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Lipid Peroxidation and Human Sperm Motility: Protective Role of Vitamin E

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ABSTRACT: Asthenospermia is the main factor of male infertility among patients consulting the Asir Infertility Center in Abha, Saudi Arabia. Lipid peroxidation occurring in both the seminal plasma and spermatozoa was estimated by malondialdehyde (MDA) concentration. Spermatozoal MDA concentration was higher in men with decreased sperm motility. The MDA concentration in the seminal plasma exhibited no relationship with sperm concentration, sperm motility, the number of immotile spermatozoa, or even the absence of spermatozoa. The MDA concentration in sperm pellet suspensions of asthenospermic and oligoasthenospermic patients was almost twice that of the normospermic males. The MDA concentration in the sperm pellet suspension from normospermic or oligospermic patients was about 10% that in the seminal plasma. However, the MDA concentration in the sperm pellet suspension of asthenospermic or oligoasthenospermic patients was about 15% that in the seminal plasma. Treatment of asthenospermic patients with oral Vitamin E significantly decreased the MDA concentration in spermatozoa and improved sperm motility. Eleven out of the 52 treated patients (21%) impregnated their spouses; nine of the spouses successfully ended with normal term deliveries, whereas the other two aborted in the first trimester. No pregnancies were reported in the spouses of the placebo-treated patients.

Key words: Asthenospermia, lipid peroxidation, vitamin E, human spermatozoa.

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Over 50 years ago, it was reported that oxygen radicals are toxic to the motility of human spermatozoa (McLeod, 1943). Since then, it has been shown that under normal physiological conditions oxygen radicals are produced by sperm (Alvarez and Storey, 1982; Alvarez et al, 1987; Aitken and Clarkson, 1987), through the leakage of electrons onto molecular oxygen from the mitochondrial electron transfer chain (Alvarez et al, 1987; Alvarez and Storey, 1989). Normal cells have a number of protective enzyme scavengers that act as antioxidants to detoxify the harmful reactive oxygen radical and prevent cell damage. One of these is superoxide dismutase (SOD), which has been reported to be in spermatozoa (Mennella and Jones, 1980; Alvarez and Storey, 1983b; Nissen and Kreysel, 1983), but its role in human sperm motility has only recently been understood (Alvarez et al, 1987; Kobayashi et al, 1991; Alvarez and Storey, 1992). Another important antioxidant enzyme is glutathione peroxidase (Alvarez et al, 1987; Alvarez and Storey, 1989, 1992).

Vitamin E is one of the most important antioxidative molecules, residing mainly in the cell membranes. It is thought to interrupt the chain reactions with lipid peroxidation and scavenge free radicals generated during the univalent reduction of molecular oxygen and during normal activity of oxidative enzymes (Ehrenkranz, 1980; Palamanda and Kehrer, 1993). These radicals, if left alone, will lead to peroxidation of phospholipids in the mitochondria of the sperm and thus to their ultimate immotility (DeLamirande and Gagnon, 1992a,b). It is also possible that vitamin E enhances the production of the scavenger antioxidant enzymes.

The objectives of this study were: 1) to identify the major abnormality in semen parameters among the patients consulting our infertility center; 2) to determine the level of lipid peroxidation in the semen of asthenospermic patients and its possible correlation with sperm motility; and 3) to compare the effects of vitamin E and a placebo on the degree of peroxidation, improvement in sperm motility, and the occurrence of pregnancy.

Materials and Methods

Semen Samples

Semen was obtained from patients consulting Asir Infertility Center (AIC), which is affiliated with Abha College of Medicine at King Saud University and Asir Health Directorate in Abha, Saudi Arabia. All semen samples were collected by masturbation after 3 days of abstinence, except in four patients, where the coitus interruptus method was used.

After liquefaction of the sample, the semen volume, sperm concentration, and morphology of sperm were determined utiliz-

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ing standard procedures (World Health Organization, 1980). Motility was assessed by use of the Makler counting chamber (Makler, 1980). Only semen samples with a leukocyte-to-spermatozoa concentration of <5% and a normal fructose concentration were used.

