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Superoxide dismutase isoenzymes in human seminal plasma and spermatozoa

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Superoxide radicals may exert both toxic and physiological regulating actions on spermatozoa. The objective of the present study was to examine the occurrence and distribution of the three superoxide dismutase (SOD) isoenzymes in human seminal plasma and spermatozoa. Human seminal plasma has previously been reported to possess high SOD activity. Here we show that the normally cytosolic CuZn-SOD remarkably accounts for 75% of the activity while the secretory extracellular SOD (EC-SOD) accounts for 25%. Studies of split ejaculates suggest that both these SOD isoenzymes are of primarily prostatic origin. The Mn-SOD activity was negligible. The total SOD activity of seminal plasma was 20 times higher than that of human blood plasma. While native EC-SOD shows high affinity for heparin and heparan sulphate, 90% of the EC-SOD in seminal plasma lacks the high affinity at ejaculation. Thus only a minor part of the seminal plasma EC-SOD has the potential to bind to cell surfaces. Human spermatozoa were found to contain exceptionally large amounts of CuZn-SOD. There was little Mn-SOD activity and the amount of EC-SOD was negligible. We conclude that spermatozoa in semen are exceptionally well protected against superoxide radicals both internally and externally. This should be of importance for both their survival and the integrity of DNA, and may also have physiological effects such as influencing capacitation.

Key words: capacitation/oxygen free radicals/superoxide dismutase/superoxide radicals

Introduction

Oxygen free radicals have been implicated in a variety of circumstances relevant to the function of spermatozoa. Spermatozoa have been shown to release hydrogen peroxide and superoxide radical (Aitken and Clarkson, 1987; Alvarez *et al.*, 1987; Griveau *et al.*, 1995; Zhang and Zheng, 1996). There is a relationship been loss of motility and peroxidation of spermatozoal lipids (Alvarez and Storey, 1982; Rao *et al.*, 1989; Kobayashi *et al.*, 1991; Aitken and Fisher, 1994; MacLeod, 1995; Storey, 1997). Also, the superoxide radical has recently been implicated in the capacitation reaction (de Lamirande *et al.*, 1993; de Lamirande and Gagnon, 1993, 1995; Griveau *et al.*, 1995; Zhang and Zheng, 1996). Reactive oxygen species may thus exert both toxic actions and participate in physiological processes.

The seminal plasma of mammals has been reported to contain large amounts of superoxide dismutase (SOD) activity, much higher than that found in other extracellular fluids (Menella and Jones, 1980; Kobayashi *et al.*, 1991; Miesel *et al.*, 1993; Zhang and Zheng, 1996). Spermatozoa have also been reported to contain SOD activity (Abu-Erreish *et al.*, 1978; Menella and Jones, 1980; Alvarez *et al.*, 1987). In mammals there are three SOD isoenzymes, the cytosolic dimeric CuZn-SOD (McCord and Fridovich, 1969), the mitochondrial matrix Mn-SOD (Weisiger and Fridovich, 1973) and the secretory tetrameric extracellular SOD (EC-SOD) (Marklund, 1982). EC-SOD shows affinity for heparin and heparan sulphate (Karlsson *et al.*, 1988), conferred by a positively charged cluster in the carboxyterminal ends of the subunits (Sandström *et al.*, 1992). The bulk of the EC-SOD in the body exists reversibly anchored to heparan sulphate proteoglycans on cell surfaces and in the tissue interstitial matrix (Marklund and Karlsson, 1989; Karlsson *et al.*, 1994). In the vasculature EC-SOD is in equilibrium between heparan sulphate proteoglycans on the endothelial cell surfaces and the plasma phase (Karlsson and Marklund, 1987; Karlsson and Marklund, 1988; Karlsson *et al.*, 1993b). Plasma also contains EC-SOD with reduced heparin-affinity resulting from truncations (Sandström *et al.*, 1993) and other modifications (Adachi *et al.*, 1991) of the carboxyterminal ends. Since the substrate, the superoxide anion radical, crosses membranes poorly (Winterbourn and Stern, 1987), the SOD isoenzymes must exert distinct protective roles in their respective compartments.

Despite the purported importance of the superoxide radical and other reactive oxygen species, no comprehensive studies regarding the SOD isoenzymes in human semen have been reported. In the present study the occurrence and distribution of CuZn-SOD, Mn-SOD and EC-SOD in human seminal plasma and spermatozoa was investigated.

