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Impaired Seminal Antioxidant Capacity in Human Semen With Hyperviscosity or Oligoasthenozoospermia

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ABSTRACT: Antioxidant capacity of seminal plasma was evaluated in 120 semen samples subdivided into asthenozoospermic and oligoasthenozoospermic specimens with normal consistency and into asthenozoospermic and oligoasthenozoospermic specimens with hyperviscosity. Semen samples ($n = 25$) from normozoospermic donors were used as a control group. Scavenger antioxidant capacity of reactive oxygen species was evaluated by superoxide dismutase and catalase activity measurements, whereas the chain-breaking antioxidant efficiency was detected by total antioxidant status assessment. In semen with normal viscosity, unaltered enzymatic and nonenzymatic antioxidant capacity was revealed in the asthenozoospermic specimens, whereas low superoxide dismutase activity was detected in oligoasthenozoospermic samples. On the contrary, impairment of both the scavenger and chain-breaking antioxidative systems was revealed in asthenozoospermic and oligoasthenozoospermic hyperviscous ejaculates, regardless of sperm count. Catalase activity and total antioxidant status values were also reduced in the 2 subgroups of hyperviscous ejaculates compared with their respective matched controls, whereas similar superoxide dismutase activities were detected in oligoasthenozoospermic samples with normal and high consistencies. These results suggest that asthenozoospermia could be related to an antioxidant deficiency only in combined ejaculate pathologies, and that a severe impairment of the low and high molecular weight seminal antioxidative capacities could be associated with semen hyperviscosity.

Key words: Superoxide dismutase, catalase, total antioxidant status, highly viscous ejaculate.

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Aerobic metabolism of human sperm produces different reactive oxygen species (ROS) (H_2O_2, O_2^-, and ·OH), which are potentially harmful to the sperm plasma membrane with its high content of polyunsaturated fatty acids (Jones et al, 1979; Storey et al, 1997). Seminal plasma contains high and low molecular weight factors that protect spermatozoa against free radical toxicity (Kovalski et al, 1992). They include enzymatic ROS scavengers such as Cu,Zn superoxide dismutase (SOD) and catalase (CAT; Alvarez et al, 1987; Jeulin et al, 1989; Aitken et al, 1996); as well as chain-breaking antioxidants such as ascorbate, urate, albumin, glutathione, and taurine (Holmes et al, 1992; Thiele et al, 1995). The imbalance between ROS production and ROS degradation has been hypothesized as a cause of oxidative stress in semen with peroxidative injury to the sperm membrane and a consequent impairment of the related functional properties, such as sperm motility (Sharma and Agarwal, 1996).

Different studies have investigated seminal antioxidant capacity and lipid peroxidative product levels in ejaculates with asthenozoospermia or other altered semen parameters, but the results are controversial (Kobayashi et al, 1991; Zini et al, 1993; Sanocka et al, 1996; Suleiman et al, 1996; Alkan et al, 1997; Jozwik et al, 1997; Lewis et al, 1997; Miesel et al, 1997; Sanocka et al, 1997) and, up to now, the relationship between the antioxidative mechanism deficiency and specific ejaculate pathologies is scarcely known.

The aim of the present work was to investigate the seminal enzymatic and nonenzymatic antioxidant capacity in semen samples that demonstrate asthenozoospermia, oligozoospermia, hyperviscosity, or a combination of these. SOD activity, CAT activity, and the total antioxidant status values were determined in asthenozoospermic and oligoasthenozoospermic samples with normal viscosity and in hyperviscous asthenozoospermic and oligoasthenozoospermic specimens.

Materials and Methods

Reagents

Unless otherwise stated, the reagents were purchased from Sigma (St Louis, Mo).

Subjects

The investigation was carried out on ejaculates from 120 patients (age range 20–40 years) attending the Sterility Treatment Center

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for evaluation of infertility. The samples were categorized on the basis of sperm motility, sperm density, and semen consistency, according to World Health Organization (WHO) criteria (1999) into the following 4 groups: A for asthenozoospermia ($n = 40$), OA for oligoasthenozoospermia ($n = 50$), VA for hyperviscous asthenozoospermia ($n = 14$), and VOA for hyperviscous oligoasthenozoospermia ($n = 16$). The ejaculates of healthy donors ($n = 25$) with normozoospermia and proven fertility (age range 20–40 years) served as a control group (C).