Semen classification was based on mean results of three semen analyses, as follows: normospermia was indicated by a sperm concentration of ≥20 × 10^6/ml, motility ≥50%, and normal morphology ≥40%; asthenospermia was indicated by a sperm concentration of ≥20 × 10^6/ml and motility <50% irrespective of morphology; and oligospermia was indicated by a sperm concentration <20 × 10^6/ml irrespective of motility or morphology. Samples with high viscosity were those that did not liquify after 60 minutes and required special treatment for liquefaction. Twenty-two normal semen samples from husbands who fathered children were used for comparative purposes.

Vitamin E Treatment

In this study, patients who were classified as asthenospermic, as a subpopulation of all patients who consulted the AIC between 1991 and 1993, participated after the aims were fully explained to them and after the approval of the Ethical Committee. Either 100 mg vitamin E or a placebo was prescribed in a random double-blind fashion, to be taken three times a day. If the semen sample improved and the patient's spouse became pregnant, the treatment was stopped; otherwise it was continued for 6 months. The placebo was given for 6 months. The criteria for patients used in this study included semen samples with a sperm motility ≤40%, a normal sperm count (as indicated above), a leukocyte concentration of <5%, and normal fructose concentration. All patients were married for ≥2 years, and the female spouse was confirmed to be free of abnormalities. A total of 110 patients were enrolled in the study, but some of the patients dropped out and some left the region and failed to continue. When the experiment was terminated, 52 patients were found to have taken vitamin E and 35 patients to have taken the placebo. Semen samples from all subjects were examined microscopically, and a portion of each sample was used to determine lipid peroxidation. Semen samples were considered improved if a ≥15% increase in sperm motility was found and not improved if the increase in sperm motility was <15%. An increase of <15% was considered to be possibly due to sample variation. Semen samples from both treatment groups were evaluated monthly.

Measurement of Lipid Peroxidation

Lipid peroxidation was measured by determining the malondialdehyde (MDA) production, using thiobarbituric acid (TBA) (Buege and Aust, 1978). The MDA level was measured in the spermatozoa and semen plasma of all patients before treatment and also monthly during treatment (as indicated above for vitamin E treatment). The sperm pellet that resulted from separating the seminal plasma from semen by centrifugation at 1,800 × *g* for 10 minutes was resuspended in 2 ml phosphate-buffered saline (PBS; pH 7.2) or a variable volume to obtain a sperm concentration of 10 × 10^6/ml. Lipid peroxides were measured in spermatozoa after the addition of 2 ml of TBA reagent (15% w/v trichloroacetic acid and 0.25 N HCl) to 1 ml of sperm suspension. The mixture was treated in a boiling water bath for 15 minutes. After cooling, the suspension was centrifuged at 1,000 × *g* for 10 minutes. The supernatant was then separated, and absorbance was measured at 535 nm. The MDA concentration was determined by the specific absorbance coefficient (1.34 × 10^5 mol/cm^3). The same procedure was performed to measure MDA in semen plasma.

Table 1. *Classification of abnormalities in the seminal fluid of male patients who attended Asir Infertility Center*

Semen abnormality	*N*	% of Total
Oligospermia	30	10.5
Asthenospermia	94	32.8
High viscosity	22	7.7
Azoospermia	38	13.2
Oligoasthenospermia	74	26
Oligospermia with high viscosity	6	2.1
Asthenospermia with high viscosity	12	4.2
Oligoasthenospermia with high viscosity	10	3.5
Total	286	100

N, the number of patients in each category who consulted the Infertility Center during the 2.5-year period (1991–1993).

Statistical Methods

Statistical analysis was done using a *t*-test (SPSS for Windows), by personal computer. *P* values <0.05 were considered significant. Regression equations and correlation factors were calculated using the least squares method.

Results

Although there were various abnormalities in the seminal fluid of male patients who consulted the AIC over the period of 2.5 years, asthenospermia appeared to be the main cause of infertility (Table 1). Asthenospermic patients alone constituted 33% of the total abnormalities. However, if oligoasthenospermic patients were added to this group, this abnormality would exceed 50% of the total abnormalities (Table 1).

