Infections in the male genital tract and reactive oxygen species

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In the male genital tract, reactive oxygen species (ROS) are generated by spermatozoa and leukocytes including neutrophils and macrophages. ROS are involved in the regulation of sperm functions such as capacitation and the acrosome reaction. Infections lead to an excessive ROS production, resulting in an ‘oxidative burst’ from neutrophils/macrophages as a first-line defence mechanism. This is modulated by several cytokines and the pro-oxidant mechanisms of bacteria and viruses. At the site of an infection, the degree of activation of leukocytes, i.e. the amount of ROS produced, and the available antioxidative systems determine whether spermatozoa are damaged or not. During an infection, an imbalance of pro- and antioxidants favouring the former results in oxidative stress which impairs the sperm functions mentioned, as well as motility and fertilization. ROS produced during infections of the testis and epididymis are especially harmful to spermatozoa due to the longer contact time and the lack of antioxidant protection. In the final ejaculate, only very high numbers of ROS-producing leukocytes are detrimental to sperm functions. An infectious injury involving ROS in the prostate gland, seminal vesicles or epididymis could impair sperm functions indirectly. Pro- and antioxidative properties of therapeutics are currently receiving more attention as part of anti-infectious therapies. At present, there are many unresolved questions concerning the exact role of ROS during infections of the male genital tract because of the difficulty of specifically assessing the site of generation and the short-lived effects of ROS. New techniques may enable specific studies to fill this gap in the near future.

Key words: lipid peroxidation/male infertility/oxidative stress/oxygen free radicals/sperm dysfunction

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Introduction

Infections can be defined as the active or passive invasion of micro-organisms into a macro-organism where they attach, multiply and induce a local or generalized reaction. The reactions brought about by different germs in testis, epididymis, prostate gland and seminal vesicles, as well as their impact on sperm functions, are the topics of other articles in this volume. The objective of this review is to discuss the possible role of reactive oxygen species (ROS) in the context of male genital tract infections.

Reactive oxygen species

Oxygen is essential for human life. Most of the body’s energy is produced by the enzymatically controlled reaction of oxygen with hydrogen in oxidative phosphorylation occurring in the mitochondria during oxidative respiration. In well-controlled reaction steps, hydrogen is provided in the form of reducing equivalents (nicotinamide adenine dinucleotide, NADH) and the energy produced is conserved in the form of high-energy phosphates (adenosine triphosphate, ATP). A four-electron reduction of molecular oxygen to water involving cytochrome oxidase occurs in the mitochondria. During this stepwise,
enzymatic reduction of oxygen, free radicals such as superoxide anion and hydroxyl radicals, as well as activated oxygen species such as hydrogen peroxide, are formed (Fridovich, 1976, 1978).

Free radicals are short-lived reactive chemical intermediates which contain one or more electrons with unpaired spin. In order to overcome this state of an unpaired electron, these products participate in hydrogen abstraction, bond scission, radical addition and annihilation reactions. Therefore, they are highly reactive and oxidize lipids in membranes, amino acids in proteins and carbohydrates, damage nucleic acids, and depolymerize hyaluronic acid. The term reactive oxidants or ROS refers to all free radicals or activated oxygen species, which may cause oxidative injury (Table I). ROS-generating processes were found to be key components in processes such as inflammation, ischaemia–reperfusion injury, ageing and carcinogenesis (Halliwell and Gutteridge, 1989; Sies, 1991; Fuchs et al., 1997).

It must be stressed that, in vivo, the reactions of these short-lived molecules are coupled in several cycles, such as the Haber–Weiss and Fenton reactions, generating highly reactive hydroxyl radicals (Figure 1). A clear attribution of biological effects to a single radical therefore is hardly possible.

At low concentrations, reactive oxidants have biopositive effects and act selectively. They are metabolic intermediates in the metabolism of prostanoids (Lands, 1985), in the regulation of vasotonus (Ignarro, 1990), in gene regulation, e.g. activation of nuclear transcription factor kappa B (NF-κB) (Schreck et al., 1992), in the regulation of cellular growth, and in the function of intra- as well as intercellular signalling and other types of signal transduction (Saran and Bors, 1989; Demple and Amabile-Cuevas, 1991; Joseph and Cutler, 1994). Furthermore, they are involved in antimicrobial defence and immunological surveillance, i.e. neutrophil oxygen burst and macrophage cytotoxicity (Test and Weiss, 1986; Klebanoff, 1992). At high concentrations, ROS react unspecifically and exert bionegative effects and damage all major classes of biomolecules, i.e. unsaturated lipids in membranes (Kappus, 1986), proteins (Pacifici and Davies, 1990), nucleic acids (Ames, 1989) and carbohydrates (Harris et al., 1971).

In order to prevent cellular damage from physiologically produced ROS, or during pathological conditions, these reactive oxidants are usually limited by compartmentalization to a strictly controlled microenvironment, or are counterbalanced by antioxidants. An imbalance of oxidants and antioxidants in favour of the former (Sies, 1991), the occurrence of peroxidation products (Spiteller, 1993), and subsequent pathological sequelae (Janssen et al., 1993) is termed ‘oxidative stress’.

Transition metal ions

Transition metal ions, in particular iron and copper, catalyse the activation of molecular oxygen in biological systems.
Infections in the male genital tract and ROS

They accelerate autoxidation of various small molecular substances, are catalysts for the Fenton reaction, and decompose lipid hydroperoxides to peroxyl radicals. In spermatozoa, ROS initiate lipid peroxidation which results in the formation of lipid hydroperoxides. These may be cleaved by phospholipase A2 and metabolized to the corresponding alcohol by glutathione peroxidase or, in the presence of transition metal ions, to peroxyl and alkoxyl radicals which propagate the lipid peroxidation cascade (Aitken and Buckingham, 1993; Alvarez and Storey, 1995; Godeas et al., 1996).

Iron—the most important transition metal ion in humans—is bound to proteins (transferrin in plasma, lactoferrin in body fluids, ferritin and haemosiderin in tissues). As these proteins are only 30% saturated with iron, the concentration of free iron in human blood is virtually zero. In pathological conditions such as inflammation, acidosis or tissue disruption, the concentrations of redox-active metal ions is increased (Boyer and McCleary, 1987; Halliwell and Gutteridge, 1990). In these situations, superoxide releases iron from ferritin, thereby potentiating the formation of other radicals such as the hydroxyl radical (Biemond et al., 1984).