Semen Collection and Analysis

Semen was collected after 3–5 days of sexual abstinence. Routine laboratory analyses (volume, pH, semen consistency, sperm concentration, sperm morphology, and sperm motility) were assessed after liquefaction, according to WHO (1999) criteria. Sperm motility was evaluated in all samples by the same technician by scoring >200 spermatozoa for each specimen. Progressive sperm motility was defined as grade a + b (spermatozoa with rapid progressive motility plus those with slow or sluggish progressive motility). Semen consistency was estimated by introducing a glass rod into the sample and measuring the length of the thread on withdrawal of the rod. Ejaculates with normal consistency had a thread length <2 cm, whereas semen classified as hyperviscous showed a thread length >2 cm. The presence of polymorphonuclear (PMN) granulocytes was assessed by the peroxidase stain using orthotoluidine.

Liquefied semen was centrifuged at 1000 × *g* for 15 minutes, and seminal plasma was stored at −80°C until assay.

Superoxide Dismutase Assay

Seminal SOD activity was measured as follows: superoxide anions generated by the xanthine/xanthine oxidase system react with 2-(p-iodophenhyl)-3-(p-nitrophenol)-5-phenyl tetrazolium chloride (INT; an electron acceptor) to form a red formazan dye (505 nm; Arthur and Boyne, 1985). SOD activity was measured by the degree of inhibition of this reaction (Gavella et al, 1996). The assay medium consisted of 50 mM sodium carbonate (pH 10.2) containing 0.1 mM EDTA, 100 μM xanthine, 25 μM INT, and seminal plasma (diluted 1:10). The reaction was started by the addition of 0.1 U/mL of xanthine oxidase, and the increase of absorbance (505 nm) was continuously monitored at 25°C for 15 minutes. The assay was calibrated using a commercial bovine erythrocyte SOD. One unit of SOD activity was defined as the amount of the enzyme causing a 50% inhibition of the INT reduction rate under assay conditions. The intra-assay and interassay coefficients of variation were 4.5% and 7.5%, respectively.

Catalase Assay

CAT activity was assayed by H_2O_2 concentration decrease after incubation with the sample (Zini et al, 1993). Hydrogen peroxide measurement was based on the horseradish peroxidase-dependent oxidation of phenol red into a blue derivative (Pick and Mizel, 1981). The assay medium, containing 0.05 mM phosphate-buffered saline (PBS) pH 8, 0.05 mM H_2O_2, and seminal plasma was incubated at 20°C for 1 hour. Horseradish peroxidase (20 U/mL) and phenol red (0.56 mM) were then added. The reaction was stopped 5 minutes later by alkalinization (NaOH 50 mM), and the absorbance was read at 630 nm. The assay was calibrated using standard bovine CAT. One unit of CAT activity was defined as the quantity of seminal plasma that was able to decrease 50% of the amount of H_2O_2 present in solution. The intra-assay and the interassay variation coefficients were 4% and 6%, respectively.

Total Antioxidant Status Assay

Nonenzymatic antioxidant activity was evaluated for the ability of seminal plasma to inhibit the reaction of ABTS (2,2′-Azino-di-[3-ethylbenzthiazolinesulphonate]) with metmyoglobin (a peroxidase) and H_2O_2 to produce the cationic radical ABTS+ (Miller et al, 1993; Gavella et al, 1996). Briefly, 0.25 mM H_2O_2 was added to the assay medium containing 80 mM PBS (pH 7.4), 0.6 mM ABTS, 0.006 mM metmyoglobin, and an undiluted sample. The absorbance was then measured (660 nm) after an incubation period of 3 minutes at 37°C (total antioxidant status [TAS] kit from Randox, Crumlin, United Kingdom). The assay was calibrated using standard Trolox, a synthetic tocopherol analogue. The arbitrary TAS units were equivalent to Trolox antioxidant capacity in mmol/L. The intra-assay and interassay coefficients of variation were 3% and 5%, respectively.