Lipid peroxidation in the seminal plasma and sperm pellets from various types of seminal fluid is shown in Table 2. The MDA concentration in the seminal plasma of normospermic subjects was 6.32 ± 0.82 (mean ± standard deviation [SD]). The MDA concentration in the seminal plasma exhibited no relationship with sperm concentration, sperm motility, the number of immotile spermatozoa, or even the absence of spermatozoa. However, the spermatozoal MDA concentration was significantly higher ($P < 0.001$) with decreased sperm motility (Table 2). Compared to that in the normospermic samples, the MDA concentration was 1.7 times greater in asthenospermic and 1.6 times greater in the oligoasthenospermic patients. Azoospermic samples, as expected, showed very low (<10%) MDA concentration in what would have been the

Table 2. *Relationship between the nature of seminal fluid and lipid peroxidation*

Type of seminal fluid	N	Lipid peroxidation MDA equivalents (nmol/ml)* Seminal plasma	Sperm pellet ($\times 10^{-1}$)
Normospermia	22	6.32 ± 0.82	5.72 ± 0.84
Oligospermia	38	6.51 ± 0.73	6.64 ± 1.22
Asthenospermia	25	6.62 ± 0.65	9.75 ± 1.41†
Oligoasthenospermia	21	6.14 ± 0.71	9.12 ± 1.23†
Azoospermia	18	5.95 ± 0.62	0.48 ± 0.23‡

* The number of sperm in the pellet suspension was adjusted to 50 × 10^6/ml.

† Statistically significant; $P < 0.001$.

‡ Not an actual sperm pellet, but a very small pellet of tissue or cellular debris.

pellet. The MDA concentration in the sperm pellet suspension of all types of seminal fluid was much less than that in the seminal plasma. The MDA concentration in the sperm pellet suspension from normospermic or oligospermic samples was about 10% of that in the seminal plasma, whereas in the asthenospermic or oligoasthenospermic samples it was 15–16% of that in the seminal plasma (Table 2).

The ages of patients who participated in the treatment and their sperm parameters were as follows: 1) vitamin E-treated subjects were 27–52 years of age (average 34.8 years), sperm concentration was 24–65 × 10^6/ml (average 41.5 × 10^6/ml), and normal sperm morphology was 54–68% (average 61.3%); 2) control (placebo group) subjects were 22–45 years of age (average 33.2 years), sperm concentration was 23–61 × 10^6/ml (average 44.2 × 10^6/ml), and normal sperm morphology was 52–66% (average 60.6%).

Treatment of asthenospermic patients with vitamin E produced very good results in reducing MDA concentration and improving sperm motility. According to the effects produced by the treatment, patients could be divided into two main categories, as shown in Table 3. The first category consisted of patients with improved sperm motility (i.e., sperm exhibited a ≥15% increase in motility). This improvement was associated with a significant decrease in the MDA concentration of the sperm pellet suspension. This category constituted 60% of the treated group, compared to 11% in the control group. The decrease in MDA concentration in this category was significantly greater in the vitamin E-treated group compared to the placebo group. The extent of improvement in sperm motility and the decrease in MDA concentration in this category were variable. Therefore, this category could be divided into two subgroups, according to the extent of improvement in sperm motility: 1) patients with improved sperm motility and whose spouses became pregnant and 2) patients with improved sperm motility whose spouses did not become pregnant during the treatment period. The improvement in sperm motility in the first subgroup exceeded 20% (25–28%), and there was a highly significant decrease in MDA concentration. This subgroup constituted 21% of the treated patients, whereas none of the patients in the placebo group achieved this improvement. The improvement in sperm motility in the second subgroup exceeded 15%, and there was also a highly significant decrease in MDA concentration. This second subgroup constituted 39% of the vitamin E-treated group and 11% of the placebo group. Although four subjects in the placebo group (11%) showed a decrease in MDA concentration and improved sperm motility (20–21%), the decrease in MDA concentration and improved sperm motility were significantly greater in the vitamin E-treated group.

The second main category (Table 3) included patients who had no improvement in sperm motility. This category exhibited a 4–13% change in sperm motility in the vitamin E-treated group, whereas there was only a 2–3% change in the placebo group. The MDA concentration in this category exhibited a slightly significant change in the vitamin E-treated group, remaining very close to the values before treatment, but it did not show any significant change in the placebo group. This category comprised 89% of the control patients compared to 40% in the treated group.