### Table I. Reactive oxygen species, including all free radicals and reactive oxidants

<table>
<thead>
<tr>
<th>Species</th>
<th>Generation</th>
<th>Half-life</th>
<th>Travelling distance</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyl-radical (OH(^{-}))</td>
<td>Metal ion-dependent breakdown of hydrogen peroxide</td>
<td>0.3 ns</td>
<td>1.8 nm</td>
<td>Very strong oxidant</td>
</tr>
<tr>
<td>Superoxide anion radical (O(_2^{-}))</td>
<td>In mitochondria/endoplasmic reticulum during electron transport; phagocytic cells during respiratory burst</td>
<td>0.4 µs–1 ms</td>
<td>55 nm–3 µm</td>
<td>Reducing or oxidizing dependent on the redox properties of its reactant; biological effect due to: a) dismutation to hydrogen peroxide and dismutation to hydroxyl radicals; b) formation of thyl radicals by reaction with endogenous thiol groups; c) formation of peroxynitrite</td>
</tr>
<tr>
<td>HO(_2^{-})</td>
<td>Protonated form of superoxide</td>
<td></td>
<td></td>
<td>Can cross biological membranes</td>
</tr>
<tr>
<td>NO(^{-})</td>
<td>Reaction product of arginine with NO synthase</td>
<td></td>
<td></td>
<td>Transmitter substance, reacts with superoxide anion radical to peroxynitrite (OONO(^{-}))</td>
</tr>
<tr>
<td>Lipid radicals</td>
<td>Abstraction of an allylic proton from a polyunsaturated lipid</td>
<td>1–10 s</td>
<td></td>
<td>Propagation of chain reaction in lipid membranes</td>
</tr>
<tr>
<td>Thyl radicals (RS •)</td>
<td>a) Non-enzymatic reaction of thiols with free radicals</td>
<td></td>
<td></td>
<td>Potential source of lipid oxidation</td>
</tr>
<tr>
<td></td>
<td>b) By xanthine oxidase, prostaglandin synthase, horseradish peroxidase, xenobiotic metabolism</td>
<td></td>
<td></td>
<td>Reaction with protein thiols to form disulphides via disulphide anion radicals</td>
</tr>
<tr>
<td>Hydrogen peroxide (H(_2)O(_2))</td>
<td>Mitochondria during oxidative phosphorylation; in peroxisomes in fatty acid metabolism</td>
<td>Persistent</td>
<td></td>
<td>Reaction with transition metal ions leads to hydroxyl radical (Fenton reaction)</td>
</tr>
<tr>
<td>Lipid hydroperoxides</td>
<td></td>
<td></td>
<td></td>
<td>Reaction with protein SH or NH(_2) groups; damage at more distant sites not directly exposed to lipid peroxidation possible</td>
</tr>
<tr>
<td>(degradation products: 2-alkenals, 4-hydroxy-alkenals)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxidizing halogens</td>
<td>Phagocytic cells, myeloperoxidase-catalysed halogenation (Br, Cl) of hydrogen peroxide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Singlet oxygen ((\text{O}_2^*))</td>
<td>Dismutation of superoxide anion radicals, reaction of hydrogen peroxide with hypochlorite</td>
<td>µs–ms</td>
<td></td>
<td>Combines chemically with a variety of biomolecules</td>
</tr>
</tbody>
</table>
In animal models, lead toxicity could be attributed to the effects of lead-induced generation of ROS (Hsu et al., 1998). Increased concentrations of metal ions were found in semen (Umeyama et al., 1986) or blood plasma (Stanwell-Smith et al., 1983) of infertile men in comparison with controls. It is unclear whether this increase was due to a higher concentration of binding proteins such as coeruloplasmin, or whether they were catalytically active and produced ROS. Transferrin (40 μg/ml) and ferritin (90 ± 68 ng/ml) were found in seminal plasma (Orlando et al., 1985; Kwenang et al., 1987). Recent studies demonstrated that seminal plasma contained several defence mechanisms against iron-mediated damage, one of those being citrate, while copper ions in physiologically relevant concentrations (1 μM) resulted in catalytically active copper concentrations leading to oxidative processes (Menditto et al., 1997). The relevance of these mechanisms could be demonstrated in vitro as some culture media, such as Ham’s F 10, which was supplemented with ferrous ions, could promote peroxidative damage in human spermatozoa (Gomez and Aitken, 1996).

**Physiological occurrence of ROS**

The generation of ROS per se occurs physiologically during normal cell metabolism. Cytosolic autoxidation of small molecular compounds includes the autoxidation of products of cell metabolism such as hydroquinones, catecholamines, thiols, flavins and tetrahydropterins, as well as the transition metal ion-catalysed cytosol monosaccharide autoxidation. Furthermore, cytosolic oxygenases such as xanthine oxidase, dihydro- orotate or flavoprotein dehydrogenases may produce superoxide anion radicals, subsequently hydrogen peroxide and the hydroxyl radical (Fuchs et al., 1997).

Mitochondrial respiration is the main biological source of superoxide anion radicals under physiological conditions (Chance et al., 1979). During the tetravalent reduction of oxygen to water by the mitochondrial cytochrome c oxidase, these radicals can leak into the cell.

\[
e^- e^- e^- e^- \rightarrow O_2 \rightarrow O_2^- \rightarrow H_2O_2 \rightarrow OH^- \rightarrow H_2O
\]

\[
2H^+ H^+ H^+
\]

Peroxisomes contain hydrogen peroxide-generating oxidases. The endoplasmic reticulum reduces oxygen to active oxygen intermediates, which in turn oxidize other substrates.

The activation of plasma membrane-bound oxidases, i.e. NADPH oxidase, cause the oxidative burst. This NADPH oxidase/myeloperoxidase system is found in inflammatory cells, which are activated by the phagocytosis of foreign materials, such as infectious agents. ROS generated by phagocytes are both defensive and noxious (Muller-Peddinghaus, 1987; Hakim, 1993).

**Formation of ROS under pathological conditions**

The following mechanisms may lead to a pathological production of ROS: ionizing radiation, solar irradiation, bioactivation of xenobiotics, inflammatory cells, increased cellular metabolism, activation of oxidases and oxygenases, disturbed endogenous electron flow, decompartmentalization of transition metal ions, and loss of antioxidant capacity. Furthermore, it was postulated that certain toxic chemicals may be generated during the metabolism of drugs and industrial chemicals by one-electron peroxidase oxidations or by cytochrome P<sub>450</sub> metabolism. These reactive oxidants may induce chronic inflammatory diseases states which could be misinterpreted as microbial infections (Parke and Sapota, 1996).

In the context of infections, either the pathogen itself may induce an increased generation of ROS (Hong et al., 1998) or the invading inflammatory cells could generate ROS during the respiratory burst, consuming the antioxidants present. Studies investigating these aspects in the male genital tract are still lacking.

**Antioxidative protection**

Antioxidants include water-soluble compounds, such as ascorbate (mainly extracellularly) and glutathione (intracellularly) (Frei et al., 1990; Reed, 1990; Ochsendorf et al., 1998), the lipid-soluble, membrane-bound antioxidants tocopherol and ubiquinol/ubiquinone (Burton and Ingold, 1989), extracellular superoxide dismutase (SOD) in epididymis and prostate (Williams et al., 1998), the intracellular enzymatic antioxidants SOD, glutathione peroxidase, glutathione reductase and catalase, and finally ancillary systems that prevent the formation or metabolism of pro-oxidants, such as NADPH ubiquinone reductase, glutathione S-transferases or glucose 6-phosphate dehydrogenase (Kehrer and Lund, 1994; Gopalakrishnan and Shaha, 1998). The principal modes of action may be divided into three stages. First, antioxidants may directly scavenge the ROS produced (prevention). Secondary reactions interfere with processes already initiated by ROS (interception). Examples are interruptions of already occurring chain reactions, such as lipid peroxidation by tocopherol. If steady-state free radical concentrations exceed this threshold, this will eventually lead to autocatalytic cell injury. The third line of defence, cell renewal, does not apply to spermatozoa. However, it could be relevant in the testis or epididymis if ROS produced locally would lead to oxidative injury. During these reactions the antioxidants interact in a complex interplay. Besides synergistic antioxidant activities, the enzymatic, water- and lipid-soluble antioxidants can regenerate oxidized antioxidants through the coupling of several reaction cycles (Buettner, 1993; Freisleben and Packer, 1993).
In summary, antioxidants are effective in protecting biological tissues below a critical threshold of ROS. A thorough review of these antioxidants, and especially in reproductive organs, can be found elsewhere (Yu, 1994; Ochsendorf et al., 1997b).