Statistical Analysis

Data were analyzed by one-way analysis of variance and the significant differences between groups, when appropriate, were established using the Duncan multiple range test. Significance was assigned at $P < .01$.

Results

Ejaculate Parameters

Ejaculate parameters of the investigated samples are shown in Table 1.

Seminal PMN Granulocytes

No significant difference was detected in the concentration of PMN granulocytes between samples with normal consistency and hyperviscous ejaculates (A = 0.7 ± 0.41 × 10^6 PMN/mL; OA = 0.8 ± 0.52 × 10^6 PMN/mL; VA = 0.72 ± 0.48 × 10^6 PMN/mL; VOA = 0.84 ± 0.59 × 10^6 PMN/mL).

Seminal Zinc and Fructose

Seminal zinc and fructose concentrations of the investigated specimens are illustrated in Table 2. As shown, there are no significant differences in prostatic and vesicular secretion marker values among the groups.

Antioxidant Capacity

Samples With Normal Consistency—SOD and CAT activities were unaltered in the A group compared with controls (SOD: A = 18 ± 7 U/mL, C = 20 ± 8 U/mL; CAT: A = 26 ± 7 U/mL, C = 29 ± 7 U/mL), whereas only a significant decrease of SOD activity was detected in the OA group (SOD: OA = 11 ± 4 U/mL vs C, $F(4/140) = 15.01$, $P < .01$; CAT: OA = 28 ± 6 U/mL; Figure 1). Seminal TAS values in the 2 groups of samples

Table 1. *Ejaculate parameters**

	Volume (mL)	Sperm Count ($\times 10^6$ mL^{-1})	Progressive Sperm Motility (%)	Normal Sperm Forms (%)
Asthenozoospermia (n = 40)	4.2 ± 1.4	70 ± 30	25 ± 10†	50 ± 20
Oligoasthenozoospermia (n = 50)	3.9 ± 1.5	12 ± 5†	15 ± 5†	25 ± 7†
Hyperviscous asthenozoospermia (n = 14)	4.2 ± 1.8	50 ± 24†	26 ± 9†	37 ± 5†
Hyperviscous oligoasthenozoospermia (n = 16)	4.1 ± 1.5	14 ± 5†	13 ± 5†	24 ± 5†
Controls (n = 25)	3.8 ± 1.5	85 ± 40	65 ± 10	54 ± 10

* Values are means ± SD.
† Indicates significantly different from controls ($P < .01$).

with normal viscosity were similar to controls (TAS: A = 1.24 ± 0.2 mmol/L, OA = 1.26 ± 0.21 mmol/L, C = 1.21 ± 0.16 mmol/L; Figure 2).

Samples With Abnormal Consistency—The VA group showed decreased activity of enzymatic and nonenzymatic antioxidant systems alike compared with controls and asthenozoospermic samples with normal viscosity. Specifically:

- SOD: VA = 12.1 ± 3.9 U/mL; vs C, F(4/140) = 15.01, $P < .01$; vs A, F(4/140) = 15.01, $P < .01$.
- CAT: VA = 18 ± 6 U/mL; vs C, F(4/140) = 14.49, $P < .01$; vs A, F(4/140) = 14.49, $P < .01$ (Figure 1).
- TAS: VA = 0.84 ± 0.14 mmol/L; vs C, F(4/140) = 30.36, $P < .01$; vs A, F(4/140) = 30.36, $P < .01$ (see Figure 2).

The VOA group displayed reduced enzymatic and nonenzymatic antioxidant capacities compared with controls, whereas when the same group was compared with the OA group, a significant reduction in CAT activity and TAS values was detected. This is shown as follows:

- SOD: VOA = 13 ± 3.1 U/mL; vs C, F(4/140) = 15.01, $P < .01$.
- CAT: VOA = 17.7 ± 5.5 U/mL; vs C, F(4/140) = 14.49, $P < .01$; vs OA, F(4/140) = 14.49, $P < .01$ (Figure 1).