Materials and methods

Collection of seminal plasma and spermatozoa

After 3 days of abstinence, ejaculates were collected from healthy men, aged 25–40 years. The first ejaculary portion and the rest of the

ejaculate were collected separately. Each specimen was immediately centrifuged (3000 *g*, 20 min) to separate spermatozoa from the seminal plasma. Next, the spermatozoa were suspended in physiological saline, followed by another centrifugation. The seminal plasma specimen and the spermatozoal pellets were stored at –70° C until assay.

Analysis of SOD isoenzymes in seminal plasma and spermatozoa

To the spermatozoal pellets was added 0.5 ml ice-cold 50 mM Na phosphate, pH 7.4, containing 0.3 M KBr, 0.5 mM phenylmethanesulphonylfluoride (Sigma, St Louis, MO, USA), 90 mg/ml aprotinin (Bayer GmbH, Leverkusen, Germany), and 10 mg/l pepstatin, 10 mg/l chymostatin, and 10 mg/l leupeptin (Boehringer Mannheim, Mannheim, Germany). The spermatozoa were homogenized by sonication under cooling with ice, followed by centrifugation (20 000 *g*, 15 min). The SOD activities, the EC-SOD, protein (Bradford, 1976) and DNA (Labarca and Paigen, 1980) contents were determined in the supernatants.

The seminal plasma specimens diluted with 20 volumes of 0.15 M NaCl were incubated overnight at 4°C with: (i) Sepharose 4B (Pharmacia Biotech, Uppsala, Sweden) (sham incubation); (ii) antiCuZn-SOD immobilized on Sepharose 4B and (iii) antiCuZn-SOD plus anti-EC-SOD immobilized on Sepharose 4B (Öhman and Marklund, 1986; Marklund, 1984). The SOD activities and EC-SOD contents were analysed in supernatants after centrifugation. The antibodies were raised in rabbits in the laboratory using human CuZn-SOD and EC-SOD. There was no unspecific binding of SOD to the Sepharose 4B, and there was no antigenic cross-reactivity between CuZn-SOD and EC-SOD (Marklund, 1984). The specific activity of EC-SOD was calculated from the differences in SOD activities and EC-SOD contents between the second and third incubations. The specific activities varied between individuals, 12.0 ± 1.5 ng/IU (mean ± SD, $n = 10$), but were remarkably similar between the two split ejaculate samples. The EC-SOD activity of the seminal plasma samples was calculated from the specific activities and the EC-SOD contents of the samples as determined by an enzyme-linked immunosorbent assay (ELISA). The Mn-SOD activity was obtained from the activity in the presence of 3 mM cyanide and finally the CuZn-SOD activity as the total SOD activity of the specimen minus the activities of the two other isoenzymes.

SOD enzymic activity was determined using the direct spectrophotometric method employing KO_2 (Marklund, 1976) as modified in (Marklund, 1985). Cyanide (3 mM) was used to distinguish between the cyanide-sensitive isoenzymes CuZn-SOD and EC-SOD and the resistant Mn-SOD. One unit corresponds to 8.3 ng of bovine Mn-SOD. The 'KO_2 assay' is performed at pH 9.5 and at relatively high superoxide concentrations. In comparison, the xanthine oxidase–cytochrome c SOD assay (McCord and Fridovich, 1969) is carried out under more physiological conditions, i.e. neutral pH and low superoxide concentrations. One unit in the 'KO_2 assay' corresponds to about 0.024 units of CuZn-SOD and EC-SOD and 0.24 units of Mn-SOD respectively in the 'xanthine oxidase assay'. Thus the 'KO_2 assay' is about 10 times more sensitive for CuZn-SOD and EC-SOD activity than MnSOD activity.

EC-SOD protein concentration was determined using an ELISA which used a monoclonal anti-human EC-SOD antibody (Karlsson and Marklund, 1988).

Size exclusion chromatography

Semen was directly ejaculated into a roughly equal volume of 0.15 M NaCl containing antiproteolytic agents (final concentrations in the mixture: Pefabloc SC 1.5 mM (Boehringer Mannheim), diethylenetriaminepentaacetic acid 3.8 mM (Sigma), aprotinin 400 mg/l (Bayer), and pepstatin, chymostatin, leupeptin all 45 mg/l (Boehringer Mannheim)). After immediate mixing, the solution was centrifuged (20 000 *g*, 15 min) and the supernatant collected and stored at –80°C. Thereafter 2 ml of the solution was chromatographed on a Superdex 200 column (1.6 × 60 cm) (Pharmacia Biotech) and eluted in 10 mM K phosphate, pH 7.4/0.15 M NaCl. The SOD activity, the EC-SOD content, and the absorbance at 280 nm were determined in collected fractions.

