

Male Genital Tract Inflammation: The Role of Selected Interleukins in Regulation of Pro-Oxidant and Antioxidant Enzymatic Substances in Seminal Plasma

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ABSTRACT: Human semen contains spermatozoa as well as populations of round nonspermatozoal cells primarily consisting of leukocytes. Activation of white blood cells present in the seminal plasma during genital tract inflammation and cellular reactions against microbial agents may provoke a release of a variety of products such as cytokines and reactive oxygen species. The aim of this study was to evaluate whether a panel of selected cytokines (interleukin [IL]-1 β , IL-6, IL-8, and tumor necrosis factor- α [TNF α]) detectable in seminal plasma during male genital tract inflammation could be considered as mediators between altered semen parameters and changed levels of pro-oxidant and antioxidant substances. Studies using chemiluminometric, spectrophotometric, and enzyme-linked

immunosorbent assay methods indicate that proinflammatory cytokines such as IL-1 β , IL-6, IL-8, and TNF α may modulate pro-oxidant and antioxidant activities in the male genital tract. The data also suggest that the function of pro-oxidant and antioxidant systems in semen may directly influence basic semen parameters. The elevated numbers of leukocytes present in semen during male genital tract inflammation without an associated contribution of cytokines and semen antioxidant capacity appear to be of little prognostic value in evaluating male fertilization potential.

Key words: Semen, leukocytes, reactive oxygen species, infertility, cytokines.

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Human semen contains spermatozoa as well as populations of round nonspermatozoal cells primarily consisting of leukocytes and immature germ cells (Fedder et al, 1993; Wolff, 1995). A relationship between the cell-mediated immunity and reproductive events has already been established (Naz et al, 1990; Aitken et al, 1991). According to the World Health Organization (1992), a number of leukocytes exceeding 1×10^6 leukocytes per milliliter of semen, classified as leukocytospermia, has frequently shown to be associated with impaired sperm function and infertility (Mankveld and Kruger, 1998; Zalata et al, 1998; Arata de Barbarella et al, 2000), although some studies appear to be controversial in this respect (Tomlinson et al, 1993).

Activation of seminal plasma white blood cells during genital tract inflammation or cellular reactions against microbial antigens may trigger the release of a variety of products such as proteolytic enzymes, cytokines, and reactive oxygen species (ROS). A negative association be-

tween excessive ROS production and human male fertility has been demonstrated in a range of earlier studies (de Lamirande and Gagnon, 1992; Griveau et al, 1995; Kurpisz et al 1996; Sanocka et al, 1996; Ochsendorf, 1999; Pasqualotto et al, 2001).

A detrimental leukocyte influence on egg fertilization has been documented (Miesel et al, 1993; Vicino et al, 1999) and it is quite possible that such a negative effect was caused either by cytokines or ROS generated by white blood cells.

Some authors have suggested that limited amounts of ROS are essential for the induction of physiological mechanisms such as capacitation and the acrosome reaction of human sperm (Zini et al, 1995; de Lamirande and Gagnon, 1997; de Lamirande et al, 1997). Hence, ROS generation under the precise control of the antioxidant system must be considered as an important factor for sperm function and to play a role in signal transduction (Joseph and Cutler, 1994). It is still not clear whether some leukocyte contamination may have a positive influence on sperm function and which factors would change this effect. By observing molecular mechanisms of infertility it has been recently speculated that cytokines and their soluble receptors have a close relationship with male infertility and sperm function at particular steps of the reproduction process (Buch et al, 1994; Huleihel et al,

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1996, 1999; Denison et al, 1999; Matalliotakis et al, 2000). Proinflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor- α (TNF- α) have been scrutinized in studies because the inflammation process is usually associated with their presence, and as a consequence, stimulates antioxidant feedback (Rajasekaran et al, 1995).

In order to evaluate the influence of cytokines, ROS, and enzymatic pro-oxidants and antioxidants on the fertility potential, we undertook measurements of selected cytokines (IL-1 β , IL-6, IL-8, and TNF α) in seminal plasma samples obtained from healthy individuals and men with genital tract inflammation; pro-oxidant and antioxidant enzymatic substances and basic seminological features (including leukocytes) in infected semen samples and in semen from healthy, uninfected subjects; and attempted to demonstrate possible interconnections between the measured substances and semen parameters in both assessed populations of men (with and without genital tract inflammation).

