the cellular

depot of antioxidants and thus makes the spermatozoa a vulnerable target of oxidative damage (35). To compensate for this lack of cytoplasm, the semen is a rich storehouse of antioxidants (36).

As briefly mentioned in the introduction section, there is evidence that suggests that in the semen, inflammation could be linked to oxidative stress. Infertile men with high levels of seminal ROS showed increased levels of pro-inflammatory cytokines and leukocyte infiltration in their semen (37). Although certain invading pathogens such as bacteria may produce ROS themselves, leukocytes are considered to be the predominant source of ROS in semen (38, 39). They do so in two ways: (i) directly, or (ii) indirectly by increasing the levels of inflammatory cytokines which then cause an increase in ROS levels (40, 41). In the "direct pathway", phagocytes are activated and as phagocytosis proceeds, there is production of large amounts of ROS (like superoxide anion, hydrogen peroxide, hydroxyl radical and hypochlorite) in oxidative bursts (42, 43). These ROS are either produced in the semen or within the leukocyte itself, and react with spermatozoal membrane. Due to the oxidative burst, oxidants outnumber antioxidants, inducing a state of oxidative stress (44). This state of oxidative stress persists even after the removal of the pathogen (23). Interestingly, in males who were infertile prior to induction of inflammation, the imbalance between oxidants and antioxidants was more drastic suggesting that inflammation induced by oxidative burst could be an important confounder of male infertility (45).

5. CYTOKINES

The second pathway of oxidative stress generation is by the generation of pro-inflammatory cytokines by leukocytes (44). Cytokines, in general, are proteins that can act as signaling molecules and that can modulate cellular reactions like inflammation (24, 43, 46). They activate the xanthine oxidase system giving rise to high levels of ROS and oxidative stress (23). Cytokines induce an inflammatory response by binding to their receptors and stimulating a signal transduction involving tyrosine or serine phosphorylation. This leads to the activation of transcription factors, which are then able to modulate gene expression. Furthermore, cytokines generally work synergistically and have a greater impact when interacting with each other to create a complex network enabling cells to react to the pathogen invasion in a systematic fashion (43, 46). There are a number of cytokines that mediate the effects of inflammation in the male reproductive tract, but the most important ones are tumor necrosis factors (TNFs) and interleukins (ILs) (47).

5.1. TNF- alpha

Tumor necrosis factor- alpha (TNF-alpha) is a 17kDa protein secreted mainly by activated T cells, monocytes, macrophages, and a few non-lymphoid cells like Sertoli and male germ cells. TNF-alpha binds to a transmembrane receptor, TNF receptor, which then recruits several cytosolic proteins and transduces the signal. In the testis, TNF-alpha regulates germ cell apoptosis, Sertoli cell-germ cell junction dynamics and Leydig cell steroidogenesis (48). TNF- alpha represses gene expression of steroidogenic enzyme in Leydig cells via activation of nuclear factor kappaB (49-51). The repression of the steroidogenic enzymes leads to a decrease in testosterone production (50). Increase in the levels of testosterone causes germ cell depletion from the epithelium (52). High levels of TNF-alpha have also been detected in the semen of sub-fertile and infertile men. In fact, a recent study reports the presence of high seminal levels of TNF-alpha in conditions of leukocyte infiltration of semen and shows that inflammation-mediated azoospermia is directly related to seminal levels of TNF-alpha (53, 54).

5.2. IL-1 alpha

Interleukin-1alpha (IL-1alpha) is a 17 kDa growth factor, acts as a co-stimulator of T cell functions, is involved in the activation and differentiation of leukocytes, and in Sertoli cell proliferation (43, 47, 55-57). IL-1alpha is secreted by Sertoli cells and germ cells in an age- and cell type-dependent manner (58). IL-1alpha is present in large amounts in secondary spermatocytes and is shed with residual body when the germ cells differentiate into spermatids (59). In response to testicular inflammation stimulated by chemicals (like zymosan) or pathogens (LPS from gram-negative bacteria), testicular macrophages secrete increased quantities of IL-1alpha (60). The secretion of this cytokine leads to inhibition of Leydig cell steroidogenesis, stimulation of Sertoli cell transferrin and IL-6 production (61-63). IL-1alpha has been shown to downregulate testicular cell adhesion and to have a role in cell-cell cross-talk. A recent report suggests that IL-1alpha may be a previously ignored regulator of the blood-testis barrier and may thus be intricately linked with the immune-privileged status of the seminiferous tubules (64).