Table 2. *Seminal zinc and fructose concentrations*

	Zinc (μg/mL)	Fructose (mg/mL)
Asthenozoospermia (n = 40)	210 ± 92	2.7 ± 1.2
Oligoasthenozoospermia (n = 50)	190 ± 80	2.8 ± 0.9
Hyperviscous asthenozoospermia (n = 14)	201 ± 93	2.7 ± 1.0
Hyperviscous oligoasthenozoospermia (n = 16)	204 ± 87	2.5 ± 1.2
Controls (n = 25)	200 ± 80	2.9 ± 1.0

Values are means ± SD. There are no significant differences among the groups.

- TAS: VOA = 0.83 ± 0.03 mmol/L; vs C, F(4/140) = 30.36, $P < .01$; vs OA, F(4/140) = 30.36, $P < .01$ (Figure 2).

Furthermore, no significant difference was detected between the VA and VOA groups for all investigated parameters.

Discussion

Physiological generation of ROS in semen affects sperm functional properties (De Lamirande and Gagnon, 1993; De Lamirande et al, 1998). In addition, ROS overproduction, due to the imbalance between pro-oxidant and antioxidant levels, is highly toxic for plasma sperm membranes and could have a role in the etiology of defective sperm function (Sharma and Agarwal, 1996; Griveau and Le Lannou, 1997). Experimentally induced high levels of ROS can damage sperm motility (Alvarez et al, 1987; Aitken et al, 1993; Griveau et al, 1995). Because of this, different clinical studies have been carried out to investigate free radical levels and antioxidant efficiency in semen that has low sperm motility, but conflicting results were obtained (Kobayashi et al, 1991; Sanocka et al, 1996; Alkan et al, 1997; Jozwik et al, 1997; Lewis et al, 1997; Miesel et al, 1997; Sanocka et al, 1997).

In this study, seminal enzymatic antioxidative properties were evaluated by the activity measurement of Cu,Zn SOD and CAT, which are O_2^- and H_2O_2 scavengers, respectively. The chain-breaking antioxidant capacity was investigated by determining the total antioxidant status, mainly due to ascorbate, urate, and albumin (Gavella et al, 1996).

Seminal plasma containing the antioxidative components derives from sex accessory glands. Therefore, prostatic and vesicular secretion marker levels (zinc and fructose) were determined in the samples in order to investigate a possible defective production of the antioxidants. Normal levels of zinc and fructose from the adnexal

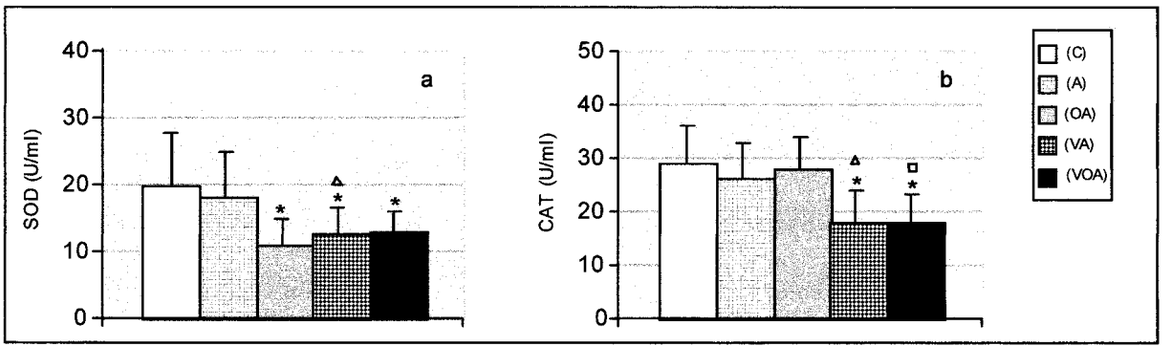

Figure 1. Enzymatic antioxidant activity in seminal plasma of asthenozoospermia (A), oligoasthenozoospermia (OA), hyperviscous asthenozoospermia (VA), and hyperviscous oligoasthenozoospermia (VOA). **(a)** SOD; **(b)** CAT. *$P < .01$ with respect to control (C); □$P < .01$ with respect to A; ▵$P < .01$ with respect to OA.

glands were detected in both samples with normal viscosity and hyperviscous specimens, as previously reported (Carpino et al, 1998; Carpino and Siciliano, 1998).