Correlations of MDA concentration with sperm motility in patients treated with vitamin E and in control (placebo) groups are presented in Figures 1 and 2. The two figures show an inverse correlation of MDA concentration in sperm pellets with sperm motility in the vitamin E-treated (Fig. 1A; $r = 0.23$, $P < 0.15$; 1B: $r = 0.79$, $P < 0.001$) and placebo (Fig. 2A: $r = 0.2$, $P < 0.18$; 2B: $r = 0.36$, $P < 0.05$) groups, respectively. There was little change in the correlation of sperm motility with MDA concentration in the placebo group. However, the correlation of sperm motility with MDA concentration in the group treated with vitamin E became highly significant after treatment. There was no clear relationship between the initial percentage of sperm motility and the extent of improvement in motility after treatment with vitamin E. However, if the initial sperm motility was <15%, then motility either did not improve or slightly improved after treatment with vitamin E. There was no significant change in the MDA concentration in the seminal plasma after treatment with vitamin E.

The 11 successful pregnancies are detailed in Table 4. Nine of these cases ended in normal term deliveries, and two pregnancies ended in abortions (Table 4). Two of the asthenospermic patients (18%) in this subgroup had 20% initial sperm motility, and the rest had 25–40% initial motility. The extent of improvement in motility in this group ranged from 21 to 40% (Table 4). None of the

Table 3. *Effect of vitamin E on sperm motility and MDA concentration in asthenospermic patients*

Category of patients	Treated group					Control (placebo) group				
	N (%)	MDA concentration ($\times 10^{-1}$)		Sperm motility (%)		*N* (%)	MDA concentration ($\times 10^{-1}$)		Sperm motility (%)	
		Before	After	Before	After		Before	After	Before	After
Total	52 (100%)	10.3 ± 1.5 (7.7–11.8)	7.1 ± 2.2 (3.4–10.8)	31.1 ± 10.4 (6–40)	48.9 ± 15.5 (10–68)	35 (100%)	9.8 ± 1.3 (7.3–11.6)	9.4 ± 1.5 (6.2–115)	30.6 ± 10.7 (5–40)	35.9 ± 12.8 (10–60)
P value			<0.001		<0.001		(NS)	NS (<0.001)	NS	NS (<0.01)
Improved sperm motility:	31 (60%)	10.1 ± 1.6 (7.7–11.8)	5.6 ± 1.2 (3.4–8.9)	33.6 ± 7.1 (15–40)	58.8 ± 6.0 (30–68)	4 (11%)	11.2 ± 0.2 (11.1–11.3)	7.7 ± 0.8 (6.2–8.1)	32.5 ± 7.6 (25–40)	54.5 ± 7.7 (46–60)
P value			<0.001		<0.001		(NS)	<0.005 (<0.001)	(NS)	<0.005 (<0.05)
1) Improved and spouses became pregnant	11 (21%)	9.1 ± 1.6 (7.9–11.8)	4.4 ± 0.6 (3.4–5.3)	32.5 ± 8.7 (20–40)	60.1 ± 6.5 (45–68)	—	—	—	—	—
P value			<0.001		<0.001					
2) Improved	20 (39%)	10.6 ± 1.5 (7.7–11.8)	6.1 ± 0.9 (5.3–8.9)	34.9 ± 6.5 (15–40)	58.1 ± 5.9 (30–65)	4 (11%)	11.2 ± 0.2 (11.1–11.3)	7.1 ± 0.8 (6.2–8.1)	32.5 ± 7.6 (25–40)	54.5 ± 7.7 (46–60)
P value			<0.001		<0.001		(NS)	<0.001 (<0.05)	(NS)	<0.005 (<0.05)
No improvement in sperm motility	21 (40%)	10.3 ± 1.2 (8.3–11.8)	9.1 ± 1.3 (8.1–10.8)	28.6 ± 12.4 (6–40)	36.5 ± 14.1 (10–53)	31 (89%)	9.7 ± 1.3 (7.4–11.6)	10.1 ± 1.1 (7.1–11.5)	29.2 ± 11.1 (8–40)	33.3 ± 11 (10–43)
P value			<0.01		NS		(NS)	NS (<0.01)	(NS)	NS (NS)

Values are means ± standard deviation (SD) and range; NS, not significant, *P* values in parentheses indicate comparison between control and corresponding treated group.