Materials and Methods

Patients

Altogether, 61 ejaculated semen samples were examined from 2 groups of men (with and without genital tract inflammation). Semen samples were obtained from patients with a genital tract inflammation ($n = 39$) and healthy individuals ($n = 22$) by masturbation after 4 days of sexual abstinence. Ejaculated samples from healthy individuals presented good sperm density, progressive motility, and morphology ($>30\%$), and constituted a control group as established by World Health Organization (1992) criteria. Leukocytes were identified by May-Grunwald-Giemsa staining, although the concentration of these cells in healthy men never exceeded $>0.1 \times 10^6$ cells/mL of semen, and bacteriological analysis did not show the presence of any pathological flora. Patients with a genital tract inflammation and who were otherwise healthy, were recruited from the outpatient andrology clinic of Poznań Hospital College. In this group of patients, semen was normozoospermic according to an assessment of sperm density (>20 million/mL of ejaculate). Leukocyte numbers in the semen of these individuals always exceeded $>0.5 \times 10^6$ cells/mL of ejaculate and bacteriological screening indicated the presence of pathological bacterial strains ($>10^3$ bacteria/mL). In some ejaculated samples of this group, leukocytes exceeded 1×10^6 /mL of ejaculate with or without the presence of pathological flora. Female partners were carefully examined for anatomical, hormonal, and immunological parameters, however, normal ovulatory profiles, patent fallopian tubes, and no signs of immunological reactions to gametes were detected. We assigned these couples as idiopathically infertile on the basis of their infertility lasting at least 2 years.

Sperm Preparations

After 30 minutes of semen liquefaction at room temperature, semen samples were subjected to the routine andrological analysis (semen volume, sperm and leukocyte densities, progressive motility, morphology, and viability). Spermatozoa from all examined samples were separated from seminal plasma by centrifugation at $300 \times g$ for 10 minutes and at $500 \times g$ in special cases when problems with semen viscosity occurred. Plasma samples were then used to determine superoxide dismutase, catalase, glutathione peroxidase, xanthine oxidase, and interleukin concentrations.

Chemicals and Equipment

Unless otherwise stated, all reagents used to determine enzymatic pro-oxidants and antioxidants were purchased from Sigma-Aldrich (Steinheim, Germany), except xanthine oxidase, which was purchased from Fluka Chemie (Buchs, Switzerland). Reagents for determining interleukin levels were purchased from Genzyme Corporation (Cambridge, Mass).

All chemiluminometric assays were performed using Luminometer Berthold LB953 (EG and Berthold, Bad Wildbad, Germany).

Superoxide Dismutase

Superoxide dismutase (SOD) activity was quantified by chemiluminescence (Miesel and Weser; 1991; Miesel and Haas, 1993) using the xanthine/xanthine oxidase lucigenin assay. A final volume of 1 mL of the sample contained the following components: 100 μ L of seminal plasma, 100 mM diethylenetriaminepentaacetic acid (DTPA), 100 mM lucigenin, 180 nM xanthine oxidase, and 50 mM xanthine in 50 mM HEPES (pH 7.4). The addition of xanthine started the reaction and the resulting photon emission was recorded in a Berthold LB 953 luminometer at 25°C. Bovine SODCuZn was used for calibration. One unit represented the concentration of SOD required to inhibit the release of superoxide by 50% and equals 5 nM copper.

Catalase

Catalase activity was determined chemiluminometrically (Aebi, 1974) in the presence of luminol. The initial enzymatic decomposition of hydrogen peroxide is the first-order reaction at low concentrations of hydrogen peroxide (less than 10 nM), and is directly proportional to the concentration of substrate and the concentration of enzyme. One milliliter contained 100 mM luminol, 100 mM DTPA, 5 mM H₂O₂, and 20 μ L of seminal plasma. The reaction commenced with the addition of H₂O₂. The resulting chemiluminescence was recorded for 3 minutes in a Berthold LB 953 luminometer at 25°C. Chromatographically purified catalase from bovine liver was used for calibration. One unit of catalase decomposes 1.0 mM H₂O₂ per minute under specified conditions.

