

# Superoxide Dismutase Activities of Spermatozoa and Seminal Plasma Are Not Correlated With Male Infertility

Yao-Yuan Hsieh,<sup>1</sup> Yu-Ling Sun,<sup>2</sup> Chi-Chen Chang,<sup>1</sup> Yi-San Lee,<sup>2</sup>  
Horng-Der Tsai,<sup>1</sup> and Chich-Sheng Lin<sup>2\*</sup>

<sup>1</sup>Department of Obstetrics and Gynecology, China Medical College Hospital, Taichung, Taiwan

<sup>2</sup>Department of Biological Science and Technology, National Chiao Tung University, Hsinchu, Taiwan

Abnormal reactive oxygen species (ROS) production is associated with defective sperm function. Superoxide dismutase (SOD) is related with the scavenging of seminal ROS. We aimed to determine the effect of SOD activities of spermatozoa and seminal plasma on sperm quality. Semen samples from infertile couples who consented to the analyses were divided into two groups: 1) normospermia (n = 20); and 2) oligoasthenozoospermia (n = 31). The SOD activities of the spermatozoa and seminal plasma were measured by determining the inhibition of pyrogallol autoxidation. The SOD activities of spermatozoa and seminal plasma in both groups were compared. The relationships between the SOD activities and the sperm qualities were determined. We noted that SOD activities of

sperm/seminal plasma in both groups were nonsignificantly different (group 1 vs. 2 =  $0.77 \pm 0.33/0.84 \pm 0.40$  U/mg protein for sperm, and  $0.66 \pm 0.36/0.83 \pm 0.47$  U/ml for seminal plasma). SOD activities of sperm/seminal plasma were positively but nonsignificantly correlated with the sperm motility (SOD of sperm =  $0.0008 \times \text{motility} + 0.67$ ; SOD of seminal plasma =  $0.0006 \times \text{motility} + 0.81$ ) and concentration (SOD of sperm =  $0.0006 \times \text{concentration} + 0.67$ ; SOD of seminal plasma =  $0.0021 \times \text{concentration} + 0.73$ ). We concluded that SOD activities of sperm and seminal plasma were nonsignificantly correlated with the seminal quality. It appears that the SOD survey is not a useful tool for determining sperm fertilization potential. *J. Clin. Lab. Anal.* 16:127–131, 2002. © 2002 Wiley-Liss, Inc.

**Key words:** antioxidant; male infertility; oligoasthenozoospermia; reactive oxygen species; sperm; superoxide dismutase

## INTRODUCTION

Reactive oxygen species (ROS) play an important role in human reproduction. Free radicals have beneficial or detrimental effects upon sperm functions, depending on their nature and concentration. The generations of ROS, such as the superoxide anion, hydrogen peroxide, and hydroxyl radical, can result in damage to cell membranes. Spermatozoa are highly sensitive to injuries caused by high ROS concentration (1). Excessive generation of ROS in semen, mainly by neutrophils but also by abnormal spermatozoa, could be a cause of infertility.

The major ROS generated by human spermatozoa is the superoxide anion. Superoxide dismutase (SOD), an ROS scavenger, catalyzes the further transformation of superoxide anion into hydrogen peroxide (2). The disorder in the regulation of the SOD may be related with the pathology of certain types of male infertility. Decreased seminal plasma antioxidant activity and increased ROS production can be responsible for idiopathic male infertility (3). Treatment with oxygen radical scavengers provides significant rescue of testicular function

(4). There are few reports in the literature concerning the effects of SOD of spermatozoa and seminal plasma upon sperm concentration and motility. This study is among the first such reports.

## MATERIALS AND METHODS

This study included 51 infertile couples who consented to the semen analyses. Semen samples were obtained by masturbation after at least 72 hr of sexual abstinence, less than 30 min after ejaculation. Samples were collected into sterile containers for immediate transportation to the laboratory. This series was approved by the Ethics Committee and Institu-

\*Correspondence to: Chich-Sheng Lin, Ph.D., Department of Biological Science and Technology, National Chiao Tung University, 1001 Ta Hsueh Road, Hsinchu, Taiwan. E-mail: lincs@cc.nctu.edu.tw

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tional Review Board of the China Medical College Hospital. Informed consents were signed by all couples who donated their semen.

After liquefaction, samples were analyzed for volume, leukocyte count, and sperm morphology according to WHO guidelines (5). Then a 6- $\mu$ l aliquot of each specimen was loaded into a 30-mm microcell slide and subjected to computer-assisted semen analysis (CASA) with the use of an HTM-S motility analyzer (Hamilton-Thorne Research, Beverly, MA). All semen samples were divided into two groups: 1) idiopathic infertility (normospermia,  $n = 20$ ); and 2) male infertility (oligo- or asthenozoospermia,  $n = 31$ , count  $<20 \times 10^6$ /ml, motility  $<50\%$ ). Then the semen was centrifuged to separate sperm from seminal plasma. Seminal plasma and homogenate sperm were loaded into an Eppendorf tube and stored in a liquid nitrogen tank before SOD analyses.