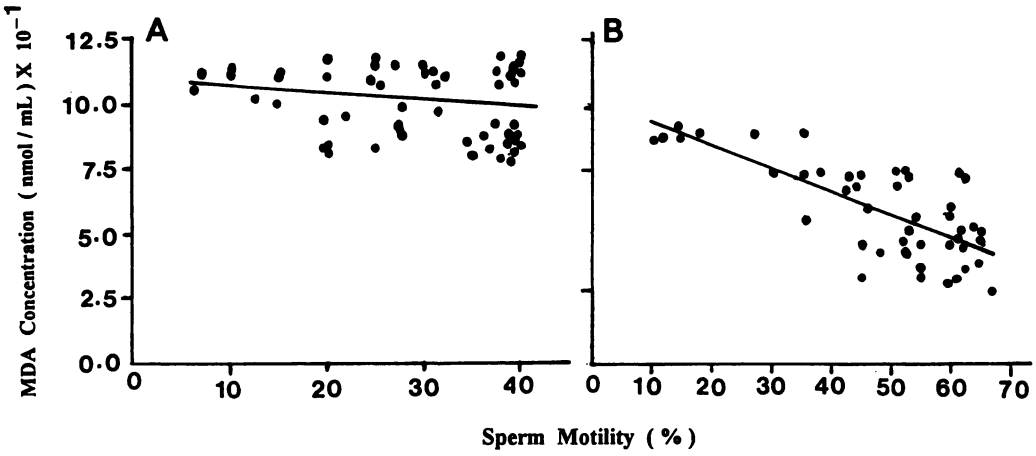

FIG. 1. Correlation between MDA concentration and sperm motility in asthenospermic patients treated with vitamin E. (A), Before treatment; (B), after treatment.

spouses of the patients who were administered placebos became pregnant.

Discussion

The results presented in this study indicate three main points. First, asthenospermia was a major factor in male infertility among the patients consulting the AIC. Second, the lipid peroxide level in semen samples correlates inversely with the percent of sperm motility. Third, the intake of vitamin E by asthenospermic patients significantly improved sperm motility and caused a significant decrease in the MDA concentration in spermatozoa.

Motility of spermatozoa depends on the integrity of the mitochondrial sheath, of which phospholipids are a major component. If fatty acids in these phospholipids are oxidized by free oxygen radicals, spermatozoa will be damaged (Jones and Mann, 1977; Alvarez and Storey, 1982), and their motility will be impaired (Alvarez and Storey, 1982, 1984a,b, 1989). Jones et al (1978) showed that spermatozoa from poor semen specimens exhibited higher peroxidation rates and were immobilized rapidly. They also showed (Jones et al, 1978) that poorly motile spermatozoa are more susceptible to lipid peroxidation than motile ones. In addition, they reported (Jones et al, 1978) that human spermatozoa became immotile within 5 minutes after the addition of 30–60 nmol of lipid peroxide. DeLamirande and Gagnon (1992a) have also shown that reactive oxygen radicals cause sperm immotility within 5–30 minutes, depending on the concentration. More recently, Griveau et al (1995) have also shown that reactive oxygen species cause a decrease in sperm motility, an increase in lipid peroxidation, and a loss of membrane polyunsaturated fatty acids. Spontaneous lipid peroxidation was also shown in rabbit and mouse spermatozoa, and a close linear correlation exists between the extent of peroxidation and the loss of sperm motility (Alvarez and

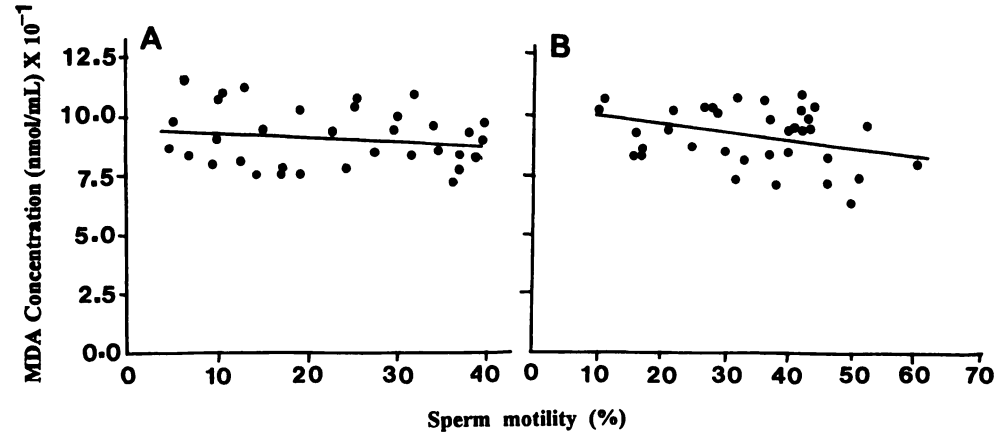

FIG. 2. Correlation between MDA concentration and sperm motility in control (placebo) asthenospermic patients. (A), Before treatment; (B), after treatment.