Measurement of oxidative stress

The direct detection of ROS in vivo is difficult as reactive oxidants are in general very short-lived intermediates (Table I). Direct detection of free radicals is possible by electron paramagnetic resonance spectroscopy (Swartz et al., 1972). To detect short-lived radicals, such as alkoxy or peroxy radicals, these measurements must be performed at low temperatures. To my knowledge, no studies using this methodology have been performed in human male reproductive organs. All other methods used indicate only indirectly the existence of ROS. An overview of these methods is given in Table II (Weber, 1990; Saran and Bors, 1991). A discussion of the specific advantages or difficulties of these tests is beyond the scope of this article. However, the multitude of tests advocated illustrates the difficulty of assessing these reactions. It must be borne in mind that the methods which provide indirect proof of radical participation in an in-vivo process lack specificity, as the radical chain responsible for the end products may have branched into different directions, depending on the local circumstances within the cells (Saran and Bors, 1991).

Table II. Synopsis of methods used to detect oxygen radicals (ROS). (Modified after Saran and Bors, 1991.)

<table>
<thead>
<tr>
<th>Methods</th>
<th>Reference(s)</th>
</tr>
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<tbody>
<tr>
<td>I. Direct measurements</td>
<td></td>
</tr>
<tr>
<td>Pulse radiolysis</td>
<td>Asmus (1984)</td>
</tr>
<tr>
<td>Electron-spin resonance spectroscopy</td>
<td>Buettner and Mason (1990)</td>
</tr>
<tr>
<td>II. Indirect methods</td>
<td></td>
</tr>
<tr>
<td>a) Detection of free radicals in vivo</td>
<td></td>
</tr>
<tr>
<td>Chemiluminescence (luminol, lucigenin)</td>
<td>Murphy and Sies (1990)</td>
</tr>
<tr>
<td>Reduction of cytochrome-c</td>
<td>Fridovich (1985)</td>
</tr>
<tr>
<td>Production of formazan from nitroblue-tetrazolium salts</td>
<td>Auclair and Voisin (1985)</td>
</tr>
<tr>
<td>Production of nitrite from hydroxylamine</td>
<td>Bors et al. (1977)</td>
</tr>
<tr>
<td>Cleavage of methionine or keto-methyl butyric acid to ethylene</td>
<td>Saran et al. (1980)</td>
</tr>
<tr>
<td>Decarboxylation of 7,14C-benzoic acid</td>
<td>Winston and Cederbaum (1985)</td>
</tr>
<tr>
<td>Deoxyribose degradation</td>
<td>Halliwell and Gutteridge (1985)</td>
</tr>
<tr>
<td>Production of sulphnic acid from DMSO</td>
<td>Babbs and Steiner (1990)</td>
</tr>
<tr>
<td>Oxidation of adrenaline</td>
<td>Bors et al. (1975)</td>
</tr>
<tr>
<td>Bleaching of carotenoids</td>
<td>Bors et al. (1984)</td>
</tr>
<tr>
<td>Electrochemical detection</td>
<td>Valentine et al. (1984)</td>
</tr>
<tr>
<td>b) Detection of chemical end-points</td>
<td></td>
</tr>
<tr>
<td>Hydroperoxides (determined with GC-MS, HPLC, chemiluminescence, fluorescence)</td>
<td>Yamamoto et al. (1990), Van Kuijk et al. (1990)</td>
</tr>
<tr>
<td>Aldehydes (i.e. TBARS)</td>
<td>Esterbauer and Cheeseman (1990)</td>
</tr>
<tr>
<td>Gaseous breakdown products (ethane, pentane)</td>
<td>Lawrence and Cohen (1984)</td>
</tr>
<tr>
<td>Cholesterol epoxides and hydroperoxides</td>
<td>Ansari and Smith (1990)</td>
</tr>
<tr>
<td>Oxidized proteins</td>
<td>Levine et al. (1990)</td>
</tr>
<tr>
<td>DNA-base degradation products</td>
<td>Dizdaroglu and Gajewski (1990)</td>
</tr>
<tr>
<td>DNA fragments, chromosome damage</td>
<td>Emerit (1994)</td>
</tr>
<tr>
<td>c) Detection of biological end-points</td>
<td></td>
</tr>
<tr>
<td>Cellular level (in vitro)</td>
<td></td>
</tr>
<tr>
<td>Viability</td>
<td>Denekamp and Rojas (1989)</td>
</tr>
<tr>
<td>Morphological and metabolic parameters (e.g. by flow cytometry or fluorescence staining and microscopy)</td>
<td>Menzel et al. (1990)</td>
</tr>
<tr>
<td>Membrane properties (potential, fluidity) (e.g. by electrode techniques, spin or fluorescence labelling)</td>
<td>Fuchs et al. (1990)</td>
</tr>
<tr>
<td>Specific mRNA production, oncogene activation, gene amplification</td>
<td>Storz et al. (1990)</td>
</tr>
<tr>
<td>Transformation studies</td>
<td>Borek (1988)</td>
</tr>
<tr>
<td>Organ level</td>
<td></td>
</tr>
<tr>
<td>Organ function measurement</td>
<td>Wendel (1990)</td>
</tr>
<tr>
<td>Oxystat techniques</td>
<td>De Groot (1990)</td>
</tr>
<tr>
<td>Perfused organ chemiluminescence</td>
<td>Murphy and Sies (1990)</td>
</tr>
<tr>
<td>Excretion of stable marker molecules</td>
<td>Shigenaga et al. (1990)</td>
</tr>
<tr>
<td>d) Inhibition studies to influence the generation of typical oxidation products</td>
<td></td>
</tr>
<tr>
<td>Addition of scavenger compounds [mannitol, formate, tert-butylic-alcohol, DMSO, butylated hydroxytoluene (BHT)]</td>
<td>Halliwell and Gutteridge (1989)</td>
</tr>
<tr>
<td>Addition of physiological antioxidants (tocopherol, ascorbate, thiols, SOD, catalase)</td>
<td>Halliwell and Gutteridge (1989)</td>
</tr>
<tr>
<td>Variation of the intracellular concentration of relevant cell constituents (e.g. glutathione by synthesis inhibition or by genetic engineering)</td>
<td>Allen et al. (1997)</td>
</tr>
</tbody>
</table>

DMSO = dimethyl sulphoxide; GS-MS = gas chromatography–mass spectrometry; HPLC = high-performance liquid chromatography; TBARS = thiobarbituric acid-reactive substances; SOD = superoxide dismutase.
With respect to infections of the male genital tract, only techniques which detect locally generated reaction products can be applied. This can be concluded from recent findings in patients with chronic prostatitis or leukocytospermia where the concentration of C-reactive protein, an indicator of bacterial infections, in seminal plasma did not correlate with its concentration in the blood (Ludwig et al., 1998). For studies investigating the role of oxidative stress in male infertility, two main approaches were used: ROS were determined by chemiluminescence or cytochrome-c reduction, and the degree of lipid peroxidation by the thiobarbituric acid method. Phorbol–myristate–acetate luminol chemiluminescence reflected the overall quality of the spermatogenic process only after removal of all contaminating leukocytes. In contrast, the level of lipid peroxidation induced by iron/ascorbate gave the same information, but did not depend on the removal of leukocytes (Gomez et al., 1998).

**Generation and relevance of ROS in the male genital tract**

As pointed out above, a direct measurement of ROS inside organs is not possible. However, there is ample indirect evidence that the impairment of sperm function by an excess generation of ROS or reduced antioxidative mechanisms is of pathophysiological importance as male infertility factor. The topic of an imbalance of pro- and antioxidants in male infertility has been reviewed elsewhere (Ochsendorf and Fuchs, 1993, 1997; Aitken and Fisher, 1994; Aitken, 1994, 1995; de Lamirande and Gagnon, 1994, 1995b; Sikka et al., 1995; Sharma and Agarwal, 1996; Griveau and Le Lannou, 1997b). Therefore, I will only briefly mention the main aspects of this oxidative stress. The reader is referred to these reviews for further details.