Table 1. Superoxide dismutade (SOD) isoenzymes in human seminal plasma

	EC-SOD U/ml	CuZn-SOD U/ml	Mn-SOD U/ml	Total SOD U/ml
First ejaculatory portion	218 ± 88	559 ± 193	2.0 ± 1.1	780 ± 269
Remaining portion	103 ± 76	318 ± 181	2.4 ± 1.3	423 ± 253
Total specimen	141 ± 65	398 ± 150	2.3 ± 0.9	541 ± 207
Blood plasma	19.6 ± 5.5	2.8 ± 1.7	4.5 ± 1.0	

Ten seminal plasma specimens were collected as split ejaculates and analysed as described under Materials and methods. The results are presented as means ± SD. For comparison the contents of the SOD isoenzymes in human blood plasma ($n = 16$) is presented (Marklund *et al.*, 1986).

Heparin–sepharose chromatography

Semen was ejaculated into a tube, and placed in a shaking thermostat bath at 37°C. After different time intervals, 1 min, 15 min, 30 min, 1 h, 3 h and 6 h, 150 µl aliquots were added to 3 ml 15 mM sodium cacodylate (Serva Feinbiochemica, Heidelberg, Germany), pH 6.50/50 mM NaCl containing antiproteolytic agents (benzamidine 1 mM (Sigma), Pefabloc SC 1 mM, aprotinin 150 mg/l and pepstatin, chymostatin, leupeptin all 17 mg/l). Immediately the solution was centrifuged (20 000 *g*, 15 min) and the supernatant was stored at –80°C until further analysis. 1 ml of the supernatant was thereafter applied to a 1 ml heparin–sepharose column and eluted with the above described cacodylate buffer. Bound components were thereafter desorbed with a linear gradient of 0–1 M NaCl in cacodylate buffer. In the collected fractions the EC-SOD protein content was determined with ELISA and the chloride content with a chloride titrator. SOD activity of the fractions could not be determined since the inclusion of benzamidine in the antiproteolytic solution interfered with the assay of the SOD activity.

Results

SOD isoenzymes in human seminal plasma

As an extracellular fluid, human seminal plasma displays a very high total SOD activity, about 20-fold higher than that of human plasma (Table I). The distribution of the isoenzyme is remarkable, with the normally cytosolic CuZn-SOD accounting for 75% and the secretory EC-SOD for 25% of the total activity. The activity of the normally mitochondrial Mn-SOD (= cyanide resistant SOD activity) is very low. In blood plasma the secretory EC-SOD predominates, whereas the presence of the small amounts of CuZn-SOD and Mn-SOD are likely to be due to leakage from the intracellular space.

To gain some insight into the source of the SOD isoenzymes, split ejaculates were studied. The activities of both CuZn-SOD and EC-SOD were higher in the first portion, suggesting that the prostate gland is the main but not necessarily sole source of the SOD isoenzymes.

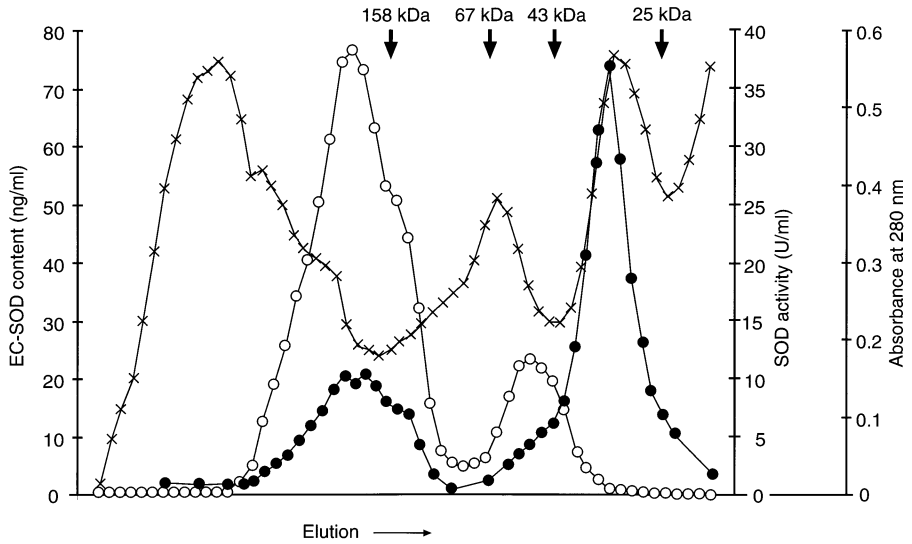

Figure 1. Size exclusion chromatography of seminal plasma. Seminal plasma was separated on a Superdex 200 column as described in the Materials and methods section. The absorbance at 280 nm was monitored (X), and the SOD activity (●) and EC-SOD content (O) determined in collected fractions. The elution positions of separately chromatographed molecular mass markers are indicated in the chromatogram; aldolase 158 kDa, bovine serum albumin 67 kDa, ovalbumin 43 kDa and chymotrypsinogen 25 kDa.