Table 4. *Details of asthenospermic patients who were treated with vitamin E and whose spouses became pregnant*

No.	Age of male (years)	Age of spouse (years)	Years of marriage	Average sperm count ($\times 10^6$/ml)	Average motility before treatment (%)	Average motility after treatment (%)	Duration of treatment
1	29	25	5	60	38	67	5 months
2	31	26	5	22	20	60	4 months
3	42	26*	4	58	41	62	2 months
4	52	29	3	61	39	61	4 months
5	32	30	4.5	60	35	65	3 months
6	35	30	3	21	40	63	4 months
7	27	26	2	52	25	55	5 months
8	31	26	3	60	37	68	4 months
9	32	29	3	61	20	45	4 months
10	34	30	2	52	40	65	6 months
11	30	24†	4	20	38	60	5 months

* Spouse aborted after 3 months.
† Spouse aborted after 4 months.
Other spouses had normal term deliveries.

Storey, 1982, 1983a,b). The results presented here show that the MDA concentration of human spermatozoa correlates inversely with the percent of sperm motility and suggest that the immotile spermatozoa underwent lipid peroxidation. Also, because the MDA concentration of the spermatozoa in asthenospermic patients was clearly increased in comparison to the MDA concentration in normospermic patients, measurement of MDA concentration could possibly be used in the assessment of male infertility.

There is some discrepancy in the literature about the level of lipid peroxidation in the testes compared to other tissues. Although Golberg et al (1962) reported that the testes produce concentrations of MDA equivalent to those in the liver or kidney, Mihara et al (1980) reported that the testes produce more MDA than the kidney but less than the liver. The results reported here concerning the concentrations of MDA in the seminal fluid are comparable to those reported for rat hepatocytes (Suleiman and Stevens, 1987). Recently, Nonokagi et al (1992) suggested that lipid peroxidation occurs variably in different parts of human male genital organs, with a maximum occurrence in the seminiferous tubules.

Bandivdekar et al (1989) suggested that the presence of lipid peroxides in the seminal fluid may be attributed entirely to the lipid fraction of the spermatozoa. This does not seem to be true, because in our results the seminal plasma of azoospermic patients exhibited concentrations of MDA similar to those in other types of seminal fluid. Furthermore, Kobayashi et al (1991) also showed that lipid peroxide exists in human seminal plasma in a concentration that compares well with our findings. This also indicates that the estimation of MDA concentration in spermatozoa is a better indicator of male infertility than the measurement of MDA concentration in the entire seminal fluid.

The present study indicates that orally administered vitamin E improves sperm motility. In the 31 subjects treated with vitamin E who achieved improved motility, the female partners of 11 became pregnant. Treatment with placebo led to improvement in sperm motility in four subjects, but there were no pregnancies reported in their partners. Improvement of sperm motility was not linked to the duration of vitamin E treatment. The overall average duration of vitamin E treatment was 5.6 months, but in the group that was able to impregnate their spouses the duration was 4.2 months. Improvement of sperm motility, however, was directly correlated with the reduction in lipid peroxidation. This strongly suggests that vitamin E plays a major role as a scavenger for reactive oxygen species, leading to a decrease in lipid peroxidation and ultimately to the protection of spermatozoa. Our results are supported by those from a previous study (Gavino et al, 1984), which showed that most rat tissues, including the testes, are more susceptible to lipid peroxidation in vitamin E-deficient animals compared to control animals fed normal diet. Recently, Palamanda and Kehrer (1993) demonstrated the involvement of vitamin E in the inhibition of lipid peroxidation. It is not clear, however, why there was no change in the MDA concentration in the seminal plasma of patients treated with vitamin E. It is also not clear why 4 of the 35 placebo-treated subjects showed a significant increase in sperm motility and a significant decrease in MDA concentration. This might be related to a possible variation in the diet of these subjects or another unknown factor. However, this change, due to an unknown effect, still indicates that the increase in sperm motility is associated with a decrease in MDA concentration. This change in MDA concentration and sperm motility was not enough to cause impregnation in the spouses of these placebo-treated subjects. In addition, the decrease in MDA concentration and the increase in sperm motility in the vitamin E-treated group were greater than those in the placebo group. This might indicate that the MDA concentration needs to be reduced to a certain level to achieve better sperm motility. It also seems that the reduction in MDA concentration that resulted from treatment with vitamin E produced a great increase in sperm motility that improved the correlation between these two parameters. Further investigation to clarify this is needed.