As early as 1943 it was observed that spermatozoa lose their motility more rapidly when incubated with oxygen (MacLeod, 1943). Due to a high content of polyunsaturated fatty acids, mainly docosahexaenoic acid (chain length: unsaturated double bonds, 22:6), the sperm plasma membranes are prone tooxidation (Jones et al., 1979; Storey, 1997; Zalata et al., 1998a). The occurrence and negative impact of this lipid peroxidation on sperm functions was clearly established by various groups (Jones et al., 1979; Alvarez et al.; 1987a; Aitken et al., 1993a,b; Griveau et al., 1995). Recent studies showed that peroxidative damage was the major cause of defective sperm motility in semen and the major contributor to complete motility loss in individual cells (Gomez et al., 1998).

In addition, ROS diminished intracellular ATP, which led to a reduction in axonemal protein phosphorylation and sperm immobilization (de Lamirande and Gagnon, 1992a,b). In recent years, DNA oxidation was also found to occur in spermatozoa (Hughes et al., 1996; Motchnik and Podda, 1997; Lopes et al., 1998; Twigg et al., 1998a). This may have an impact on intracytoplasmic sperm injection (ICSI) procedures where the normal functions of spermatozoa are no longer essential (Twigg et al., 1998c). Finally, ROS could impair the antioxidant mechanisms of the cells (Griveau et al., 1995).

Several studies using different techniques demonstrated an increased ROS generation in the ejaculates of infertile men, especially in patients with oligozoospermia (for example Iwasaki and Gagnon, 1992; Mazzilli et al., 1994). An excess generation of ROS was associated with loss of motility, the outcome of spermatozoa–oocyte fusion tests, and fertility (Aitken and Clarkson, 1987; Alvarez et al, 1987b; Aitken et al., 1991; de Lamirande and Gagnon, 1992a).

It was shown that ROS could be generated by spermatozoa or contaminating leukocytes. The reason for increased ROS levels was not a lack of antioxidants, but an excessive production of ROS in spermatozoa, especially from those with excess residual cytoplasm (Zini et al., 1993; Aitken et al., 1994a). As generating systems, a NAD(P)H oxidase located in the sperm plasma membrane (Aitken and Clarkson, 1987; Aitken et al., 1989, 1997; de Lamirande and Gagnon, 1995b; Leclerc et al., 1997), and a sperm diaphorase, integrated in the mitochondrial respiratory system, i.e. a NAD(P)H-dependent oxidoreductase in the midpiece, were discussed (Gavella and Lipovac, 1992). The former mechanism was supported by studies showing an increased generation of superoxide anions after addition of NADPH, which acts as electron donor. Evidence for the latter mechanism were: (i) a correlation of midpiece defects with increased membrane lipid peroxidation (Rao et al., 1989); (ii) retention of excess residual cytoplasm as a result of defective spermogenesis in the midpiece, resulting in enhanced cytoplasmic space (Aitken et al., 1994a; Gomez et al., 1996); and (iii) a negative correlation of the cytoplasmic enzymes lactic acid dehydrogenase, creatine phosphokinase and glucose 6-phosphate dehydrogenase, which stimulated the generation of NADPH as electron donor, with defective sperm functions (Aitken et al., 1994a, 1997; Casano et al., 1991; Huszar and Vigue, 1994). Increased activities of xanthine oxidase, which generate ROS, have been reported in spermatozoa of infertile men (Sanocka et al., 1996, 1997).

The ROS production rate of leukocytes was 1000 times higher than that of spermatozoa at capacitation (de Lamirande and Gagnon, 1995a). Accordingly, leukocytes were identified to be the main producers of ROS in semen (Aitken and West, 1990; Aitken et al., 1992, 1995a; Kessopoulou et al., 1992; Ochsendorf et al., 1997a; Hipler et al., 1998; Zalata et al., 1998b).

Some studies showed that NO, formed by reactions of L-arginine by the calcium-dependent and cytokine-inducible nitric oxide (NO) synthase decreased sperm motility (Rosselli et al., 1995; Weinberg et al., 1995; Perera et al., 1996). Therefore, reactions between superoxide anions (produced by neutrophils) and this NO, forming the highly reactive peroxynitrite, have also to be considered. Although the existence of NO synthase in mammalian testis was demonstrated, and the reaction mechanism well established, no
data on peroxynitrite in human semen or genital organs have yet been reported (Gagnon et al. 1998; Zini et al., 1998). To date, only an increased rate of peroxynitrite production has been determined in spermatic veins of patients with varicocele. This was due to an increased activity of NO synthase and xanthine oxidase, with resulting formation of NO and (possibly) superoxide and their highly reactive product, peroxynitrite (Mitropoulos et al., 1996).

In contrast to these damaging effects due to excessive generation of ROS, the role of ROS in signal transduction processes is one primary interest of current research.

**Molecular effects of oxidants during reproduction**

With respect to sperm function, experimental evidence is emerging that ROS are involved in several fundamental mechanisms of sperm physiology. At present, the precise mechanisms are under investigation, but there is evidence that the capacity for generation of ROS and antioxidative mechanisms change during epididymal transit (Fisher and Aitken, 1997; Tramer et al., 1998) and are part of the regulatory processes (Markey et al., 1998).

The data available show that an increase in ROS generation at the beginning of capacitation is followed by an increase in tyrosine phosphorylation. This suggests a link between ROS production and signal transduction processes. Besides ROS, other factors are involved and necessary for capacitation to occur, such as calcium, adenyl cyclase/cAMP, glucose and bicarbonate in the extracellular fluids (Bize et al., 1991; Griveau et al., 1994; Aitken et al., 1995b, 1996; Fraser, 1995; Aitken, 1997; de Lamirande et al., 1997, 1998a; Leclerc et al., 1997, 1998; de Lamirande and Gagnon, 1998). One essential event of capacitation appears to be the hyperactivation of spermatozoa which allows the latter to break free from epithelial binding and to penetrate the zona pellucida (de Lamirande et al., 1997). The acquisition of this form of motility was associated with the generation of superoxide anions and a progressive phosphorylation of tyrosine residues (Leclerc et al., 1996, 1997). Not only superoxide anions, but also low concentrations of NO, could promote human sperm capacitation (de Lamirande and Gagnon, 1993; Zini et al., 1995; Yeoman et al., 1998). Recently, the acrosome reaction was also associated with an extracellular superoxide anion generation of spermatozoa (de Lamirande et al., 1998b).

The induction of lipid peroxidation was associated with an increased binding of spermatozoa to both homologous and heterologous zona pellucida, an effect which could be reversed by the addition of vitamin E (Aitken et al., 1989; Kodama et al., 1996). Increased rates of spermatozoa-oocyte fusion could be induced by low concentrations of hydrogen peroxide, possibly by an increase of tyrosine phosphorylation (Aitken et al., 1995b). Likewise, increased hydrogen peroxide-scavenging capacities of spermatozoa were associated with poor fertilization rates in an in-vitro fertilization (IVF) programme (Yeung et al., 1996).

These effects could be reversed by the addition of membrane-impermeable antioxidants (SOD, catalase). Therefore, it was postulated that the ROS-target should be located at the external side of the sperm plasma membrane and subject to oxidation/reduction cycles. Possibly, external fluids or cells from the female reproductive tract may be responsible for these effects. However, it must be borne in mind that these processes are highly complex and of paramount importance for reproduction. A mere reduction in oxidative events seems improbable and, as in other cell types, a redundancy of the main pathways may be present (de Lamirande et al., 1997).