Analysis by size exclusion chromatography

Upon separation on a Superdex 200 column, the SOD activity of seminal plasma is divided into two main peaks (Figure 1). The smaller, which elutes at an apparent molecular mass of 176 kDa, coincides with the main peak of EC-SOD protein. There is also a smaller EC-SOD protein peak eluting at about 54 kDa, which may represent proteolytically degraded enzyme in a mono- to dimeric state. The main SOD activity peak elutes at 33.5 kDa. The four fractions containing the largest amounts of SOD activity in this peak were incubated overnight with anti-CuZn-SOD immobilized on Sepharose 4B; <0.45 U/ml SOD activity remained in the non-bound fraction, showing that this peak represents CuZn-SOD. The SOD isoenzymes thus elute at the expected positions in the chromatogram. There was no SOD activity at the void volume showing that the enzymes are free in solution.

Heparin-affinity of EC-SOD in seminal plasma

Figure 2 shows the result of a heparin–sepharose chromatography of a seminal plasma specimen, followed 1 min after ejaculation by dilution of the semen into a solution containing antiproteolytic agents. Already at this early time only 10% of the enzyme shows the high heparin affinity of native EC-SOD, which elutes at 0.55 M NaCl. 20% of the EC-SOD shows a minimal heparin-affinity, but 70% lacks affinity. Samples treated with antiproteolytic agents after longer incubation at 37°C showed similar patterns and the high heparin-affinity peak still accounted for 6.5% of the total amount of EC-SOD after 6 h (chromatograms not shown). To test the efficacy of the antiproteolytic treatment, a small amount (final concentration 200 ng/ml) of recombinant human EC-SOD (Tibell *et al.*, 1987) was added to the diluted 1 min seminal plasma sample and incubated for 60 min at 25°C. No reduction in the heparin-affinity of the added EC-SOD could be detected by heparin–sepharose chromatography (not shown).

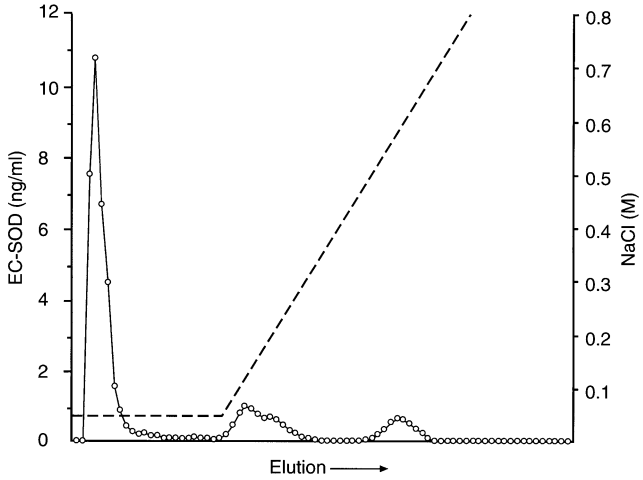

Figure 2. Separation of seminal plasma on heparin–sepharose. Seminal plasma treated with antiproteolytic agents 1 min after ejaculation was chromatographed as described in the Materials and methods section. O = EC-SOD content and – – – = NaCl gradient.

SOD isoenzymes in human spermatozoa

The content of SOD isoenzymes in spermatozoa are presented in Table II, together with results for some tissues extracted and analysed in the same manner (Sandström *et al.*, 1993). The activity of the cytosolic CuZn-SOD of spermatozoa is exceptionally high, but the activity of the mitochondrial Mn-SOD is low. The content of the secretory EC-SOD is very low.

Discussion

The seminal plasma contains a high SOD activity, and surprisingly CuZn-SOD accounts for 75% of the activity. CuZn-SOD is not synthesized with a signal peptide, is mainly located in the cytosol and nucleus (McCord and Fridovich, 1969), and

Table II. Superoxide dismutade (SOD) isoenzymes in human spermatozoa. Eleven sets of normal human spermatozoa were collected and analysed as described under Materials and methods. The data are presented as means ±SD. For comparison mean values for a number of human tissues extracted and analysed similarly (Sandström *et al.*, 1993) are presented.

