Mechanisms and effects of male genital tract infection on sperm quality and fertilizing potential: the andrologist’s viewpoint

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There are several mechanisms acting in synergism that can impair sperm characteristics of patients with accessory gland infection. In some cases, conventional sperm variables are disturbed with oligo and/or asthenozoospermia. In other patients, these sperm variables may appear normal, but the functional capacity of spermatozoa may be impaired. In particular, changes in the composition of the sperm membrane may result in reduced acrosome reactivity and capacity to fuse with the oolemma, and oxidative damage of the sperm DNA may induce mutagenesis. Changes in the biochemical make-up of seminal plasma can also reduce the in-vivo fertilizing capacity of spermatozoa, and infection-related disruption of the blood–testis barrier can induce the generation of anti-sperm antibodies and immunological infertility. Many of these functional abnormalities will not become evident upon ‘basic semen analysis’, which explains why some authors are unable to link infection of the accessory sex glands to subfertility. Also, functional and anatomical damage acquired as a result of infection is often permanent and not reversible by (antibiotic) treatment. Clearly, there are many more aspects of male accessory gland infection that require investigation. Available data should stimulate clinicians to place more emphasis on the prevention of infection-related infertility than on its treatment, as the latter is often unsuccessful.

Key words: anti-sperm antibodies/cytokines/fertility/reactive oxygen species

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Introduction

The andrologist is confronted with male accessory gland infection (MAGI) as a common occurrence in subfertile men (WHO, 1987). Although the prevalence of MAGI among men with abnormal semen quality varies widely in different regions of the world (Rowe, 1988), it is generally accepted to cause couple infertility (Rowe et al., 1999). This is related to possible direct effects of MAGI on the fertilizing capacity of the spermatozoa, but is probably also due to effects of male genital tract infection on the female partner (Eggert-Kruse et al., 1997).

Some controversies remain regarding this point of view (Tomlinson et al., 1993), but these may be related to problems in defining the diagnosis of MAGI and the fact that antibiotic treatment of infertile men with MAGI may not always restore fertility (Comhaire et al., 1986; Branigan and Muller, 1994), rather than to disagreement on the impact of the disease in terms of the biochemistry and functional quality of the spermatozoa. In addition, infection of the male genital tract rarely affects the testis, and this has been ascribed to its immune-privileged situation (Mahl-Brown et al., 1988; Bellgrau et al., 1995).

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The ‘primum movens’ and the role of cytokines

The initiators of infection are pathogens (Wilson et al., 1998) which may originate from either the urinary tract or be sexually transmitted (Ness et al., 1997). This has long been known, and among urinary invaders the usual pathogens are commonly found: Escherichia coli, Proteus species, Klebsiella and Streptococcus group D (Comhaire et al., 1980). In European countries, sexually transmitted diseases with gonococcus have become rare, and the prevalence of infection with Chlamydia (trachomatis) seems to present an increasing trend, though detection is difficult (Wolff et al., 1994). The role of mycoplasma remains controversial (Kalugdan et al., 1996).

Cytokines play an important role in the immunoinflammatory mechanisms which underlie the host response to infection, the most appropriate subdivisions being those that modulate leukocytes to produce proinflammatory responses, and those that have the capacity to downregulate inflammatory cells. However, it is now realized that cytokines rarely, if ever, act in isolation but rather act to induce (or inhibit) other cytokines, creating a population or network of cytokines to which cells respond (Wilson et al., 1998).

The infiltrating pathogen stimulates the production of interleukin 8 (IL-8) by macrophages (Yoshimura et al., 1987). This cytokine has been reported to exert a negative effect on the fertilizing potential of spermatozoa (Rajasekaran et al., 1995), and others consider it as a potential marker for the diagnosis of MAGI (Koumantakis et al., 1998).