As the amount of superoxide anion produced by spermatozoa during capacitation is more than three orders of magnitude lower than that produced by activated neutrophils (de Lamirande and Gagnon, 1995a), it is apparent that during an infection these fine-tuned reactions may be disturbed and the unspecific oxidation of molecules may prevail.

**ROS in infections of the male genital tract**

*Generation of ROS during urogenital infections*  

As described in the Introduction, the invasion of micro-organisms leads to a defence reaction in the respective tissues, i.e. unspecific and specific immune reactions. An early and effective mechanism is the killing of microbes via the oxidative burst of polymorphonuclear leukocytes (PMNL) and macrophages (Roos, 1991; Döring and Wörlitzsch, 1995; Saran et al., 1999). As described, these cells are the main producers of ROS in the male genital tract, and their role in the context of male infertility is discussed controversially (Bar-Chama et al., 1994; Wolff, 1995; Fedder, 1996; Trum et al., 1998).

There are several independent studies indicating the generation of ROS in semen (Aitken and Clarkson, 1987; Aitken et al., 1989; D’Agata et al., 1990; Iwasaki and Gagnon, 1992; Mazzilli et al., 1994; Ochsendorf et al., 1994; Zalata et al., 1995a; Ford et al., 1997). Due to the significant correlation with leukocyte numbers, the origin of these ROS could be attributed to leukocytes, even in non-leukocytospermic samples (Aitken et al., 1992; Kessopoulou et al., 1992; Ford et al., 1997). The detection of ROS by chemiluminescence, a method often used for these studies, was therefore advocated as a reliable means of detecting leukocytospermia (Leino and Virkkunen, 1991).

Only a few studies related the high ROS concentrations with infection. In one study (Mazzilli et al., 1994), significantly elevated superoxide anion generation was found in patients with sperm cultures positive for aerobic bacteria (in comparison with the remaining study population and fertile controls). In another study, higher ROS generation was found in chronic non-bacterial inflammation (D’Agata et al., 1990),
though no correlation between ROS concentrations and the number of peroxidase-positive cells was described. The amount of ROS generated by spermatozoa after Percoll gradient separation was increased in patients with male accessory gland infections, and correlated to the leukocyte concentration in semen (Depuydt et al., 1998). It was demonstrated that the proportions of polyunsaturated fatty acids, the double bond index of docosahexaenoic acid in the sperm plasma membranes, were lower in samples with peroxidase-positive cells >1×10^6/ml than in samples <1×10^6/ml. The former samples showed lower concentrations of thiobarbituric acid-reactive substances (a marker of lipid peroxidation) after incubation with iron/ascorbate. This was probably due to the decreased availability of polyunsaturated fatty acids in the membrane after destruction by ROS generated by the leukocytes (Zalata et al., 1998b).

Spermatozoa of spinal cord-injured males show a marked reduced motility and function (Linsenmeyer and Perkasli, 1991). Urinary tract infections were reported to be the leading cause of impaired infertility in these patients (Tollon et al., 1997). However, in these men elevated ROS concentrations were also detected after Percoll separation in the sperm fraction (de Lamirande et al., 1994). Elevated ROS concentrations were negatively correlated with sperm motility, and were independent of the method of ejaculation (Padron et al., 1997).

**Generation of ROS by leukocytes and macrophages**

One problem which remains unsolved is that of the relevance of the leukocytes under normal conditions. It is well known that leukocytes are present in semen, albeit in varying numbers (Barratt et al., 1992). With respect to all non-sperm cells, the majority of these so-called ‘round cells’ consist of immature germ cells with <5% white blood cells under normal conditions (Eggert-Kruse et al., 1992a). The predominant cell types are granulocytes (50-60%), macrophages (20-30%) and T lymphocytes (2-5%) (Wolff and Anderson, 1988b; Fedder et al., 1993; Wolff, 1995). The former two cell types are powerful producers of ROS.

According to WHO criteria, leukocyte concentrations >10^6/ml are regarded as pathological, as former studies showed a correlation with the isolation of >10^9/ml pathogenic or non-pathogenic bacteria, a correlation with granulocyte elastase levels >1000 ng/ml, and an inflammation coefficient set up by Comhaire and co-workers (Comhaire et al., 1980; Jochum et al., 1986; Wolff and Anderson, 1988a; World Health Organization, 1992). Other studies reported adverse effects on biochemical (Depuydt et al., 1998) or clinical parameters, such as sperm motility or results of intrauterine insemination, if the number of peroxidase-positive cells exceeded 2×10^6/ml of semen (Milingos et al., 1996; Yanushpolsky et al., 1996) and for IVF if the number of leukocytes exceeded 6×10^6/ml ejaculate (De Geyter et al., 1994). Another study reported reduced sperm count, morphological quality and ability of spermatozoa to penetrate cervical mucus if the percentage of leukocytes in round cells exceeded 15%. In this study, no correlation was found with microbial colonization of semen samples (Eggert-Kruse et al., 1992a). Others concluded, based on their studies, that high elastase levels did not always reflect bacterial infection (Cumming et al., 1990; Eggert-Kruse et al., 1992b). Therefore, the relevance of leukocytospermia is discussed controversially (for an overview, see Wolff, 1995).

It was postulated that seminal leukocytes might have the physiological role of the removal of dead and/or abnormal spermatozoa, as this could be demonstrated to be the case in the human uterine cervix following insemination (Thompson et al., 1991; Tomlinson et al., 1992b).

Ample evidence was provided that leukocytes in the male genital tract could adversely affect sperm function, depending on the cell type, the site of inflammation, the immunological activation status of the leukocyte, i.e. with respect to granulocytes the amount of ROS produced, and the anti-inflammatory properties of the individual (Wolff, 1995; Fedder, 1996). It is obvious that it is hardly possible to determine these variables in vivo. In seminal plasma, spermatozoa are in contact with leukocytes from the time-point of ejaculation until entry into the cervix, a rather short time period. In addition, seminal plasma possesses several highly effective defence mechanisms that prevent cellular damage if the leukocyte numbers are not excessive. These include prostasomes, which interact with granulocyte membranes (Saez et al., 1998) and antioxidants (Ochsendorf et al., 1997b).

Due to the antioxidants present in spermatozoa and seminal plasma, such as SOD (Alvarez et al., 1987b; Williams et al., 1998), catalase (Jeulin et al., 1989), glutathione (Ochsendorf, 1998), glutathione peroxidase (Griveau et al., 1995), vitamin E (Thérond et al., 1996) or ascorbate (Thiele et al., 1995), damage of spermatozoa was prevented even in the presence of leukocyte counts >1×10^6/ml (Aitken et al., 1994b).

The following circumstances could, nevertheless, lead to sperm damage due to ROS generated by these leukocytes: higher leukocyte concentrations could overwhelm these defence mechanisms (Yanushpolsky et al., 1996), the first contact with ROS could occur in epididymis or testis during infection/inflammation without protecting antioxidants (see below), or if seminal plasma was removed during sperm preparation for assisted reproduction techniques.

There is evidence that the generation of hydrogen peroxide before IVF reduced the potential to promote embryo development (Kuribayashi and Gagnon, 1996). It was demonstrated that sperm functions were significantly impaired if repeated centrifugation was used before the separation of leukocytes from sperm suspensions. Centrifugation increased the levels of ROS by 20- to 50-fold (Aitken and Clarkson, 1988; Iwasaki and Gagnon, 1992). The removal of the seminal plasma leaves spermatozoa unprotected, leading to the impairment of motility (Plante et al., 1994; Baker et al., 1996).
Recently, it was demonstrated that DNA oxidation was also increased during these procedures (Twigg et al., 1998b). As a consequence, antioxidant supplementations during sperm preparation are presently under investigation (Griveau and Le Lannou, 1994; Baker et al., 1996; Kuribayashi and Gagnon, 1996; Parinaud et al., 1997; Armstrong et al., 1998; Hughes et al., 1998; Twigg et al., 1998a). However, as some reducing agents have an adverse potential, antioxidants cannot be used uncritically (Yu and Anderson, 1997).