	EC-SOD		CuZn-SOD		Mn-SOD	
	U/mg protein	U/mg DNA	U/mg protein	U/mg DNA	U/mg protein	U/mg DNA
Spermatozoa	2.11 ± 1.25	51 ± 37	518 ± 129	14600 ± 5500	2.4 ± 2.7	64 ± 37
Kidney (n = 4)	12	297	377	8700	24	593
Lung (n = 2)	17	231	192	1280	8.4	111
Prostate (n = 3)	40	714	193	3540	25	350
Testis (n = 1)	14	263	214	4040	1.9	36
Uterus (n = 2)	49	967	130	3530	3.3	59
Placenta (n = 3)	7.8	175	197	4690	0.8	27

has never been shown to be secreted. In comparison the blood plasma content is negligible (Table I). The prostate gland appears to be the main source of the CuZn-SOD according to the split ejaculate study. The activity in the final seminal plasma is ~10% of that of prostate gland cytosol (Sandström *et al.*, 1993). Some apocrinic secretion may occur in the prostate gland epithelium, which might explain the presence of CuZn-SOD in the seminal plasma (Aumuller and Seitz, 1990). However, other not yet characterized secretion mechanisms in the prostate and in other parts of the genitourinary system cannot be excluded. The enzyme is not associated with, for example, prostasomes (Ronquist and Brody, 1985) according to the size exclusion chromatography study (Figure 1). The presence of large amounts of the signal peptide-containing secretory EC-SOD is less surprising, and the main source may again be the prostate gland. The gland itself contains comparatively large amounts of EC-SOD (Table II). In the rat, epididymis contains extremely large amounts of mRNA encoding EC-SOD and the cauda epididymal plasma has a very high SOD activity (Perry *et al.*, 1993). This suggests that epididymis may also be an important source in man. The seminal plasma content of EC-SOD is much larger than that seen in blood plasma (Table I).

The most functionally distinguishing property of EC-SOD is its high affinity for heparin and heparan sulphate. The major portion of EC-SOD in the human body, exists bound to heparan sulphate on cell surfaces and in tissue interstitial matrix (Marklund and Karlsson, 1989; Karlsson *et al.*, 1994). Native recombinant EC-SOD as well as EC-SOD isolated from tissues including the prostate gland (Tibell *et al.*, 1987; Sandström *et al.*, 1993) shows high affinity for heparin and elutes at 0.55 M NaCl from a heparin–sepharose column (Figure 2). Such high affinity is necessary for efficient binding to the weaker ligand heparan sulphate (Sandström *et al.*, 1993). In contrast, 1 min post-ejaculation only 10% of the EC-SOD in the seminal plasma showed high heparin affinity. Incubation of the semen at 37°C for up to 6 h resulted in only minor additional loss of heparin affinity. The carboxyterminal heparin-binding domains of EC-SOD are highly susceptible to truncations by proteases such as trypsin and plasmin, but the rest of the molecule is relatively resistant (Karlsson *et al.*, 1993a). The low heparin-affinity of seminal plasma EC-SOD is likely caused by the trypsin-like prostate-specific antigen which is secreted in very high concentration by the prostate. Extensive proteolysis appears to occur during storage before ejaculation. Because of the low heparin affinity of the major part of the EC-SOD in semen, there should be little binding of EC-SOD to cell surfaces in vagina and other locations, and to spermatozoa.

Spermatozoa have previously been shown to contain CuZn-SOD activity (Abu-Erreish *et al.*, 1978; Menella and Jones, 1980), and the presence of some Mn-SOD activity has also been indicated (Menella and Jones, 1980). Here we show that by comparison with a number of human tissues, the CuZn-SOD activity of human spermatozoa is exceptionally high. The activity is also several-fold larger than the activities we have previously found in more than 50 different cultured human cell lines (Marklund *et al.*, 1982; Marklund, 1990). On the other hand the Mn-SOD activity is very low, despite ample occurrence of mitochondria in spermatozoa (Saaranen *et al.*, 1989). There was very little EC-SOD associated with the spermatozoa. It is not known whether spermatozoa contain surface-associated heparan sulphate that could mediate binding of EC-SOD, but the small portion of EC-SOD with high heparin affinity in the seminal plasma would still allow only minimal binding.