It has been reported that the presence of abnormal spermatozoa and leukocytospermia can increase ROS production in semen, but their damaging effects on sperm properties are still controversial (Aitken et al, 1988; Rao et al, 1989; Wolff et al, 1990; Gonzales et al, 1992; Kovalski et al, 1992; Tomlinson et al, 1993; Aitken et al, 1994; Plante et al, 1994; Wolff, 1995; Fedder, 1996). According to some authors, ROS generated by leukocytes can be harmful for sperm cells only in the absence of seminal plasma scavenger systems, as in acute testicular or epididymal infections (Wolff, 1995).

PMN granulocytes are the most prevalent type of white blood cells in semen (Fedder, 1996). Their determination in semen samples indicates that PMN concentration was similar in all investigated groups of samples; therefore, it was not considered a discriminating parameter among semen with normal or high consistency and between the asthenozoospermic and oligoasthenozoospermic subgroups within each category.

The ejaculates with normal consistency revealed unaltered seminal enzymatic and nonenzymatic antioxidant capacity in asthenozoospermic samples, whereas only the deficiency of SOD was detected in oligoasthenozoospermic specimens. This suggests that the lowering of sperm motility is not related to the antioxidant systems when it is present as a single altered semen parameter. On the other hand, when asthenozoospermia is combined with oligozoospermia, the low seminal O_2^- scavenging capacity could support an inadequate seminal defense against free radical toxicity, which, in turn, could affect sperm motility. However, with the multifactor etiology of oligoasthenozoospermia, only a limited influence of this single antioxidase deficiency on sperm motility can be hypothesized.

Hyperviscosity of liquefied semen is a biophysical alteration of an ejaculate whose biochemical etiology is scarcely known, despite the different studies that have been carried out on this topic (Gonzales et al, 1993; Mendeluk et al, 1997a,b; Carpino and Siciliano, 1998; Munuce et al, 1999). Only recently, Mendeluk suggested the existence of a highly organized network with an oligosaccharide-peptide core in semen with high consistency (Mendeluk et al, 2000).

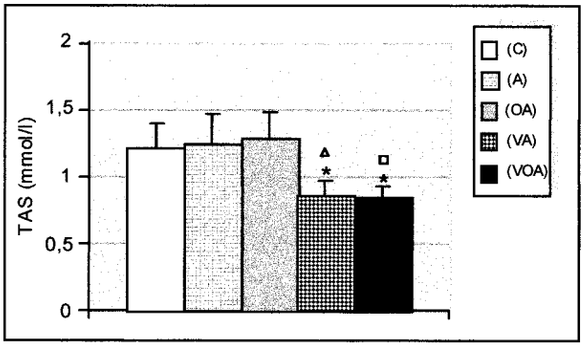

Figure 2. Total antioxidant status values in seminal plasma in asthenozoospermia (A), oligoasthenozoospermia (OA), hyperviscous asthenozoospermia (VA), and hyperviscous oligoasthenozoospermia (VOA). *$P < .01$ with respect to control (C); □$P < .01$ with respect to A; ▵$P < .01$ with respect to OA.

The impairment of the scavenging and chain-breaking antioxidative systems has been demonstrated in the 2 groups of hyperviscous ejaculates, irrespective of sperm count. The same results were obtained when hyperviscous asthenozoospermic and hyperviscous oligoasthenozoospermic specimens were compared with the normally viscous asthenozoospermic and oligoasthenozoospermic ejaculates, with the exception of SOD activity, which was similar in oligoasthenozoospermic samples with normal and abnormal consistencies. Therefore, the lowering of CAT activity and TAS values appeared to be associated with seminal hyperviscosity, whereas the low seminal O_2^- scavenging capacity could be related either to oligoasthenozoospermia or to seminal hyperviscosity.

As reported above, no prostatic or vesicular hypofunction was revealed in highly viscous semen. Therefore, in the absence of available data about the ROS levels in these samples, further investigations are needed to explain the cause of their damaged defenses against ROS injuries.

This study has demonstrated, for the first time, a severe impairment of both the high and low molecular weight antioxidative systems in semen with hyperviscosity. A negative influence of the probable oxidative stress on low sperm motility cannot be excluded. This suggests that treatment with antioxidants could be useful in patients showing abnormal semen consistency to protect sperm cells by peroxidative damage and to improve their functional properties.

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