The generation of ROS was dependent on the oxygen tension. Higher oxygen tensions increased ROS generation, mainly from leukocytes (Griveau and Le Lannou, 1997a; Whittington and Ford, 1998). Low oxygen tensions improved the survival rate and penetration capacity, especially of spermatozoa from oligozoospermic patients (Griveau et al., 1998), a finding which may be of clinical value in assisted reproduction programmes.

Different opinions exist on the clinical significance of the ROS detected. Some authors found ROS levels or leukocyte counts not to be prognostically helpful both for in-vivo (Tollinson et al., 1993) and in-vitro reproduction (Tollinson et al., 1992a), but according to others ROS were of discriminatory prognostic value both in vivo (Aitken et al., 1991) and in vitro (Krausz et al., 1994; Sukcharoen et al., 1994).

Finally, in the artificial situation of cryopreservation it could be shown that leukocyte counts >0.5×10⁹/ml were associated with increased ROS generation during cryopreservation (Mazzilli et al., 1995; Wang, A. et al., 1997b); however, this was not associated with increased lipid peroxidation (Wang, Y. et al., 1997).

Type and location of white blood cells

Different leukocyte subpopulations were detected in most parts of the male reproductive tract, both in health and disease. Monocytes/macrophages were present in most compartments, especially in the epididymis after vasostomy, but also in normal fertile men (Phadke and Phadke, 1961; El-Demiry and James, 1988; Barratt et al., 1990). In the testis, these cells produced superoxide anion radicals during experimentally induced bacterial infections (Wei et al., 1988). These experimental studies did not report the presence of granulocytes in the epididymis. It is unclear why these cells apparently did not participate in the inflammatory processes. The real situation in vivo is not known due to lack of available material. The data available showed a marked reduction in the numbers of seminal leukocytes after vasectomy. It was concluded therefore that seminal lymphocytes and macrophages appear to originate mainly from the epididymis and rete testis, while granulocytes seem to be contributed largely by the prostate and seminal vesicles (Holstein 1978; Wang and Holstein, 1983; Olsen and Shields, 1984; El-Demiry et al., 1985; El-Demiry and James, 1988; Anderson et al., 1990). The leukocyte reduction after vasectomy might be caused by the lack of chemoattraction through spermatozoa (McClinton et al., 1990).

In the prostate gland, the presence of leukocytes could be associated with inflammation, while secretions of the normal prostate very rarely contained granulocytes (Schaeffer et al., 1981). Although leukocytes in the prostate correlated with inflammation of the prostate (>40 leukocytes per microscopic field after massage), only the ejaculate volume was decreased in leukocytes in semens, as was the viability of cells after 3 h in one study (Colpi et al., 1988), while others found reduced motility, normal morphology and increased volume in chronic abacterial prostatitis (Leib et al., 1994). It was concluded that in leukocytes the prostate might be the major source of white blood cells in semen, rarely an early sign of acute epididymitis (Wolff, 1995).

If increased concentrations of activated granulocytes were present in the epididymis, prostate gland or the seminal vesicles during a silent genital tract infection, the release of ROS might not impair ejaculate parameters, but could damage normal functioning of the respective organ. This could be demonstrated for the prostate gland, as a negative correlation existed between the seminal levels of granulocyte elastase (an enzyme indicating activation of granulocytes) and citric acid (a marker of prostate function) (Wolff et al., 1991a). Recent studies demonstrated more activated peritoneal mononuclear phagocytes from rats with experimental autoimmune prostatitis, indicating the involvement of oxygen radicals in the pathogenesis of this autoimmune disease (Orsilles et al., 1993, 1995). These findings were supported by recent studies which showed decreased catalase concentrations in this condition (Orsilles and Depiante-Depaoli, 1998) as well as positive effects of antioxidant treatment of chronic prostatitis (Tarasov et al., 1998).

Some studies did not report adverse effects of genital tract inflammation on the function of the seminal vesicles (Cooper et al., 1990; Wolff et al., 1991a), while in another study (Gonzales et al., 1992) an increased incidence of antisperm antibodies, decreased sperm count, motility and vitality were found if leukocytes in semen were present. A positive semen culture in combination with leukocytes in semen was related to lower levels of corrected fructose, indicating hypofunction of the seminal vesicles (Gonzales et al., 1989).

Due to the lack of antioxidants in the surrounding fluid and the longer contact time, inflammation of the epididymis should theoretically be more deleterious to spermatozoa. At present, it is not possible to define the original production site of ROS found in human semen. However, the analysis of the amount of lipid peroxidation present in sperm cells, or the concentration of polynsaturated fatty acids, might be indirect evidence of prior oxidative damage (Gomez et al., 1998; Zalata et al., 1998b). The normal function of the epididymis is difficult to assess. Decreased concentrations of the enzyme alpha-glucosidase or carnitine indicate an obstruction. In case
of acute epididymitis, the glucosidase and carnitine concentrations were decreased in men with clinical symptoms of acute epididymitis in comparison with those in normozoospermic controls (Cooper et al., 1990). An inverse correlation of alpha-glucosidase and ROS generation or number of peroxidase-positive white blood cells was described ($r = -0.3$; Depuydt et al., 1998; Mahmoud et al., 1998), while other authors did not find such an association (Wolff et al., 1991a). Acute infections appear to impair the function of the epididymis, while the impact of silent inflammation remains to be clarified.

**Infectious agents**

The causal role of infectious agents in human semen for male infertility remains a controversial subject. This may be the result of different terminology, i.e. colonization versus infection, different cut-off values, i.e. significant bacteriospermia versus detection of germs, and different kinds of infectious agents (Corradi et al., 1992; Bar-Chama and Fisch, 1993; Purvis and Christiansen, 1993; Carmeli et al., 1994; Ludwig et al., 1994; Eggert-Kruse et al., 1995a,b, 1996; Ness et al., 1997; Xu et al., 1997). Furthermore, the pathological mechanisms of bacteria and viruses which lead to an impaired sperm function are largely unknown. Thereby, the involvement of ROS during infection of different bacteria and viruses have entered the focus of interest.

*Rickettsia rickettsii* infections led to an intracellular increase of ROS production within 5 h. The major oxidant produced was hydrogen peroxide, and the increase of intracellular oxidant generation or ROS and pro-oxidant cytokines, and increase viral replication via ROS-mediated activation of the host cell NF-κB

In some viral infections, human immunodeficiency virus (HIV), hepatitis B and C, oxidative stress was postulated as a possible relevant causal factor of the respective pathology (Fuchs et al., 1991, 1994, 1998; Larrea et al., 1998). Some studies demonstrated that T lymphocytes and macrophages—but not motile spermatozoa—were infected by the HIV virus (Quayle et al., 1997), while others found HIV-1 proviral DNA in the nuclei of germ cells at all stages of their differentiation without germ cell damage or signs of an immune response (Muciaia et al., 1998).