Glutathione Peroxidase

Glutathione peroxidase activity was determined by spectrophotometric assay as described by Flohe and Gunzler (1984) and Rice-Evans et al (1991). The following reagents were used: 0.1 M potassium phosphate buffer (pH 7.0) containing 0.1 mM eth-

ylenediamine tetraacetic acid (EDTA), 2.4 U/mL glutathione reductase, 10 mM reduced glutathione (GSH) in water, 1.5 mM NADPH in 0.1% NaHCO₃, 12 mM *t*-butylhydroperoxide, 1.5 mM H₂O₂ in water. Test enzyme samples contained 0.05–1.0 U/mL. The following reagents were pipetted into a semimicrocuvette set at 37°C: 500 µL of 0.1 M phosphate buffer (pH 7.0), 100 µL of seminal plasma sample, 100 µL of glutathione reductase (0.24 U); and 100 µL of 10 mM glutathione solution. The H₂O₂-independent oxidation of NADPH was measured for 3 minutes in order to give a baseline at 340 nm. The reaction began with the addition of 100 µL of a solution of either *t*-butylhydroperoxide or 1.5 mM H₂O₂ that had been prewarmed to 37°C. The decrease in absorbance was monitored for 5 minutes. Replacing the glutathione peroxidase-containing sample with the buffer enabled an assessed the nonenzymatic reaction.

Xanthine Oxidase

The xanthine oxidase activity was determined by the chemiluminometric assay described by Miesel and Weser (1991) and Rice-Evans et al (1991) with application of xanthine. A final volume of a 1-mL sample contained the following mixture: 100 µL of seminal plasma, 10 mM DTPA, 100 mM lucigenin, and 50 mM xanthine in 50 mM HEPES (pH 7.4). The addition of xanthine initiated the reaction and the resulting photon emission was recorded in a Berthold LB 953 luminometer at 25°C.

Interleukin-1β, Interleukin-6, Interleukin-8, TNFα

The Predicta (Cambridge, MA) IL-1β, IL-6, IL-8, and TNFα enzyme immunoassay kits contain a 96-well microtiter plate pre-coated with monoclonal antibody to a proper cytokine. A measured volume of the studied samples, either standard substance or control buffer, was added to each test well and incubated to allow any cytokine present to be captured by antibodies on the microtiter plate. The wells were then washed, and a biotin-labeled polyclonal antibody to the tested cytokine was added to bind the captured IL-1β, IL-6, IL-8, or TNFα. The wells were washed again and a peroxidase-labeled avidin reagent was added to attach the biotin (in the immune complex) on the plate. After incubation the wells were washed and a peroxidase-labeled goat anti-rabbit immunoglobulin G was added to attach the polyclonal antibody (in the immune complex) on the plate. After a third wash, a substrate buffer (peroxide) and chromogen (tetramethylbenzidine) were added to the wells, thereby producing a blue color in the presence of peroxidase. The color reaction was stopped by the addition of sulfuric acid, which converted the blue color to yellow. The intensity of the colorimetric reactions was in a direct proportion to the amount of tested cytokine present in the studied sample or standard. The absorbance was read with Multiscan Plus (Labsystems, Helsinki, Finland) at 450 nm, and a standard curve was constructed to quantitate cytokine concentrations.

Respective values for detection limit, reproducibility range, and source of antibodies are as follows:

Human IL-1β: detection limit, 3 pg/mL; intra-assay value, 8.2%; interassay value, 9.6%; source of antibody, biotinylated rabbit anti-human (Yang et al, 1993);

Human IL-6: detection limit, 18 pg/mL; intra-assay value,

Table 1. Concentrations of pro-oxidant and antioxidant substances, cytokines, and the oxidosensitive index in seminal plasma of infertile men with genital tract inflammation and good sperm density, and in healthy controls