5.3. IL-1beta

IL-1beta is involved in regulation of spermatogenesis and spermiogenesis but in high amounts is cytotoxic to germ cells (43). An interesting characteristic of IL-1beta is that it is a paracrine as well as an autocrine substance that serves as an important mediator of immunologic and pathologic responses to conditions of stress, antigenic challenges, and infection (65). For example, when bacterial infections were simulated by treating with LPS, there was an increase in the expression IL-1beta concomitant with a decrease in testosterone levels. Thus, the ensuing loss of spermatogenesis could be either due to the direct effect of inflammation or due to inflammation-induced decrease in testosterone production (43, 66). IL-1beta has been shown to decrease semen quality, sperm count and motility (67). IL-1beta signals an increase in expression of adhesion molecules and receptors so that the leukocytes can attach to the blood vessel and reach the site of infection (57). It is interesting to note that high IL-1beta levels were detected in semen although it is completely sealed off from circulation (68).

6. SEMINAL MARKERS OF INFLAMMATION

According to the World Health Organization human semen is normal if the leukocyte count is below one

million per milliliter (69). If the number of leukocytes exceeds one million, spermatozoa are damaged and the condition is called leukocytospermia (27). The main leukocytes that infiltrate semen are granulocytes and T lymphocytes (70). Granulocytes generally make up the largest leukocyte type in seminal fluid (50-60%), followed by T lymphocytes (2-5%) (41, 71). Leukocytospermia has been associated with the impairment of various seminal parameters such as decreased sperm motility, ability of sperm to penetrate zona pellucida, impaired fertilizing capability, and decreased viability due to their ability to induce ROS (72, 73)}. Leukocytocytes and macrophages migrate to the semen when there is pathogen- or tissue damage-induced inflammation (24, 41, 74). The oxygen metabolism of the leukocytes accelerates and is connected with the production and release of large amounts of superoxide anion and hydrogen peroxide (43). Thus, leukocytes are the most obvious markers of inflammation in the semen.

Inflammation in the male reproductive tract is an equally important indicator of inflammation in the semen. As previously discussed, cytokines can induce inflammation directly and are also a part of leukocyte-mediated inflammatory defense. A positive correlation between the levels of different cytokines and the presence of leukocytes have recently been demonstrated in semen. Increased levels of IL-8 and TNF-alpha were detected in semen contaminated with pathogens and showing a high leukocyte count (53). However, high IL-6 levels were seen in leukocyte-infiltrated semen, even in the absence of pathogens suggesting that IL-6 could also be used as an indicator of non-pathogenic inflammation in the semen (53). Most interesting is the study by Seshadri *et al* who show that men with different seminal defects have different cytokine profiles. For example, sub-fertile men who were oligospermic (low sperm count) had increased levels of IL-6 and IL-10 levels, whereas those who were asthenospermic (low sperm motility) had increased IL-8 and IL-10 in their semen. In sub-fertile men with obstructive azoospermia (due to blockage in the reproductive tract) the levels of IL-6, IL-10 and TNF-alpha were high (54). These studies clearly show that cytokine profiling in the semen could be used as a method to detect inflammation in the reproductive tract and infections especially in the scenarios like assisted reproductive therapies (ART), where it is necessary to minimize genetic defects transmitted due to selection of poor quality sperm.

7. INFLAMMATION AND ASSISTED REPRODUCTION

When men opt for assistance for sub-/ infertility, they are examined for general health and infections. Reports suggest that even if the male reproductive tract is under inflammation, viable sperm may be selectively harvested and used for ART like *in vitro* fertilization (IVF) or intra-cytoplasmic sperm injection (ICSI) (75). However, the accidental selection of damaged sperm is high and can have the following consequences: (i) sperm with improper DNA organization or chromatin condensation could cause defects in the fetus and in the offspring (28), (ii) sperm with

fragmented DNA, which are able to fertilize the oocyte could lead to embryonic development stops resulting in failed pregnancy or spontaneous abortion when paternal genes are switched on (76), (iii) sperm with damaged DNA could lead to poor quality of blastocysts, sub-optimal pregnancy rates, and an increase in miscarriage (22), (iv) fertilization of oocyte with sperm damaged by ROS (seen during inflammation) could be a possible cause of neonatal cancers in the offspring (9). However, it is still not clear if this is due to the direct effects of leukocyte infiltration-induced inflammation or is a result of ROS-induced apoptosis (76-78). Despite the number of studies which show the detrimental effect of inflammation in the male reproductive tract on ART, currently there are no definite methods to overcome this setback and subjects are thus advised against ART till they are normal.

8. CONCLUSIONS

Inflammation of the male reproductive tract is mediated chiefly by cytokines like TNF-alpha and Interleukins. In the testis, inflammation affects Sertoli cells (causing a loss of blood-testis barrier function and immune-privilege status) and Leydig cells (causing a decrease in testosterone production) and resulting in "arrested spermatogenesis". Seminal contamination by pro-inflammatory agents impairs sperm morphology and physiology leading to sub/ infertility. The use of assisted reproductive technologies could give sub/ infertile men suffering from infection-induced inflammation a chance for a successful fatherhood. However, a number of long-term and holistic studies are needed before such therapies can be prescribed.

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