It could be demonstrated that low levels of superoxide and NO metabolites could facilitate viral replication and also induce the generation of pro-inflammatory cytokines and lipid mediators (Peterhans, 1997b). Viruses could also activate phagocytic cells to release ROS and pro-oxidant cytokines, and increase viral replication via ROS-mediated activation of the host cell NF-κB (Schwarz, 1996; Ciriolo et al., 1997; Shibutani, 1997). Another mechanism was the production of autoantibodies against antioxidants such as SOD (Semrau et al., 1998). It could be demonstrated that these autoantibodies produced during Epstein–Barr virus infections functionally impaired SOD (Ritter et al., 1994) and resulted in oxidative damage with lipid peroxidation, producing sequelae such as cholestasis or spleen rupture (Semrau et al., 1996; Schaade et al., 1998). As a consequence, the use of antioxidants in the treatment of acute and chronic viral diseases is presently discussed (Dolganova and Sharonov, 1997; Giuliano et al., 1997; Peterhans, 1997a).

Interestingly, dehydroepiandrosterone (DHEA) sulphate prevented the oxidative damage of murine retrovirus-infected aged mice (Araghi-Niknam et al., 1998), while testosterone induced lipid peroxidation and decreased the SOD, catalase and glutathione peroxidase activities in the testis of rats, thus leading to oxidative stress (Chainy et al., 1997). It is apparent from these conflicting data that more research is needed in this field to elucidate the exact pathological mechanisms of infectious agents and their relationship to physiological hormone actions.
Cytokines and oxidative stress

Cytokines are regulatory peptides that are produced and secreted by leukocytes and other cells, and which influence the growth, differentiation or function of cells via autocrine or paracrine mechanisms. Cytokines are synthesized and secreted on demand, for example during a viral infection. They bind to receptors at target cells in the vicinity of the cytokine-producing cells. This binding results in a signal transduction cascade involving tyrosine or serine phosphorylation, and leading to an activation of transcription factors. These migrate to the nucleus and regulate gene expression. The different cytokines interact with each other, which results in a complex network that enables the cells to react to pathogenic stimuli in a well-controlled manner (Cicco et al., 1990; Meda et al., 1993; Yie et al., 1994). Furthermore, these processes are controlled on multiple levels of signal transduction (Derevianko et al., 1996).

Cytokines are also involved in gonadal and sperm functions. A variety of cytokines was shown to be present in human semen, such as transforming growth factor (TGF) -α (Nocera and Chu, 1993; Yie et al., 1994), epidermal growth factor (Hirata et al., 1987), TNF-alpha (Hussenet et al., 1993), different interleukins and their soluble receptors and antagonists (Liabakk et al., 1993; Huleihel et al., 1996; see Tables III and IV). These studies often yielded conflicting results, perhaps due to the different test systems used, to different time points of testing in the evolution of certain conditions, and to a lack of any clearly defined pathological entities, such as 'oligo-astheno-teratozoospermia (OTA) syndrome' or 'genital infection'.

In some in-vitro studies, cytokines such as interferon (IFN)-gamma and TNF-alpha were shown to decrease the motility of spermatozoa (Hill et al., 1987; Eisermann et al., 1989; Fedder and Ellerman-Eriksen, 1995; for an overview, see Fedder, 1996), while other authors questioned this (Wincek et al., 1989; Fedder et al., 1991; Haney et al., 1992). No effect of IFN-gamma, TNF-alpha or IL-8 on the motility of spermatozoa in the female genital tract (Srivastava et al., 1987; Eisermann et al., 1989; Fedder et al., 1991; Haney et al., 1992). No effect of IFN-gamma, TNF-alpha or IL-8 on the motility of spermatozoa in the female genital tract (Srivastava et al., 1987; Eisermann et al., 1989; Fedder et al., 1991; Haney et al., 1992). However, cytokines seemed to increase the generation of ROS by human spermatozoa (Buch et al., 1994; Rajaskaran et al., 1995), thus increasing oxidative stress in the male genital tract. IFN-gamma appeared to enhance the priming effects of TNF-alpha on the respiratory burst of PMNL (Meda et al., 1994). The cytokines IL-1-alpha, IL-1-beta and TNF-alpha stimulated the ROS generation of cells from the ejaculate of fertile donors at levels occurring physiologically in human seminal plasma (Buch et al., 1994). Concentrations of IL-6, but not IL-1-alpha, were elevated in the serum of patients with acute bacterial infections (Chen et al., 1995). This confirms the results of several studies indicating elevated IL-6 concentrations in the ejaculate of men with infections of the reproductive tract, and its correlation with leukocytospermia (Table III). IL-6 is known to stimulate the migration and oxidative burst of PMNL, thus increasing the ROS load in the male reproductive organs and the ejaculate. The high concentrations of TGF-beta may provide immune protection for spermatozoa in the female genital tract (Srivastava et al., 1996).
### Table IV. Cytokines detected in human seminal plasma

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Disorder</th>
<th>Findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1</td>
<td>fertile, azoo, OTA w/wo infection</td>
<td>=</td>
<td>Huleihel et al. (1997)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>astheno, OTA</td>
<td>=</td>
<td>Shimonovitz et al. (1994)</td>
</tr>
<tr>
<td>MAGI</td>
<td>positive bacterial sperm culture</td>
<td>=</td>
<td>Comhaire et al. (1994)</td>
</tr>
<tr>
<td></td>
<td>fertile, azoo, OTA w/wo infection</td>
<td>=</td>
<td>Huleihel et al. (1996)</td>
</tr>
<tr>
<td></td>
<td>infertile men w/wo infection</td>
<td>▼↑ in azoo</td>
<td>Dousset et al. (1997)</td>
</tr>
<tr>
<td>IL-2</td>
<td>bacteria in semen</td>
<td>O</td>
<td>Hussenet et al. (1993)</td>
</tr>
<tr>
<td></td>
<td>leukocytospermia</td>
<td>=</td>
<td>Rajaskaran et al. (1995)</td>
</tr>
<tr>
<td></td>
<td>infertile/fertile men</td>
<td>=</td>
<td>Paradisi et al. (1995)</td>
</tr>
</tbody>
</table>

Correlation: 0 leukocytes
+ sperm count; motil.; morphol.

- Huleihel et al. (1996)
- Dousset et al. (1997)
- Shimonovitz et al. (1994)
- Comhaire et al. (1994)
- Gruschwitz et al. (1996)
- Huleihel et al. (1996)
- Rajaskaran et al. (1995)
- Paradisi et al. (1995)

**Abbreviations:** = no difference; ▼↑ = increased; ▼ = decreased; 0 = no correlation; + = positive correlation; astheno = asthenozoospermia; azoo = azoospermia; C3 = complement; fertile = fertile men; infection = genital infections; infertile = infertile men; leuko = leukocytospermia; MAGI = male accessory gland inflammation; motil. = motility; morphol. = morphology; MCAF = monocyte chemotactic and activating factor; oligo = oligozoospermia; OTA = oligo-astheno-teratozoospermia; PMN-elastase = polymorphonuclear elastase; SOD = superoxide dismutase; sRIL = soluble receptor interleukin; varico = varicocele; w = with, w/wo = with or without.
The origin and regulation of cytokines in the male genital tract is still under investigation. Cytokines produced by the testis are part of an intrinsic system regulating germ cell proliferation (Pöllannen et al., 1989; Boockfor and Schwarz, 1991; Saez, 1994) and the differentiation of Leydig cells and their steroidogenic properties (Calkins et al., 1988; Guo et al., 1990). IL-1 and IL-6 were both identified in Sertoli cells and germ cells. The expression was modulated during the seminiferous epithelium cycle. Furthermore, cytokines appeared to be secreted by the epididymis and the prostate (Boockfor and Schwarz, 1991; Twillie et al., 1995). Finally, cytokines could be released by immunocompetent cells present in the male genital tract, even in the absence of an infection. IFN-gamma and IL-2 were secreted by CD4+ T-helper cells, type Th1, participating in cell-mediated immune responses, while Th-2 cells effected humoral responses by the secretion of IL-4, IL-5 and IL-10. In the absence of white blood cells, IL-8 appeared to be produced constitutively and correlated with sperm concentration (Depuydt et al., 1996; Eggert-Kruse et al., 1998a), while IL-6 correlated with alpha-glucosidase (Depuydt et al., 1996). During infections and leukocytospermia, IL-2 and IL-8 concentrations in seminal plasma were found to be significantly higher than those in fertile men, while IL-4 concentrations were lowered under these conditions (Omuro et al., 1998).