The SOD activity of mammalian seminal plasma is, since it is an extracellular fluid, overall very high although it varies between species (Menella and Jones, 1980). However, when compared with human tissues he activity of human seminal plasma is ~10-fold lower per unit volume (Marklund *et al.*, 1982; Sandström *et al.*, 1993). A high SOD activity may have been conserved during evolution, since it has been observed also in the filarium *Brugia malayi* (Ou *et al.*, 1995). Seminal plasma also contains large amounts of other antioxidants including ascorbate, urate (Aitken and Fisher, 1994) and thiols (Miesel *et al.*, 1993). Finally, the spermatozoa are exceptionally well equipped with CuZn-SOD. This comprehensive multifactorial antioxidant armament may be important for reducing mutagenic effects of reactive oxygen species during spermatogenesis, storage and post-ejaculation. The addition of SOD has been shown to preserve the viability of spermatozoa (Kobayashi *et al.*, 1991), and a high endogenous content of SOD delays onset of lipid peroxidation and loss of motility (Alvarez *et al.*, 1987; Storey, 1997). Also, the ratio between seminal plasma SOD activity and the activity of the oxygen radical-producing enzyme xanthine oxidase is positively correlated with the spermatozoal progressive motility (Kurpisz *et al.*, 1996). The presence of a large SOD activity in seminal plasma

may serve an additional function. Superoxide radicals have been shown to induce hyperactivation and capacitation (de Lamirande *et al.*, 1993; De Lamirande and Gagnon, 1995; Zhang and Zheng, 1996), and spermatozoa have been shown to release the radical particularly in the hyperactivated state (Aitken and Clarkson, 1987; Griveau *et al.*, 1995; Zhang and Zheng, 1996). External SOD activity suppresses hyperactivation (De Lamirande *et al.*, 1993; de Lamirande and Gagnon, 1995; Zhang and Zheng, 1996). The capacitation process is transient and can only occur once in spermatozoa (Cohen-Dayag *et al.*, 1995). The high SOD activity of seminal plasma would suppress that reaction in the male genitourinary system and in the vagina. After movement to the upper female genital tract, the spermatozoa will encounter a much lower external SOD activity allowing a timely hyperactivation and capacitation. Of interest in this context is the marked increase in oxygen tension in the oviduct that occurs upon ovulation (Maas *et al.*, 1976). The reason for the occurrence of large amounts of both the normally cytosolic CuZn-SOD and the secretory EC-SOD in seminal plasma is obscure, particularly since the distinguishing cell-surface affinity of EC-SOD is essentially absent. The isoenzymes may serve as backups for each other; indeed male mice lacking EC-SOD (Carlsson *et al.*, 1995) or CuZn-SOD (Reaume *et al.*, 1996) are fully fertile. We conclude that spermatozoa in semen are exceptionally well protected against superoxide radicals, both internally and externally. This should be of importance for both their survival and DNA integrity, and may also have physiological effects including an influence on capacitation.

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References

Abu-Erreish, G., Magnes, L. and Li, T.-K. (1978) Isolation and properties of superoxide dismutase from ram spermatozoa and erythrocytes. *Biol. Reprod.*, **18,** 554–560.

Adachi, T., Ohta, H., Hirano, K. *et al.* (1991) Nonenzymic glycation of human extracellular-superoxide dismutase. *Biochem. J.*, **279,** 263–267.

Aitken, R.J. and Clarkson, J.S. (1987) Cellular basis of defective sperm function and its association with the genesis of reactive oxygen species by human spermatozoa. *J. Reprod. Fertil.*, **81,** 459–469.

Aitken, R.J. and Fisher, H. (1994) Reactive oygen species generation and human spermatozoa: the balance of benefit and risk. *BioEssays*, **16,** 259–267.

Alvarez, J.G. and Storey, B.T. (1982) Spontaneous lipid peroxidation in rabbit epididymal spermatozoa. Its effect on sperm motility. *Biol. Reprod.*, **27,** 1102–1108.

Alvarez, J.G., Touchstone, J.C., Blasco, L. and Storey, B.T. (1987) Spontaneous lipid peroxidation and production of hydrogen peroxide and superoxide in human spermatozoa. Superoxide dismutase as major enzyme protectant against oxygen toxicity. *J. Androl.*, **8,** 338–348.

Aumuller, G. and Seitz, J. (1990) Protein secretion and secretory processes in male accessory glands. *Int. Rev. Cytol.*, **121,** 127–231.

Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72,** 248–254.