SOD activity was measured by determining the inhibition of pyrogallol autoxidation (6). A 0.1-ml sample was added to 2.7 ml of 50 mM Tris-cacodylic acid buffer with 1 mM EDTA (pH 8.2). Then 0.2 ml of 0.2 mM pyrogallol was added to immediately determine changes in absorbance at 420 nm for 5 min. The rate of pyrogallol autoxidation was taken from the decrease in  $A_{420}$  per min. An inhibition of 50% by sample was defined as 1 unit SOD. Results were reported as U/ml for seminal plasma or U/mg of protein for sperm.

The SOD activities in both seminal plasma and sperm in the two groups were determined and compared. The relationships between the SOD activities and the sperm motility or concentrations were determined. The SAS system with *t*-test and a linear regression model were utilized for statistical analyses. A *P*-value of  $<0.05$  was considered statistically significant.

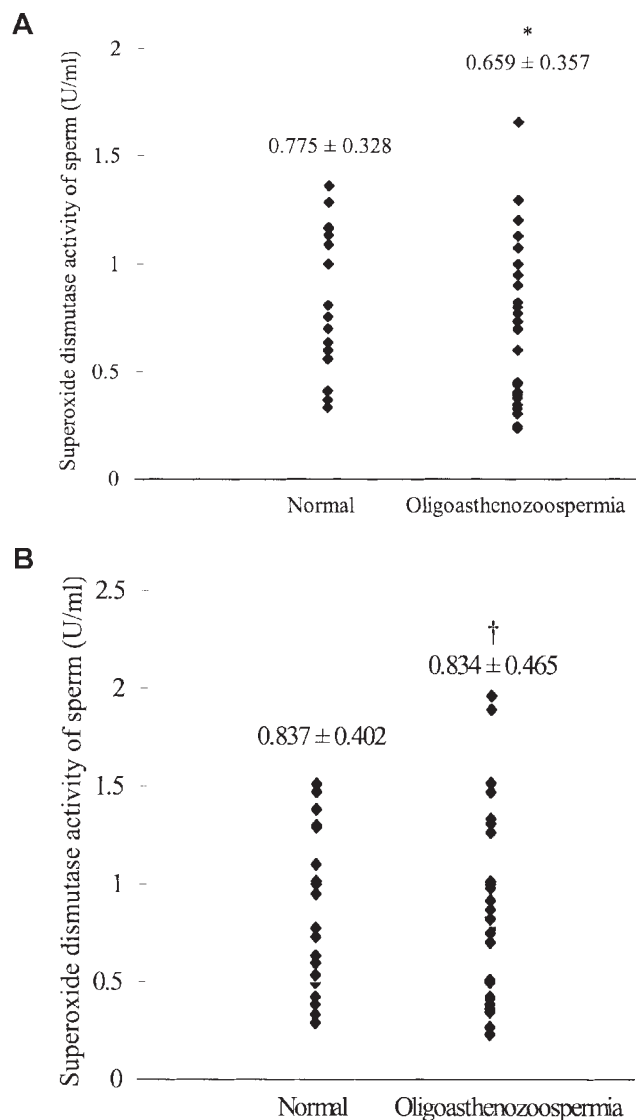
## RESULTS

SOD activities of sperm/seminal plasma in both groups were nonsignificantly different (*P*-value = 0.25/0.99). SOD activities of sperm/seminal plasma in groups 1 and 2 were  $0.77 \pm 0.33/0.84 \pm 0.40$  U/mg protein vs.  $0.66 \pm$  and  $0.36/0.83 \pm 0.47$  U/ml, respectively (Fig. 1A and B). We observed a positive but nonsignificant correlation between the sperm motility and SOD activities of sperm/seminal plasma. We established the formula of their relationship as:  $SOD = 0.0008 \times \text{motility} + 0.67/SOD = 0.0006 \times \text{motility} + 0.81$  (*P*-value = 0.54/0.57) (Fig. 2A and B).

We also noted the positive but nonsignificant correlation between the sperm concentrations and the SOD activity of sperm/seminal plasma. The formula for their relationship was:  $SOD = 0.0006 \times \text{concentration} + 0.67/SOD = 0.0021 \times \text{concentration} + 0.73$  (*P*-value = 0.43/0.26) (Fig. 3A and B).