Tissue damage caused by the infection provokes an inflammatory reaction, and stimulates the generation of interleukin 1 (IL-1) which induces several effects in the surrounding environment (Arend and Dayer, 1994). It stimulates polymorphonuclear neutrophils which secrete, among other substances, IL-8 and reactive oxygen species (ROS). IL-1 also stimulates macrophages, which are the major source of IL-8 but also secrete IL-6 and hepatocyte growth factor (HGF) (Depuydt et al., 1998). IL-6 interacts with B lymphocytes that become antibody-producing cells (Hirano et al., 1990); the latter are directed against the invading pathogens, but can interfere with sperm function (Figure 1) (Depuydt et al., 1998).

It has been documented that particular cytokines, e.g. IL-6, can cause membrane damage (Yamauchi-Takahara et al., 1995), which may decrease the functional capacity of spermatozoa.
Over the past few years, various growth factors and cytokines have been measured in seminal plasma: HGF, IL-1α, IL-1β, IL-2, IL-6, IL-8, IL-10, tumour necrosis factor (TNF)α, soluble TNF (sTNF) receptor types I and II, soluble receptors (sR) sR IL-2, sR IL-6, and IL-1 receptor antagonist (RA) (Hussein et al., 1993; Rajasekaran et al., 1995; Depuydt et al., 1996; Gruschwitz et al., 1996; Huleihel et al., 1996; Dousset et al., 1997; Denison et al., 1999). However, previous studies on the effects of cytokines and growth factors on sperm function have provided somewhat controversial outcomes (for a review, see Depuydt and Comhaire, 1998).

The development of appropriate cytokine networks to combat infections will depend on the nature of the infecting organism and on the genetic make-up of the individual. A number of cytokine genes have now been found to have polymorphisms in non-coding regions, which can control the rate of production of the cytokine. It is assumed that these different cytokine networks would render individuals more or less resistant to particular infections (Westendorp et al., 1997).

**White blood cells and reactive oxygen species**

Pathogens and tissue damage attract white blood cells (WBC) to the site of infection. The phagosomes of polymorphonuclear granulocytes (PMN) contain the enzyme peroxidase that can generate highly aggressive oxygen species from hydrogen peroxide (H₂O₂), such as singlet oxygen (¹O₂), hypochlorous acid (HOCl), and hydroxyl (OH⁻) and superoxide (O₂⁻) radicals. These are essentially aimed at destroying the pathogen in the phagolysosome, but they can leak out of the (disintegrating) PMN into the extracellular environment. It is these oxygen radicals (also called ROS) and nitric oxide (NO) (Rosselli et al., 1995) that cause damage to the surrounding tissues. The ROS are counteracted by antioxidants that are normally present in the seminal plasma (Alvares et al., 1987; Zini et al., 1993) and in fluids secreted along the genital tract. In normal circumstances, there is an equilibrium between the generation of ROS and antioxidants (Alvarez and Storey, 1989; Kovalski et al., 1992; Griveau and Le Lannou, 1997), leaving enough ROS available as is required to bring about fusion with the oocyte membrane (Aitken et al., 1993; Salgo et al., 1993).

Similarly, membrane fusion occurring at the time of the acrosome reaction requires the availability of a critical amount of ROS (de Lamirande and Gagnon, 1993b; Chernomordik et al., 1995). In case of WBC activation or infiltration, the amount of ROS generated is increased and it cannot be counterbalanced by the antioxidant capacity of the genital fluids (de Lamirande and Gagnon, 1993a). The latter may, in fact, be decreased because of the functional impairment of the accessory sex glands brought about by the infection (Lewis et al., 1995, 1997).

The excessive ROS exerts major destructive effects on both the sperm membrane and the sperm DNA. The sperm membrane is rich in polyunsaturated fatty acids (PUFA) (Zalata et al., 1998a), which makes it highly sensitive to ROS. This high content of PUFA is necessary for optimal membrane fluidity required for tail movement, but also for the acrosome reaction and membrane fusion (Aitken et al., 1993). Chemical systems that generate ROS—such as the xanthine oxidase system—and oxidative agents such as Fe²⁺ (Shivaswamy et al., 1993) change the phospholipid composition of the sperm membrane, with decreased PUFA and increased saturated fatty acids, thus reducing membrane fluidity and function (Zalata et al., 1998b). Simultaneously produced primary lipid peroxidation products decompose into secondary oxidation products, some of which are detectable as thiobarbituric acid-reactive substances (TBARS) (Aitken et al., 1993; Zalata et al., 1998b).