Carlsson, L.M., Jonsson, J., Edlund, T. and Marklund, S.L. (1995) Mice lacking extracellular-superoxide dismutase are more sensitive to hyperoxia. *Proc. Natl. Acad. Sci. USA*, **92,** 6264–6268.

Cohen-Dayag, A., Tur-Kaspa, I., Dor, J. *et al.* (1995) Sperm capacitation in humans is transient and correlates with chemotactic responsiveness to follicular factors. *Proc. Natl. Acad. Sci. USA*, **92,** 11039–11043.

de Lamirande, E. and Gagnon, C. (1993) A positive role for the superoxide anion in triggering hyperactivation and capacitation of human spermatozoa. *Int. J. Androl.*, **16,** 21–25.

de Lamirande, E. and Gagnon, C. (1995) Capacitation-associated production of superoxide anion by human spermatozoa. *Free Radic. Biol. Med.*, **18,** 487–495.

de Lamirande, E., Eiley, D. and Gagnon, C. (1993) Inverse relationship between the induction of human sperm capacitation and spontaneous acrosome reaction by various biological fluids and the superoxide scavenging capacity of these fluids. *Int. J. Androl.*, **16,** 258–266.

Grivevau, J.F., Renard, P. and le Lannou, D. (1995) Superoxide anion production by human spermatozoa as a part of the ionophore-induced acrosome reaction process. *Int. J. Androl.*, **18,** 67–74.

Karlsson, K. and Marklund, S.L. (1987) Heparin-induced release of extracellular-superoxide dismutase to human blood plasma. *Biochem. J.*, **242,** 55–59.

Karlsson, K. and Marklund, S.L. (1988) Plasma clearance of human extracellular-superoxide dismutase C in rabbits. *J. Clin. Invest.*, **82,** 762–766

Karlsson, K., Lindahl, U. and Marklund, S.L. (1988) Binding of human extracellular-superoxide dismutase C to sulphated glycosaminoglycans. *Biochem. J.*, **256,** 29–33.

Karlsson, K., Edlund, A., Sandström, J. and Marklund, S.L. (1993a) Proteolytic modification of the heparin-binding affinity of extracellular-superoxide dismutase. *Biochem. J.*, **290,** 623–626

Karlsson, K., Sandström, J., Edlund, A. *et al.* (1993b) Pharmacokinetics of extracellular-superoxide dismutase in the vascular system. *Free Radic. Biol. Med.*, **14,** 185–190

Karlsson, K., Sandström, J., Edlund, A. and Marklund, S.L. (1994) Turnover of extracellular-superoxide dismutase in tissues. *Lab. Invest.*, **70,** 705–710

Kobayashi, T., Miyazaki, T., Natori, M. and Nozawa, S. (1991) Protective role of superoxide dismutase in human sperm motility. Superoxide dismutase activity and lipid peroxide in human seminal plasma and spermatozoa. *Hum. Reprod.*, **6,** 987–991.

Kurpisz, M., Miesel, R., Sanocka, D. and Jedrzejczak, P. (1996) Seminal plasma can be a predictive factor for male infertility. *Hum. Reprod.*, **11,** 1223–1226.

Labarca, C. and Paigen, K. (1980) A simple, rapid and sensitive DNA assay procedure. *Anal. Biochem.*, **102,** 344–352.

Maas, D.H.A., Storey, B.T., and Mastroianni, L., Jr. (1976) Oxygen tension in the oviduct of the rhesus monkey (*Macaca mulatta*). *Fertil. Steril.*, **27,** 1312–1317.

MacLeod, J. (1995) The role of oxygen in the metabolism and motility of human spermatozoa. *Am. J. Physiol.*, **138,** 512–518.

Marklund, S.L. (1976) A spectrophotometric study of the spontaneous disproportionation of the superoxide anion radical, and a sensitive direct assay for superoxide dismutase. *J. Biol. Chem.*, **251,** 7504–7507.

Marklund, S.L. (1982) Human copper-containing superoxide dismutase of high molecular weight. *Proc. Natl. Acad. Sci. USA*, **79,** 7634–7638.

Marklund, S.L. (1984) Properties of extracellular superoxide dismutase from human lung. *Biochem. J.*, **220,** 269–272.

Marklund, S.L. (1985) Direct assay of superoxide dismutase with potassium superoxide. In Greenwald, R.A. (ed.), *Handbook of Methods for Oxygen Radical Research.* Boca Raton, CRC Press Inc, pp. 249–255.