Cytokines may induce different but localized biological effects, including the synthesis of other cytokines. Studies indicated local production of these factors in the secondary sex glands, independently of spermatogenesis (Huleihel et al., 1996; Matalliotakis et al., 1998a). Thus, at present it is difficult to deduce from global cytokine measurements in whole semen either what the local regulatory concentrations of these cytokines in a specific organ may be, or what their specific effects are (Doussel et al., 1997). However, the association of increased concentrations of interleukins in inflammatory disorders and infections (see Table III) may modulate the generation of ROS in these states (Depuydt et al., 1996). It was shown that ROS production was enhanced by bacterial products and cytokines during bacterial infections of the urogenital tract (Wang, A. et al., 1997a).

Finally, the balance of cytokines can be modulated by prostaglandins which occur in high concentrations in semen. Prostaglandins stimulate IL-10 and inhibit the secretion of IL-12, leading to the tolerance to antigens, such as spermatozoa, and also to viruses (Kelly, 1997; Kelly and Critchley, 1997).

In the context of inflammation of accessory sex glands, it must be borne in mind that infections of the female genital tract may also interfere with physiological sperm functions. This topic cannot be discussed here in detail, but was investigated in recent studies and reviews (Riley and Behrman, 1991; Ho et al., 1997; Lapointe et al., 1998).

**Therapy and ROS**

The beneficial role of drug therapies with respect to their interference with ROS has received some attention (Cash, 1997; Kang et al., 1998). There are drugs which stimulate endogenous defence mechanisms and those which inhibit ROS formation. They may be grouped into antioxidant substances as substitutive therapy (SOD, thiol groups, vitamins E and C), chelating agents (desferrioxamine) which lower the level of transition metal ions, inhibitors of superoxide ions generation (allopurinol), superoxide scavengers (flavonoids), eliminators of hydrogen peroxide (glutathione), and scavengers of hydroxyl radicals (Robak and Marcinkiewicz, 1995). In contrast, some drugs induce ROS production, possibly as part of their therapeutic action (Walubo et al., 1995; Postma et al., 1996). As part of their microbial killing armory, antibiotics may use oxidative processes similar to that which has evolved within phagocytic cells (Gutteridge et al., 1998). Others, such as the antihyperlipidaemic agent, gemfibrozil, led to side effects by enhancing the induction of ROS production by blood phagocytes (Scatena et al., 1997). Thus, the outcome of ROS generation may be beneficial or harmful, depending on the balance of pro- and antioxidants.

In order to counteract the damaging effects of ROS, antioxidative supplementation therapy may be beneficial. Supplementation of the diet with vitamins E and/or C reduced the lead-induced generation of ROS in rats, prevented loss of motility, and sustained the capacity of oocyte penetration in lead-exposed rats. No effect was seen in animals not exposed to lead (Hsu et al., 1998). In humans, the effects of such antioxidant therapies are unresolved. Although antioxidants are effective against ROS damage to spermatozoa in vitro, in animal models and some in-vivo trials (for reviews, see Geva et al., 1998; Lenzi et al., 1998; Tarin et al., 1998), its definitive role in clinical practice is far from clear and remains a matter of debate (Ford and Whittington, 1998; Rolf et al., 1999). At present, no data are available with respect to antioxidative therapy in male genital tract infections. However, in systemic infections, such as septic shock or HIV infection, antioxidants could have a major impact on clinical infectious diseases (Keusch, 1993).

It is known that, at therapeutic concentrations, antibiotics such as tetracycline, oxytetracycline, minocycline and erythromycin inhibited ROS production by polymorphonuclear granulocytes. However, they did not suppress the hydrogen peroxide concentrations produced in vitro by xanthine–xanthine oxidase. Thus, their effects originated from their effects on PMNL cell function and not by their ROS-scavenging properties. Antibiotics such as cephalaxin, penicillin G, chloramphenicol or streptomycin exerted no effect on ROS concentrations (Miyachi et al., 1986), a finding which could also be confirmed for azithromycin (Levert et al., 1998).
Antibiotic treatment with co-trimoxazole or ciprofloxacin of patients with positive bacterial cultures did not change ejaculate parameters, but did modify the function of the accessory glands (Merino and Carranza-Lira, 1995). This is in line with the above-outlined possible pathological mechanisms of infections. The generation of ROS after stimulation with formyl-methionyl-leucyl-phenylalanine (FMLP) was normalized after sufficient antibiotic therapy of bacterial prostatitis. This parameter correlated with a negative bacterial influence of bacteria and/or leukocytes during IVF or ICSI (Michelmann, 1998). However, there appeared to be no negative influence of bacteria and/or leukocytes during IVF or ICSI (Michelmann, 1998). Antibiotic treatment of asymptomatic patients was even detrimental to IVF outcome (De Geyter et al., 1994; Liversedge et al., 1996). At present, there is no evidence that spermatozoa act as a vector for the transportation of bacteria into the ooplasm (Michelmann, 1998).

Conclusions

Reactive oxygen species are highly reactive intermediates of normal cell metabolism. Their generation is well controlled and serves physiological purposes, such as energy generation, defence mechanisms and signal transduction. Counterparts are several low-molecular weight and enzymatic antioxidants. An imbalance of pro- and antioxidants in favour of the former is termed ‘oxidative stress’. In the male genital tract, ROS are generated by spermatozoa, granulocytes and macrophages. It could be established that oxidative stress severely impairs sperm function, such as motility or fertilization. On the other hand, ROS are involved in physiological processes such as capacitation and the acrosome reaction. Excessive generation of ROS, for example during infections, could disturb these physiological processes.

At present, there are many unresolved questions concerning the role of ROS during infections of the male genital tract. Reasons for this are the difficulty to assess specifically the generation and effects of the short-lived ROS, and therefore the lack of specific studies. During infections, an oxidative burst occurs as a first-line defence mechanism, this being modulated by several cytokines; thus, the roles of neutrophils and macrophages as producers of ROS are of primary interest. The site of an infection, the activation of leukocytes, i.e. the amount of ROS produced, and the available antioxidative systems determine whether or not spermatozoa are damaged. In particular, infections of the testis and epididymis must be regarded as being potentially harmful to spermatozoa. The longer contact time and the lack of adequate protection at this site may facilitate injury to spermatozoa. Due to the high concentration of antioxidants in seminal plasma only very high numbers (at least $>2\times10^6/\text{ml}$) of ROS-producing leukocytes should be detrimental to sperm function in the ejaculate.

Recent studies have shown that bacteria and viruses might mediate their damaging effects via oxidative mechanisms. Thus, the microorganisms themselves, or the resulting defence reactions, i.e. the oxidative burst of neutrophils, could indirectly impair sperm functions through injury to prostate gland, seminal vesicles or epididymis. Moreover, it has been shown that some anti-infectious drugs may have pro- or antioxidative properties as part of their action. Based on these findings, antioxidants could receive more attention as a part of anti-infectious therapies.

At present, these mechanisms are clinically relevant during epididymitis or for the in-vitro preparation of spermatozoa for assisted reproduction. Future studies are required to deal with the questions of whether, and under what situations, granulocytes in the ejaculate can impair sperm functions via ROS, and whether the production of ROS is relevant if these are generated in the female genital tract during an infection.

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