Parameter	Healthy controls†	Infertility and genital tract inflammation†
Superoxide dismutase (SOD) (U/mL)	3.9 (3.75–4.1)	3.95 (3.4–4.2)
Catalase (U/mL × 10 ³)	4.3 (4.1–4.5)	3.0* (0.1–4.5)
Glutathione peroxidase (U/mL × 10 ⁻³)	5.5 (2.5–5.5)	6.5 (5.5–6.6)
Xanthine oxidase (XO) (U/mL × 10 ⁻³)	3.25 (1.0–5.0)	8.0** (5.0–10.0)
IL-1β (pg/mL)	10.0 (2.75–20.0)	155.0* (52.0–195.0)
IL-6 (pg/mL)	10.5 (0.0–110.0)	107.5*** (0.0–450.0)
IL-8 (pg/mL)	575.0 (410.0–790.0)	1100.0* (940.0–2645.0)
TNFα (pg/mL)	6.5 (0.0–11.5)	8.0 (0.0–24.0)
Oxidosensitive SOD/XO	1.11	0.49**

† Figures are expressed as the median; interquartile values are indicated in parentheses.

* *P* < .05

** *P* < .01

*** *P* < .001

6.6%; interassay value, 8.8%; source of antibody, biotinylated rabbit anti-human (Wong et al, 1988);

Human IL-8: detection limit, 1 pg/mL; intra-assay value, 6.6%; inter-assay value, 11%; source of antibody, biotinylated rabbit anti-human (Gesser et al, 1996);

Human TNFα: detection limit, 3 pg/mL; intra-assay value, 6%; inter-assay value, 10%; source of antibody, biotinylated rabbit anti-human (Braegger et al, 1992).

Undiluted antibodies were applied in the all cases (no titration therefore was performed).

Statistical Analysis

The results are presented as a median. The statistical significance of the results was assessed using the Kruskal-Wallis nonparametric analysis of variance test, the Mann-Whitney *U*-test, and Spearman rank order correlations.

Results

SOD in Seminal Plasma

The revealed SOD levels in seminal plasma samples of healthy men (*n* = 22) reached a median of 3.91 U/mL (Table 1). In the group of patients with infertility and genital tract inflammation, the determined SOD activity was similar to that of control individuals, with a median of 3.59 U/mL (Table 1).

Catalase in Seminal Plasma

Median catalase seminal plasma levels obtained in a cohort of healthy donors was $4.3 \text{ U/mL} \times 10^3$. In a group of men with infertility and genital tract inflammation, the activity of catalase presented as a median was $3.0 \text{ U/mL} \times 10^3$. This difference was found to be statistically significant ($P < .05$, see Table 1).

Glutathione Peroxidase in Seminal Plasma

Seminal plasma samples from healthy controls ($n = 22$) presented as a median of $5.5 \text{ U/mL} \times 10^{-3}$ of glutathione peroxidase. In men with infertility and genital tract inflammation, the activity of glutathione peroxidase was elevated; the median was $6.5 \text{ U/mL} \times 10^{-3}$. The difference was not statistically significant ($P = .1$, see Table 1).

Xanthine Oxidase in Seminal Plasma

Xanthine oxidase activity was elevated in a group of men with infertility and semen inflammation (median $8.01 \text{ U/mL} \times 10^{-3}$) in comparison with that of healthy controls (median $3.25 \text{ U/mL} \times 10^{-3}$). This difference was found to be statistically significant ($P < .01$, see Table 1).

IL-1 β in Seminal Plasma

Seminal plasma from healthy individuals contained IL-1 β with a median value of 10.0 pg/mL . Seminal plasma samples from normozoospermic but infertile men with genital tract inflammation contained clearly elevated levels of IL-1 β compared with that of controls (median 155.0 pg/mL). This difference was found to be statistically significant ($P < .05$, see Table 1).

IL-6 in Seminal Plasma

Seminal plasma from healthy, normozoospermic controls contained moderate levels of IL-6 at a median of 10.5 pg/mL . However, seminal plasma samples from patients with genital tract inflammation contained very high IL-6 levels with a median value of 107.5 pg/mL , which was considered to be statistically significant ($P < .001$, see Table 1).