Semen contains scavenger systems to counteract the effects of lipid peroxidation and prevent spermatozoal damage. These systems include superoxide dismutase (SOD) (Mennella and Jones, 1980; Nissen and Kreysel, 1983; Marklund, 1986; Alvarez et al, 1987; Alvarez and

Storey, 1992), glutathione peroxidase (Alvarez et al, 1987; Alvarez and Storey, 1989, 1992), and catalase (Bandivdekar et al, 1989; Jeulin et al, 1989). According to various investigators, the SOD system seems to be the most effective natural protective system in the seminal fluid. Alvarez et al (1987) showed that the SOD activity of a semen sample appears to be a good predictor of the lifetime of that particular sample. Furthermore, Kobayashi et al (1991) showed that addition of exogenous SOD to semen samples led to improved sperm motility and caused a decrease in MDA concentration. Griveau et al (1995) have shown that reactive oxygen species inactivated these enzymes. Although we did not measure SOD activity in the semen samples in our study, it is possible that the supplementation of vitamin E compensated for deficient or low SOD activity in our semen samples. Further investigation to clarify this is needed.

In conclusion, vitamin E seems to protect against the loss of sperm motility by lipid peroxidation. Supplementation of vitamin E improved sperm motility and increased the possibility of fertilization in asthenospermic subjects, even when the original sperm motility was 20%. A great decrease in the MDA concentration in spermatozoa towards normospermic levels in response to vitamin E treatment therapy seems to be a good predictor for which patients will be able to successfully impregnate their spouses. Further studies may elucidate the details of the action of vitamin E on seminal fluid and also determine whether there is a possibility of using vitamin E as a protectant in the cryopreservation of spermatozoa for *in vitro* fertilization.

Since the end of the initial study using vitamin E, of the 11 successful patients, 3 returned desiring more children. Following further vitamin E treatment, they were once again able to impregnate their spouses. Also, following the completion of the study, 26 of the placebo patients were switched to vitamin E and, during its course, 4 were then able to successfully impregnate their spouses.

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References

Aitken RJ, Clarkson JS. Cellular basis of defective sperm function and its association with the genesis of reactive oxygen species by human spermatozoa. *J Reprod Fertil* 1987;81:459–469.

Alvarez JG, Storey BT. Spontaneous lipid peroxidation in rabbit epididymal spermatozoa: its effect on sperm motility. *Biol Reprod* 1982;27: 1102–1108.

Alvarez JG, Storey BT. Taurine, hypotaurine, epinephrine and albumin inhibit lipid peroxidation in rabbit spermatozoa and protect against loss of motility. *Biol Reprod* 1983a;29:548–555.

Alvarez JG, Storey BT. Role of superoxide dismutase in protecting rabbit spermatozoa from O_2 toxicity due to lipid peroxidation. *Biol Reprod* 1983b;28:1129–1136.

Alvarez JG, Storey BT. Assessment of cell damage caused by spontaneous lipid peroxidation in rabbit spermatozoa. *Biol Reprod* 1984a;30: 323–331.

Alvarez JG, Storey BT. Lipid peroxidation and the reactions of superoxides and hydrogen peroxide in mouse spermatozoa. *Biol Reprod* 1984b;30:833–841.

Alvarez JG, Storey BT. Role of glutathione peroxidase in protecting mammalian spermatozoa from loss of motility caused by spontaneous lipid peroxidation. *Gamete Res* 1989;23:77–90.

Alvarez JG, Storey BT. Evidence for increased lipid peroxidative damage and loss of superoxide dismutase activity as a mode of sublethal cryodamage to human sperm during cryopreservation. *J Andro* 1992;13: 232–241.