## DISCUSSION

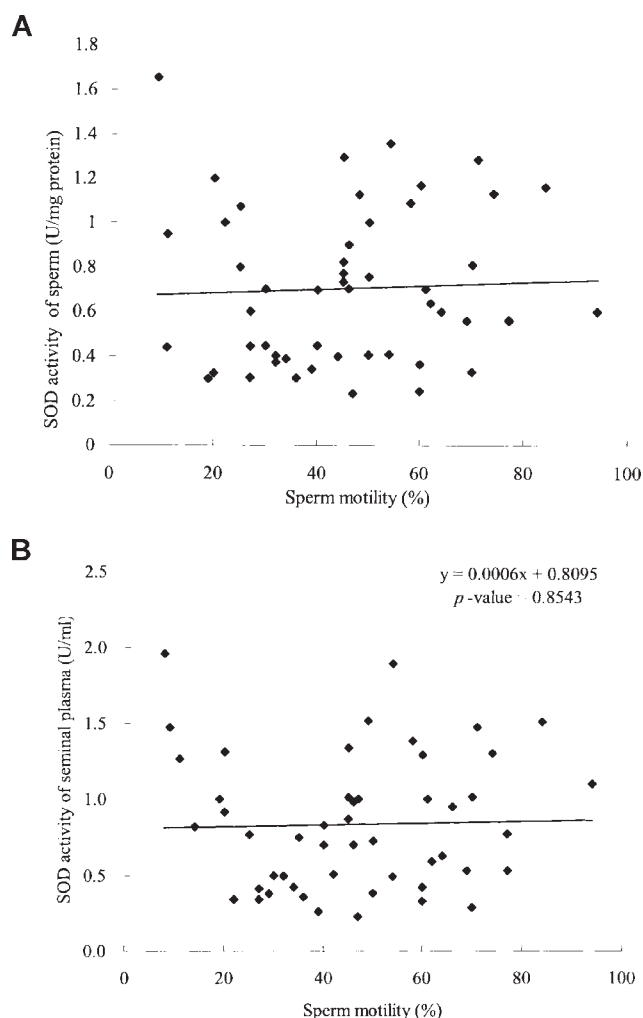
Excessive generation of ROS in semen, mainly by neutrophils but also by abnormal spermatozoa, could be a cause for infertility. Abnormal ROS production is associated with de-



**Fig. 1.** Comparisons of superoxide dismutase (SOD) activities of (A) spermatozoa and (B) seminal plasma in two groups of semen samples (\**P*-value = 0.25; †*P*-value = 0.99).

fective sperm function (2,7,8). The incidence of spontaneous pregnancy was negatively correlated with ROS production (9). Low concentrations of ROS do not affect sperm viability but do cause sperm immobilization, mostly via depletion of intracellular ATP and the subsequent decrease in the phosphorylation of axonemal proteins. Polyunsaturated fatty acids in the phospholipids of human spermatozoa are highly susceptible to peroxidation generated by ROS (10). In fact, ROS have beneficial or detrimental effects on sperm functions depending on the nature and concentration of the ROS involved, as well as the moment and the location of exposure (11).

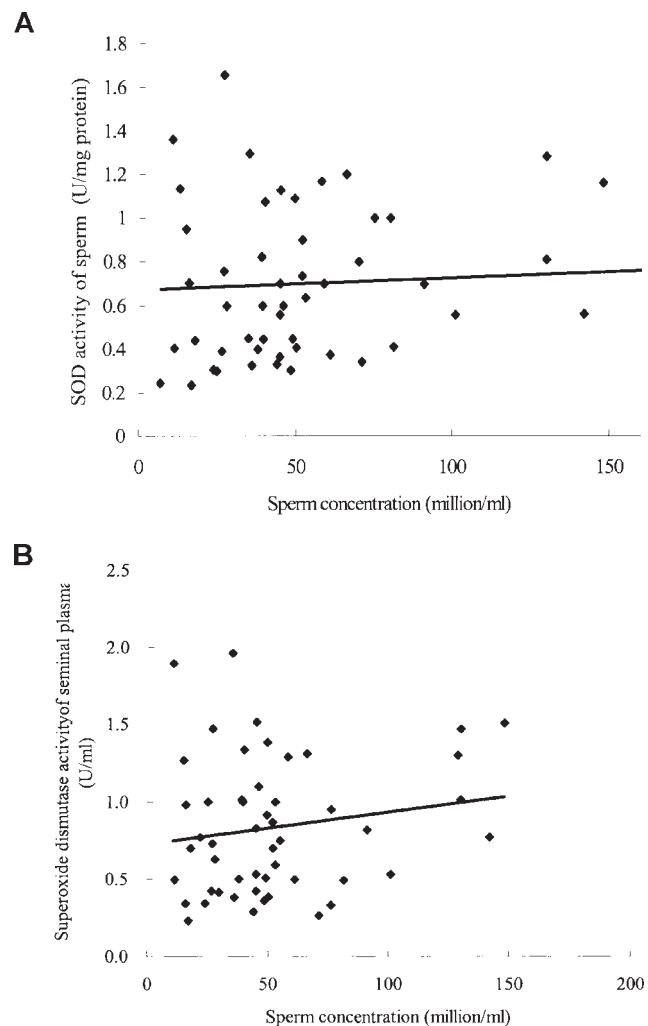
ROS are related to spermatozoa hyperactivation, capacitation, and acrosome reaction (12,13). ROS production is negatively related with the sperm-oocyte fusion capacity of human