IL-8 in Seminal Plasma

Concentrations of IL-8 in seminal plasma of normozoospermic men with infertility and genital tract inflammation (median 1100.0 pg/mL) was significantly different from values detected in seminal plasma samples of healthy men (median 575.0 pg/mL , $P < .05$, see Table 1).

TNF α in Seminal Plasma

TNF α was detectable in seminal plasma samples obtained from healthy men, with a median value of 6.5 pg/mL . Higher concentrations of this cytokine (median value 8.0 pg/mL) was observed in a group normozoospermic infer-

Table 2. *Seminological analysis of ejaculates from healthy men and infertile men with genital tract inflammation*

Semen parameters	Healthy men†	Genital tract inflammation†
Concentration of sperm cells ($1 \times 10^6/\text{mL}$)	60.5 (22.0–120.0)	25.0** (1.0–69.5)
Rapid and slow progression (%)	52.0 (31.0–62.0)	20.0*** (0.0–42.0)
Nonprogression (%)	18.5 (16.0–38.0)	34.0 (10.0–59.0)
Morphology (%)	32.2 (30.0–68.0)	26.0* (5.0–35.0)
Vitality (%)	79.0 (71.0–85.0)	65.0 (35.0–75.0)

† Figures are expressed as the median; interquartile values are indicated in parentheses.

* $P < .05$

** $P < .01$

*** $P < .001$

tile men with genital tract inflammation, although it was not statistically significant ($P > .05$, see Table 1).

Oxido-Sensitive Index

The Oxido-sensitive index (SOD/xanthine oxidase) of seminal plasma was also determined according to our previous observation, indicating the importance of balance between pro-oxidant and antioxidant enzymatic substances (Kurpisz et al, 1996). On average, the oxido-sensitive index in ejaculated samples from healthy controls (1.11) was higher than in samples from infertile men (0.45) with genital tract inflammation, and the difference was statistically significant ($P < .01$, Table 1). A diminished oxido-sensitive index value may indicate that pro-oxidant activity overwhelms antioxidant protection with respective consequences (eg, peroxidation of lipids) for sperm membranes (plasmalemma dysfunction).

Semen Parameters

The seminological parameters determined in samples from patients with genital tract infection (sperm density, progressive motility, nonprogressive motility, morphology, and viability) differed from those in semen from healthy controls, however, statistical significance was observed in sperm density, progressive motility, and morphology (Table 2).

Spearman Rank Order Correlation Among Semen Parameters, Antioxidant and Pro-Oxidant Activities, and Concentration of Studied Cytokines

In seminal plasma from healthy donors we observed only one correlation that occurred between xanthine oxidase and catalase ($r = -.599$, $P = .018$, Table 3), whereas there were as many as six statistically significant ($P < .05$) correlations in seminal plasma from infertile men with genital tract inflammation; of these, 2 were positive

Table 3. Spearman rank order correlations among semen parameters, pro-oxidant and antioxidant activities, and studied cytokines in controls and infertile men with genital tract inflammation

Examined individuals	Correlations	Spearman rank (<i>r</i>)	<i>P</i>
Healthy men (n = 22)	↑ XO → CAT ↓	-.599	.018
Infertile men with genital tract inflammation (n = 39)	↑ IL-1β → CAT ↓	-.781	.037
	↑ IL-6 → CAT ↓	-.745	.05
	↑ IL-8 → CAT ↓	-.823	.012
	↑ IL-8 → SOD ↑	.644	.04
	↑ TNFα → XO ↑	.784	.007
	↑ XO → sperm morphology ↓	-.367	.04