Similar changes are observed if spermatozoa are exposed to excess ROS generated by WBC, and spermatozoa of patients with infection of the accessory sex glands were found to have reduced PUFA but increased saturated fatty acids composition of membrane phospholipids, lower membrane fluidity and higher concentrations of TBARS (Zalata et al., 1998a). Although these changes do reduce the acrosome reactivity and fusogenic capacity of the spermatozoa, they do not seem to influence their conventional characteristics.

Indeed, patients with infection of the accessory sex glands do not usually present with lower sperm concentration, poorer motility and increased abnormal morphology than do men with other causes for sperm deficiency (Yanushpolsky et al., 1996). In contrast, the presence of a high number of WBC (Zalata et al., 1998a) and E.coli (Diemer et al., 1996) may reduce the linear velocity of spermatozoa in men with asthenozoospermia by depleting adenosine triphosphate (ATP) (de Lamirande and Gagnon, 1992a,b).

Oxidative stress caused by excess ROS and by lipid oxidation products also affects membrane proteins, with changes of the tertiary structure and expression of certain receptors (e.g. the c-met receptor for HGF) (Depuydt et al., 1998), and of membrane transport proteins, with resulting disturbance of ionic balance. The function of certain enzymes with SH-groups may also be altered by oxidation of the latter.

Mature spermatozoa have almost no cytoplasm and therefore are particularly sensitive to the damaging effects of ROS on their DNA. This then causes DNA fragmentation that can be made visible by means of the modified alkali single cell gel electrophoresis (COMET) assay (Hughes et al., 1996). Also, 2-deoxyguanosine, a normal DNA base, is oxidized to 8-OH-2-deoxyguanosine (8-OH-2-dG), resulting in damaged DNA. Whereas the former nucleotide binds to cytosine, the latter will form a base-pair with thymine during DNA replication. Thymine will bind with adenosine, so that a point mutation is introduced in the DNA (Cheng et al., 1992). The extent of such DNA damage will depend on the degree of oxidative stress, and can reliably be estimated by measuring the proportion of 8-OH-2-dG in sperm DNA (Fraga et al., 1991).
DNA mutation induced by oxidation probably has little importance in case of natural conception, intrauterine insemination and conventional in-vitro fertilization (IVF), as the oxidative changes to the sperm membrane will strongly reduce the probability of spermatozoa-oocyte fusion and fertilization. In case of intracytoplasmic sperm injection (ICSI), however, the steps of the acrosome reaction and membrane fusion are bypassed, and the damaged DNA is introduced directly into the oocyte. It will depend on the capacity of DNA repair of the oocyte whether or not mutation-related damage will be expressed in the embryo. In case the DNA repair capacity of the oocyte is surpassed, oxidation-induced damage may potentially result in minor gene defects such as deletions in the Y-chromosome (Vogt, 1998). These may ‘come to expression’, whereas this would perhaps be avoided by minimizing such oxidative DNA damage. Some aspects of sperm dysfunction may thus be counteracted by antioxidants either added to the semen or within the semen (in vitro (Oeda et al., 1997) or given orally (Lenzi et al., 1993; Kessopoulou et al., 1995; Tarin et al., 1998), even in case of persistence of leukocytospermia.

**Infection-related impairment of accessory gland function**

Tissue damage caused by infection and inflammation can impair the secretory function of the accessory sex glands, namely the epididymis, the prostate and the seminal vesicles.

Functional deficiency of the epididymis may cause poor sperm motility (asthenozoospermia), and that can be evidenced by measuring the secretory products of these glands in seminal plasma. Epididymal markers that have been suggested are glycerylphosphorylcholine, l-carnitine and alpha-glucosidase (Garcia-Diez et al., 1992). The latter seems to provide the most reliable information, and it can be measured by means of a simple colorimetric test (Episcreen; FertiPro, Beernem, Belgium) (Mahmoud et al., 1998). The activity of alpha-glucosidase in semen is decreased in case of obstruction of sperm transport between the epididymis and the ejaculatory duct (Fourie et al., 1998), in hypoandrogenism, and during or after infection/inflammation.