Marklund, S.L. (1990) Expression of extracellular-superoxide dismutase by human cell lines. *Biochem. J.*, **266,** 213–219.

Marklund, S.L. and Karlsson, K. (1989) Binding of human extracellular-superoxide dismutase C to cultured cell lines and to blood cells. *Lab. Invest.*, **60,** 659–666.

Marklund, S.L., Westman, G., Lundgren, E. and Roos, G. (1982) Copper- and zinc-containing superoxide dismutase, manganese-containing superoxide dismutase, catalase, and glutathione peroxidase in normal and neoplastic human cell lines and normal human tissues. *Cancer Res.*, **42,** 1955–1961.

Marklund, S.L., Westermarck, T. and Santavuori, P. (1986) Superoxide dismutase isoenzymes in cerebrospinal fluid and plasma from patients with neuronal ceroid-lipofuscinoses. *Clin. Sci.*, **71,** 57–66.

McCord, J.M. and Fridovich, I. (1969) Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). *J. Biol. Chem.*, **244,** 6049–6055.

Menella, M.R.F. and Jones, R. (1980) Properties of spermatozoal superoxide dismutase and lack of involvement of superoxides in metal-ion-catalysed lipid-peroxidation reactions in semen. *Biochem. J.*, **191,** 289–297.

Miesel, R., Drezejczak, P.J.E. and Kurpisz, M. (1993) Oxidative stress during the interaction of gametes. *Biol. Reprod.*, **49,** 918–923.

Öhman, M. and Marklund, S.L. (1986) Plasma extracellular-superoxide dismutase and erythrocyte CuZn superoxide dismutase in alcoholics treated with disulfiram. *Clin. Sci.*, **70,** 365–369.

Ou, X., Tang, L., McCrossan, M. *et al.* (1995) *Brugia malayi*: localisation and differential expression of extracellular and cytoplasmic CuZn superoxide dismutases in adults and microfilariae. *Exp. Pathol.*, **80,** 515–529.

Perry, A.C.F., Jones, R. and Hall, L. (1993) Isolation and characterization of a rat cDNA clone encoding a secreted superoxide dismutase reveals the epididymis to be a major site of its expression. *Biochem. J.*, **293,** 21–25.

Rao, B., Soufir, J.C. and David, G. (1989) Lipid peroxidation in human spermatozoa as related to midpiece abnormalities and motility. *Gamete Res.*, **4,** 127–134.

Reaume, A.G., Elliott, J.L., Hoffman, E.K. *et al.* (1996) Motor neurons in CuZn superoxide dismutase-deficient mice develop normally but exhibit enhanced cell death after axonal injury. *Nat. Genet.*, **13,** 43–47.

Ronquist, G. and Brody, I. (1985) The prostasome: its secretion and function in man. *Biochim. Biophys. Acta*, **2,** 203–218.

Saaranen, M., Suistomaa, U. and Vanha-Perttula, T. (1989) Semen selenium content and sperm mitochondrial volume in human and some animal species. *Hum. Reprod.*, **4,** 304–408.

Sandström, J.M., Carlsson, L., Marklund, S.L. and Edlund, T. (1992) The heparin-binding domain of extracellular-superoxide dismutase, and formation of variants with reduced heparin affinity. *J. Biol. Chem.*, **267,** 18205–18209.

Sandström, J., Karlsson, K., Edlund, T. and Marklund, S.L (1993) Heparin-affinity patterns of extracellular-superoxide dismutase from human tissues and plasma. *Biochem. J.*, **294,** 853–857.

Storey, B.T. (1987) Biochemistry of the induction and prevention of lipoperoxidative damage in human spermatozoa. *Mol. Hum. Reprod.*, **3,** 203–213.

Tibell, L., Hjalmarsson, K., Edlund, T. *et al.* (1987) Expression of human extracellular-superoxide dismutase in chinese hamster ovary cells and characterization of the product. *Proc. Natl. Acad. Sci. USA*, **84,** 6634–6638.

Weisiger, R.A. and Fridovich, I. (1973) Mitochondrial superoxide dismutase: site of synthesis and intramitochondrial localization. *J. Biol. Chem.*, **248,** 4793–4796.

Winterbourn, C.C. and Stern, A. (1987) Human red cells scavenge extracellular hydrogen peroxide and inhibit formation of hypochlorous acid and hydroxyl radical. *J. Clin. Invest.*, **80,** 1486–1491.

Zhang, H. and Zheng, R.-L. (1996) Promotion of human sperm capacitation by superoxide anion. *Free Rad. Res.*, **24,** 261–268.

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