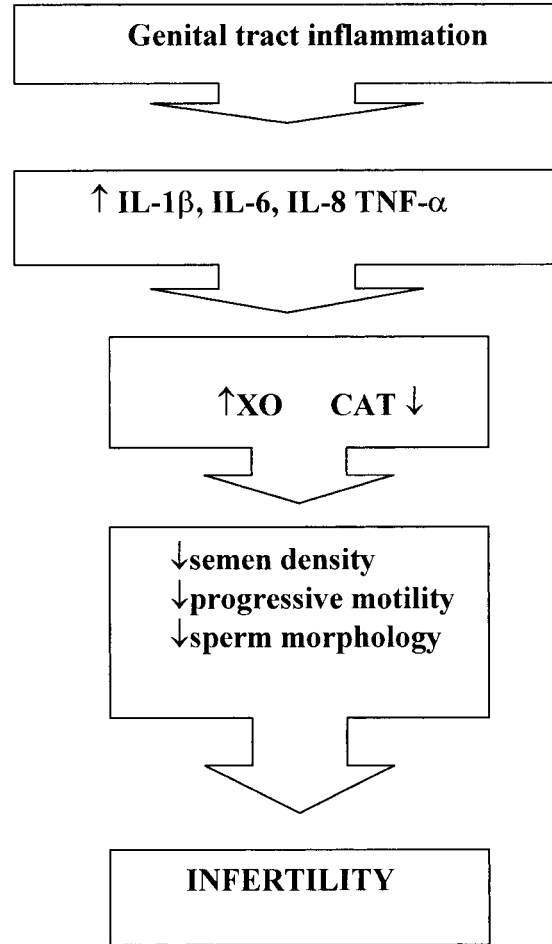
CAT indicates catalase; XO, xanthine oxidase

(Table 3). These correlations indicate the relationship between the studied cytokines and pro-oxidant and antioxidant substances as well as pro-oxidant and antioxidant enzymes and measured seminological parameters. When the examined individuals (n = 61) were analyzed statistically (Table 4), the range of the observed correlations was extended and reached more than 20 significant correlations. Of those, 7 were positive and 11 were negative (although the general nature of correlations was similar to the one revealed in a subgroup of infertile men with genital tract inflammation). It should be further emphasized that elevation of xanthine oxidase was always associated with a deterioration of sperm parameters, whereas proinflammatory cytokines up-regulated xanthine oxidase (Table 4). On the other hand, in a group of studied

Table 4. Spearman rank order correlations among semen parameters, pro-oxidant and antioxidant activities, and studied cytokines in all examined individuals

Correlations*	Spearman rank	Statistical significance
↑ IL-1β → CAT ↑	<i>r</i> = .731	<i>P</i> = .0005
↑ IL-1β vs XO ↑	<i>r</i> = .56	<i>P</i> = .005
↑ IL-1β vs GPx ↓	<i>r</i> = -.047	<i>P</i> = .017
↑ IL-6 vs XO ↑	<i>r</i> = .506	<i>P</i> = .0003
↑ IL-6 vs GPx ↑	<i>r</i> = .456	<i>P</i> = .002
↑ TNFα vs CAT ↓	<i>r</i> = -.447	<i>P</i> = .007
↑ TNFα vs XO ↑	<i>r</i> = .359	<i>P</i> = .016
↑ CAT vs XO ↓	<i>r</i> = -.660	<i>P</i> = .0001
↑ CAT vs GPx ↓	<i>r</i> = -.4	<i>P</i> = .029
↑ XO vs GPx ↓	<i>r</i> = -.470	<i>P</i> = .004
↑ IL-6 vs semen density ↓	<i>r</i> = -.48	<i>P</i> = .015
↑ IL-6 vs progressive motility ↓	<i>r</i> = -.47	<i>P</i> = .016
↑ TNFα vs semen density ↓	<i>r</i> = -.328	<i>P</i> = .031
↑ CAT vs semen density ↑	<i>r</i> = .450	<i>P</i> = .002
↑ CAT vs progressive motility ↑	<i>r</i> = .331	<i>P</i> = .036
↑ XO vs semen density ↓	<i>r</i> = -.436	<i>P</i> = .001
↑ XO vs progressive motility ↓	<i>r</i> = -.338	<i>P</i> = .018
↑ XO vs morphology ↓	<i>r</i> = -.318	<i>P</i> = .015

* Correlations reflect the transition stage between the pro- and antioxidant balance and oxidative stress in the semen.



Relationship among the cytokines, antioxidants, pro-oxidants, and semen parameters.

individuals, catalase usually has been negatively associated with IL-1β, IL-6, IL-8, and TNFα (Tables 3 and 4). Low catalase activity can be associated with low semen quality (sperm density and progressive motility), as can be implied from Table 4. This noted appropriately in the Figure.