**Fig. 2.** The lack of correlation between sperm motility and SOD activity is shown: (A) sperm motility vs. SOD activities of spermatozoa ( $y = 0.0008x + 0.6679$ ,  $P\text{-value} = 0.7471$ ); and (B) sperm motility vs. SOD activities of seminal plasma ( $y = 0.0006x + 0.8095$ ,  $P\text{-value} = 0.8543$ ).

spermatozoa (2). ROS at low concentrations may inhibit sperm-egg fusion via oxidation of the sulfhydryl (SH) proteins in the sperm membrane (14). High lipid peroxidation may reduce the capacity of the sperm to undergo acrosomal reaction and fertilization (2). The capability of the spermatozoa for oocyte fusion was impaired with the addition of hydrogen peroxide (15).

Significantly higher levels of superoxide anion have been observed in infertile males (8). The positive correlation between superoxide anion level and sperm morphology abnormalities has been demonstrated (7,8). Lipid hyperoxidation produced by ROS is strongly related with the morphological abnormalities of spermatozoa, primarily in tail defects (16). This could be due to loss of membrane fluidity (17) or to selective inactivation of some of the biochemical pathways leading to acrosomal reaction, such as second messenger sys-



**Fig. 3.** The positive but nonsignificant correlation between sperm concentration and SOD activities is shown: (A) sperm concentration vs. SOD activities of spermatozoa ( $y = 0.0006x + 0.6714$ ,  $P\text{-value} = 0.4339$ ); and (B) sperm concentration vs. SOD activities of seminal plasma ( $y = 0.0021x + 0.7263$ ,  $P\text{-value} = 0.2555$ ).

tems (18). Lipid peroxidation triggers the loss of membrane integrity, causing increased cell permeability, enzyme inactivation, structural damage to DNA, and cell death (19).

There is controversy about the role of SOD activity in sperm viability. Different studies have reported that: 1) SOD could prevent oxidative damage in sperm (20); 2) diminished SOD activity in seminal plasma was associated with male infertility (21); 3) decreased SOD was responsible for decreased sperm motion and viability (22); 4) high concentrations of SOD prevented the loss of motility in mouse sperm (23); and 5) the addition of SOD to the sperm suspension significantly improved sperm motility (24).

In contrast, some investigators demonstrated that ROS activity was not influenced by the presence of SOD (15). In fact, in one study sperm SOD activities varied widely between individuals (25). The SOD activity in seminiferous

epithelium is regulated over a wide range during spermatogenesis. Another study reported no difference in the SOD activities for spermatozoa or seminal plasma in samples that either did or did not produce ROS (26). Nonsignificant differences in SOD levels were detected in seminal plasma of fertile and infertile men (27). Higher ROS production in infertile patients may be due to increased ROS production rather than defective ROS scavenging activity (28). High SOD activities reflect errors in spermatogenesis associated with germ cell exfoliation and the retention of excess residual cytoplasm by the spermatozoa (29).

Hydrogen peroxide is the primary toxic ROS for human spermatozoa (30). High concentrations of hydrogen peroxide induce lipid peroxidation and result in cell death. Therefore, the balance of the SOD in semen and sperm is important for maintaining sperm motility. Some agents used for improving sperm quality can influence the SOD activity. Brezezinska-Slebozinska et al. (31) observed increased SOD and decreased ROS levels after vitamin E treatment. This suggests that vitamin E may improve the sperm motility and fertilization rates by reducing the ROS.

In this study, we observed that the SOD level was positive but nonsignificantly correlated with sperm mobility and concentration. Higher SOD may scavenge the generation of ROS, which may lower the cytotoxicity to human spermatozoa. Higher concentrations of spermatozoa may produce higher levels of SOD. This could interpret their correlation. Although their correlation in this study was nonstatistically different, it may be better defined after larger series are studied. In comparing the SOD activities between both groups, we observed that the difference in spermatozoa was more significant than that in seminal plasma. This may be due to fact that the SOD and ROS were produced mainly by abnormal spermatozoa and then released into the seminal plasma (32).

In conclusion, the SOD activities in sperm and semen were not significantly correlated with seminal quality. A seminal SOD survey may not be a useful tool for determining sperm fertilization potential. However, the results of this study could provide a database for further research into the effects of SOD on sperm. SOD dysfunction is not the single determiner for sperm regulation, which is a complex process involving many factors. The real roles played by SOD and other antioxidants in sperm quality merit further investigation.

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