Alvarez JG, Touchstone JC, Blasco L, Storey BT. Spontaneous lipid peroxidation and production of hydrogen peroxide and superoxide in human spermatozoa: superoxide dismutase as major enzyme protectant against oxygen toxicity. *J Androl* 1987;8:338–348.

Bandivdekar AH, Devasagayam TPA, Pradhan DS, Joseph DR, Hurkadli KS. Suppression of lipid peroxidation in human spermatozoa by prostatic inhibin. *Arch Androl* 1989;22:217–221.

Buege JA, Aust SD. Microsomal lipid peroxidation. *Methods Enzymol* 1978;52:302–310.

DeLamirande E, Gagnon C. Reactive oxygen species and human spermatozoa. I. Effects on the motility of intact spermatozoa and on sperm axonemes. *J Androl* 1992a;13:368–378.

DeLamirande E, Gagnon C. Reactive oxygen species and human spermatozoa. II. Depletion of adenosine triphosphate plays an important role in the inhibition of sperm motility. *J Androl* 1992b;13: 379–386.

Ehrenkranz R. Vitamin E and the neonate. *Am J Dis Child* 1980;134: 1157–1168.

Gavino VC, Dillard CJ, Tappel AL. Release of ethane and pentane from rat tissue slices: effect of vitamin E, halogenenated hydrocarbons, and iron overload. *Arch Biochem Biophys* 1984;233:741–747.

Goldberg L, Martin LE, Batchelor A. Biochemical changes in the tissues of animals injected with iron. 3. Lipid peroxidation. *Biochem J* 1962; 83:291–298.

Griveau JF, Dumont E, Renard P, Callegari JP, Le Lannou D. Reactive oxygen species, lipid peroxidation and enzymatic defence systems in human spermatozoa. *J Reprod Fertil* 1995;103:17–26.

Jeulin C, Soufir JC, Weber P, Laval-Martin D, Calvayrac R. Catalase activity in human spermatozoa and seminal plasma. *Gamete Res* 1989; 24:185–196.

Jones R, Mann T. Damage to ram spermatozoa by peroxidation of endogenous phospholipids. *J Reprod Fertil* 1977;50:261–268.

Jones R, Mann R, Sherins R. Adverse effects of peroxidized lipid on human spermatozoa. *Proc R Soc Lond B Biol Sci* 1978;201:413–417.

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

McLeod J. The role of oxygen in the metabolism and motility of human spermatozoa. *Am J Physiol* 1943;138:512–518.

Makler A. The improved ten micrometer chamber for rapid sperm count and motility evaluation. *Fertil Steril* 1980;33:337–338.

Marklund SL. Superoxide dismutase in human tissues, cells and extracellular fluids: clinical implications. In: Johnson JE, et al, eds. *Free*

Radicals, Aging, and Degenerative Diseases. New York: Alan R Liss; 1986:509–526.

Mennella MR, Jones R. Properties of spermatozeal superoxide dismutase and lack of involvement of superoxides in metal-ion-catalysed lipid peroxidation reaction in semen. *Biochem J* 1980;191:289–297.

Mihara M, Uchiyama M, Fukuzawa K. Tribarbituric acid value on fresh homogenate of rat as a parameter of lipid peroxidation in aging, CCl_4 intoxication and vitamin E deficiency. *Biochem Med* 1980;23:302–311.

Nissen HP, Kreysel HW. Superoxide dismutase in human semen. *Klinische Wochenschrift* 1983;61:63–65.

Nonokagi T, Noda Y, Narimoto K, Shiotani M, Mori T, Matsuda T, Yoshida P. Localization of CuZn-superoxide dismutase in the human male genital organs. *Hum Reprod* 1992;7:81–85.

Palamanda JR, Kehrer JP. Involvement of vitamin E and protein thiols in the inhibition of microsomal lipid peroxidation by glutathione. *Lipids* 1993;28:427–431.

Suleiman SA, Stevens JB. Bipyridylin-herbicide toxicity: effect of paraquat and diquat on isolated rat hepatocytes. *J Environ Pathol Toxicol Oncol* 1987;7:73–84.

World Health Organization. *WHO Laboratory Manual for the Examination of Human Semen and Semen–Cervical Mucus Interactions.* Singapore: Press Concern; 1980:7–32.