Discussion

Herein we have shown that excessive generation of ROS by seminal leukocytes during genital tract infection may change the profile of oxidants and antioxidant activity in seminal plasma. In some cases, the antioxidants may already be overwhelmed due to the kinetics of inflammation, and even less than 1.0×10^6 leukocytes/mL of semen may cause a serious change in oxidative metabolism of semen (Tables 1 and 2). Moreover, proinflammatory cytokines such as IL-1β, IL-6, IL-8, and TNFα seem to modulate the activity of pro-oxidant and antioxidant enzymes and can severely influence basic semen parameters.

Of all the cytokines analyzed in this experimental design, IL-1 β , IL-6, and IL-8 were statistically significant indicators of pathological process in semen (Table 1).

Leukocytic infiltration into the human ejaculate is believed to be a clinically significant factor in the etiology of infertility (Aitken et al, 1999). Existing controversies in recent literature do not sufficiently emphasize the role of genital tract inflammation in transient or persistent male infertility.

In our previous studies (Kurpisz et al 1996; Sanocka et al, 1996; Miesel et al 1997) we suggested that a non-equilibrated antioxidant system in semen might be one of the important reasons for these conflicting results. When a general antioxidant capacity of semen (total trapping) is characterized, a fundamental change in the action of particular enzymatic factors in semen can be omitted (Sanocka et al 1997).

A study by Aitken et al (1999) postulated that leukocyte infiltration (to the point of leukocytospermia $>1 \times 10^6$ /mL of semen) had no influence on the morphology of sperm cells or their motility. On the other hand, a profound influence of the leukocytes on the functional capacity of the washed sperm preparations at the stage of sperm-oocyte fusion was observed. In general, whenever leukocytes were added to the purified gametes, a sperm-oocyte fusion cannot occur. The ROS threshold of ROS generation influencing sperm-oocyte fusion was previously presented (Miesel et al, 1993). On the other hand, Tomlinson et al (1993) suggested that measurement of seminal plasma leukocytes in a routine semen analysis appeared to be of little prognostic value in male fertilization potential. Furthermore, these authors postulated that elevated concentrations of neutrophils and macrophages improved sperm morphology and semen density. Those results are outwardly incompatible with our observations because an efficient antioxidant system is sometimes able to scavenge even very high amounts of ROS. Therefore, both arms of the equation (pro-oxidants and antioxidants) must be carefully analyzed.

Genital tract inflammation reflected in leukocytospermia must certainly enhance the levels of free radicals, and its effects on semen parameters might be strictly dependent on initial antioxidant capacity. It is not a surprise that a comparison of leukocytospermic samples (1×10^6 /mL of semen) with nonleukocytospermic samples revealed significant differences at the levels of ROS, however, the antioxidant status was not precisely characterized (Tomlinson et al, 1993). In another study (Kessopoulou et al, 1992), the authors did not find a significant correlation between sperm morphology and ROS; only the beat cross-frequency of the sperm tail was negatively correlated with ROS levels. In our previous studies (Kurpisz et al, 1996; Sanocka et al, 1996; Miesel et al, 1997) we demonstrated that an inefficient semen antioxidant

system correlated with infertility and semen pathology, and was especially associated with asthenozoospermia. Furthermore, we observed that elevated levels of peroxidized lipids in the cell membranes of spermatozoa hinted at significant generation of ROS (Sanocka et al, 1997).

A study by Leib et al (1994) examined a relationship between chronic abacterial prostatitis and the development of male infertility. Statistical evaluation of these 2 groups, normal fertile men and patients with longstanding (1–20 years) chronic abacterial prostatitis, showed that sperm motility parameters, sperm morphology, prostate markers, and white blood cells were out of the normal range in the group with chronic, abacterial prostatitis. In addition, a correlation was found between the duration of the disease and 2 important semen variables: increased prostatic markers and appearance of sperm morphological defects. Furthermore, the authors suggested that the other reasons for such observed pathological symptoms could be linked to persistent generation of ROS in semen of patients with nonbacterial inflammation. During genital tract inflammation, beside leukocyte contamination, quite often (but not always), pathological bacterial strains may appear in semen. Hence, another group of authors concentrated mainly on bacterial semen infections and its relation to infertility (Monga and Roberts, 1994; Merino et al, 1995; Keck et al, 1998; Potts et al, 2000) and indicated that bacterial infections might cause visible alterations in semen characteristics, volume, sperm motility, and viability. Immobilization or death of spermatozoa can be a biological response to the action of bacterial toxins. The influence of genital inflammation on fertility was mediated through diminished sperm motility due to the adherence of dialyzable factors in semen samples (Monga and Roberts, 1994).