Impaired secretion of the seminal vesicles results in a decreased volume of the ejaculate (<2 ml) and diminished concentration of fructose in seminal plasma. The ejaculate volume has the highest power to assess seminal vesicular function in the semen of men with or without infection (Comhaire et al., 1989). Neither the measurement of fructose concentration nor the calculation of the total fructose content per ejaculate increases the discriminatory accuracy over that of the ejaculate volume. Therefore, measurement of fructose has little value for andrological diagnosis.

The prostate is often functionally affected in accessory gland infection. This results in decreased secretion of the proteolytic kallikrein-like enzyme prostate-specific antigen (PSA) (Shibata et al., 1997) with decreased liquefaction of seminal plasma. Increased viscosity or non-liquefaction of seminal plasma are significantly more common in ejaculates of patients with abnormal sperm characteristics and infection, than in men with increased number of WBC in semen but normal sperm characteristics, or in men with normal semen (WHO, 1987).

Furthermore, in accessory gland infections the secretion of other enzymes, such as acid phosphatase and gamma-glutamyltransferase is decreased. The concentration of citric acid is diminished, causing the pH of semen to increase. In semen, the best markers of prostate function are the total output of citric acid and gamma-glutamyltransferase per ejaculate (Comhaire et al., 1989). In addition, semen of men with accessory gland infection contains lower concentrations of the bivalent ions of calcium and zinc, the latter ion having been shown to be involved in chromatin stability and DNA condensation (Kvist and Bjorndahl, 1985; Kjellberg et al., 1992; Kundu and Rao, 1995).

The prostate fluid of normal men exerts inhibition of lymphocyte-mediated antibacterial activity (De Simone et al., 1988), but also has a bacteriostatic effect which is correlated with the concentration of muramidase in semen. Patients with chronic prostatitis have a lower muramidase activity in seminal plasma, and the bacteriostatic effect is decreased (Comhaire et al., 1980). As a result, these patients are more susceptible to recurrence of infection of the genitourethral tract.

**(Sub)obstruction of sperm transport and anti-sperm antibodies**

Both acute and chronic infection and/or inflammation, especially of the epididymis, may cause partial or complete obstruction of sperm transport with, respectively, oligozoospermia or azoospermia. Also, pressure-induced tears of the distal segments of the epididymal duct or ductuli efferentes may occur and the blood–testis barrier can be breached (Witkin, 1988). The immunological defence will then be activated and production of anti-sperm antibodies initiated (Dondero et al., 1984; Munoz and Witkin, 1995; Witkin et al., 1995). First, IgM antibodies will be produced, but these are not secreted into the genital tract because their size is too large to pass the epithelial barrier. Shortly afterwards, antibodies of the IgG class appear, and these can enter the genital tract. The anti-sperm antibodies of the IgG class come into contact with the spermatozoa, and attach to these (Nikolaeva et al., 1993). In some cases—and more commonly indeed during infection—secretory IgA anti-sperm antibodies are produced locally in the genital tract (probably the epididymis) (Meinertz et al., 1991). Antibodies of the IgA class can then be detected on the ejaculated spermatozoa, but not in serum, and this is associated with an additional reduction of their fertilizing capacity. Methods for the detection of anti-sperm antibodies include the Immunobead test (Dondero et al., 1991; Haas et al., 1991) and the Latex mixed antiglobulin reaction (MAR) test (Jager et al., 1978; Vermeulen and Comhaire, 1983)
(SpermMar), both of which can be used directly on semen and indirectly on serum. It has been documented the Latex MAR test is more sensitive and specific than the Immunobead test, and that the Immunobead test for IgA class anti-sperm antibodies may give false-positive results (Andreou et al., 1995).

Much attention has also been given to the role of immunomodulators in seminal plasma as a protection in the presence of infection (for a review, see Kelly, 1999).

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