Our results point to another mechanism of male infertility. In this hypothetical situation, pathological bacterial strains present in semen (even without contaminating leukocytes) may cause an increase of cytokine levels that in turn may abolish the activity of catalase and up-regulate the xanthine oxidase action (see the Figure). Those changes are the direct consequence of exposure to ROS (in what may create a vicious circle), progressively exhausting the antioxidant scavenging capacity and thereby impairing the membrane structures of spermatozoa.

We believe that the crucial role in perpetuation of the inflammatory process may belong to cytokines, and that these bioactive substances may constitute an important link between inflammation and male infertility. Cytokines released by various cell populations can be involved in proliferative and differentiating responses of a variety of cell subsets (including germ cells) and are capable of markedly influencing the biological activities of these cells (Fedder, 1996; Dousset et al, 1997).

In addition, proinflammatory cytokines such as: IL-1 β ,

IL-6, and TNF α are involved in the reduction of the ability of spermatozoa to penetrate. Grushwitz et al (1996) determined the cytokine content in seminal plasma of patients with unexplained infertility and correlated these results with urogenital infections and sperm parameters. They observed that IL-1 β , IL-6, and TNF α levels in seminal plasma were negatively correlated with the number of progressively motile sperm, but there was no correlation with a total sperm count, viability, pH, morphological sperm abnormalities, or hormonal parameters. Cytokine levels (seminal plasma) were significantly elevated, indicating bacterial or mycoplasmal infections of the urogenital tract. Fedder and Ellermann-Ericsen (1995) more closely investigated the effect of cytokines TNF α , IL-8, and IFN- γ on sperm motility and the acrosome reaction. Among the cytokines examined, only IFN- γ showed an ability to inhibit sperm motility, and this phenomenon was observed only when high concentrations of the cytokine were applied.

Sikka et al (2001) in their study suggested that combinations of lipopolysaccharides and interferon- γ are detrimental to human spermatozoa and may contribute to male infertility in patients with chronic genitourinary inflammation. In the present study, we have strongly indicated that the activity of the antioxidant system is dependent on particular interleukins (Tables 3 and 4), which can be potent modulating factors of antioxidant semen capacity. A clear relationship between basic semen parameters and oxidative stress was well observed.

It has been recently established that ROS may act as intracellular signaling molecules to mediate the biological effects of cytokines. One of the main targets of ROS is transcription factor κ B (nuclear factor- κ B [NF κ B]). NF κ B-dependent transcription is inhibited by antioxidants and its activation is induced or potentiated by ROS (Boulares et al 2000; Bowie et al 2000; Kwon et al, 2000). It has been known that TNF α may increase IL-6 gene expression through the activation of NF κ B, and that the antioxidants can suppress TNF α -dependent IL-6 expression, thereby inhibiting the activation of the transcriptionally active NF κ B (Kikumori et al, 1998).

The precise molecular mechanisms for regulating the antioxidant response to male genital tract inflammation remains unclear, however, results generated so far indicate that cytokines may play an important role during the inflammatory reactions and are connected with oxidative metabolism.

In summary, we provide further evidence that 1) proinflammatory cytokines such as IL-1 β , IL-6, IL-8, and TNF α modulate pro-oxidant and antioxidant activities; 2) the function of pro-oxidant and antioxidant systems in semen may directly influence semen parameters and that long-term genital tract inflammations may lead to male infertility; 3) measurement of seminal plasma leukocytes

during male genital tract inflammation without an associated contribution of cytokines or semen antioxidant capacity appears to have little prognostic value in the evaluation of male fertilization potential. Taking into account the results presented, it should be considered the application of selected antioxidants in supplementary treatments for men with genital tract inflammations.

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