Free radicals, by definition, harbor only one electron in an orbital rather than two. Because having an unpaired electron is an unstable state, free radicals tend to be chemically reactive. Two radicals can combine to form a covalent bond; or the radical may react with a non-radical either by donating its unpaired electron, by acquiring an electron from a non-radical, or by directly joining onto the non-radical. Because of the nature of their chemical interactions, more radicals tend to be generated as a free radical interacts with a non-radical.

Biologically, free radicals are formed from many sources. However, one source common to all eukaryotic cells is the electron transport chain in the mitochondria. In the process of reducing O2 to H2O, the first step involves the addition of one electron to O2 to from superoxide, O2.-. Usually, superoxide is reduced to water via addition of more electrons and hydrogen. However, there is a slow and basal level of leakage of superoxide anions, and this contributes to the level of free radicals present biologically. There are other sources of free radicals including enzymes which specifically generate free radicals, UV rays which promote hydroxyl radical formation, etc. The chemistry of superoxide (and free radicals) is rich; and via complex reactions, other reactive oxygen species (ROS-free radicals or compounds like hydrogen peroxide which can become free radicals) such as hydrogen peroxide (H2O2) and hydroxyl radical (.OH) can be generated.

Because ROS and their derivatives are reactive, they can damage various biomolecules including lipids, proteins and DNA. In efforts to curtail these potentially damaging effects, cells have evolved complex antioxidant defense mechanisms including those which enzymatically neutralize many of these ROS and those which use small molecule scavengers like vitamin E and ascorbate. Enzymes dedicated to protecting the cell from these ROS include superoxide dismutase (SOD), which converts superoxide anions into oxygen and hydrogen peroxide; and glutathione peroxidaseand catalase, both of whcih catalyze the conversion of hydrogen peroxide into water and oxygen. Although the presence of these enzymatic protectants is extensive intracellularly, their protective roles extracellularly are limited as their levels are low in extracellular compartments. Instead, small molecule scavengers like vitamin C (ascorbate) and vitamin E (tocopherols) seem to play a more important role in the extracellular milleu. Tocopherols are membrane soluble lipids which react with free radicals and reactive oxygen species. In the process of reacting with a free radical, tocopherols themselves become a free radical, albeit a very non-reactive one. Therefore, they act to scavenge free radicals by chemically reacting with them preferentially, and thereby sparing other biomolecules. Subsequently, the modified tocopherol reacts with ascorbate, which is water soluble, and this converts ascorbate into a free radical; this reaction also regenerates the unmodified tocopherol.

The free radical form of ascorbate is then either excreted in urine or regenerated to the unmodified form by other chemicals; ultimately, the chain terminator in this process is NADH which donates an electron and hydrogen to neutralize the free radical. Ascorbate also has the ability to scavenge free radicals independent of vitamin E.

Reactive oxygen species have been implicated as causative agents in various pathological conditions in which their levels are elevated. Some implicated pathologies include diseases associated with chronic inflammation (rheumatoid arthritis, hepatitis, etc.), neurodegenerative illnesses (Alzheimer's and Parkinson's) and some cancers. There is now evidence suggesting that free radicals and reactive oxygen species (ROS) play a significant role in many cases of male infertility. Furthermore, in many of these situations, the dynamic struggle between antioxidant defense systems and ROS generation sems to be imbalanced such that the levels of ROS increase. Scientists are currently studying the effects of vitamin E and C supplementation (along with other antioxidants) on sperm quality and fertility.

II. Levels of ROS Are Elevated in Infertile Patients; High Levels of ROS Are Correlated with Non-functioning Spermatozoa

In one study (4), comparisons of the levels of ROS were made between infertile men (n=172) and fertile men (n=10). 40% of the infertile men had elevated levels of ROS (`elevated' was considered a peak signal equal to or greater than twice the background on a chemiluminescent assay), whereas none of the fertile men, and none of the azoospermic infertile men (n=6) had detectable levels of ROS. The levels of ROS

were also compared to other variables, and a negative correlation was established between levels of ROS and percentage of motile sperm, and the linearity with which the sperm traveled (p values are all less than 0.05).

Experiments have established that the source of ROS in semen is the spermatozoa (1, 4, 13). Furthermore, by partitioning out the spermatozoa subpopulations from samples of infertile men, researchers were able to show that morphologically abnormal sperm with low motility produced the highest levels of ROS. Other studies also seem to support this observation (5, 6, 15). Studies also show that these abnormal spermatozoa are less capable of oocyte-spermatozoa fusion in vitro (15).

In another study (6), spermatozoa from fertile donors (n=6) were incubated with xanthine and xanthine oxidase, which are chemicals known to induce an oxidative burst of superoxide anions (and subsequently other ROS as a result of the chemistry aforementioned). Within one and a half hours, the beat frequency of the flagella and the motility of the spermatozoa had decreased to `0%' of the control values. This lends strong support to the view that ROS can have a dramatic effect on spermatozoa functioning, and therefore, on male fertility; other experiments addressing the effects of ROS on beat frequency and motility show similiar results (7, 9, 15). In the same study (6), the protective effects of adding superoxide dismutase and catalase were also assessed. Whereas superoxide dismutase conferred little protection, the addition of catalase offered dramatic protection against the effects of the oxidative burst elicited by xanthine and xanthine oxidase (X + XO). Thus, although X + XO addition resulted in a burst of superoxide anion formation, it is the byproduct, hydrogen peroxide (and ROS derived from it), which seems to have the most effect on spermatozoa functioning.

The results of these experiments suggest that abnormal sperm with low motility produce high levels of ROS which can then subsequently damage and affect the motilty of otherwise normal sperm within the semen population. Questions which still need to be addressed in the scientific community include : why and how these abnormal sperm release so much ROS, and how these abnormal sperm form in the first place.

III. The Antioxidant Carrying Load is Different in Fertile and Infertile Men

Given that ROS have a detrimental effect on spermatozoa functioning and given that a large fraction of infertile men seem to have elevated levels of ROS relative to fertile men, another topic that needs to be addressed is the antioxidant status of fertile versus infertile men. In one study (8), the total antioxidant capacity (both enzymatic and non-enzymatic) was compared in seminal plasma from fertile and infertile men. Although the study's main limitation was in its small sample sizes, the trends in the result suggest that the levels of total antioxidants are reduced in infertile men compared to fertile men. This situation brings up the question of causality. Are the levels of antioxidants in these infertile men depleted becuase they produce higher levels of ROS which are then scavenged by the antioxidants (thereby depleting their levels)? Or do these men lack normal capacities for antioxidants in the seminal plasma, which then allows their levels of ROS to be elevated? Could both of these mechanisms occur in vivo?

IV. The Effects of Vitamin E and C Supplementation in Spermatozoa Functioning In Vivo and In Vitro

Many studies which look at the protective effects of antioxidant supplementation in sperm samples focus their attention on enzymatic antioxidants. Although the addition of enzymatic antioxidants to sperm samples is feasible and practical from an in vitro fertilization perspective, it is not as practical from an in vivo and from a nutritional perspective. Because of these limitations of enzymatic antioxidants, it is more practical (from a nutritional perspective) to consider studies aimed at the beneficial effects of dietary-derived antioxidants on spermatozoa functioning. In particular, vitamin C (ascorbate) and vitamin E (tocopherol) are of interest because of their well documented abilities in scavenging and terminating free radicals.

In one study, the protective effects of vitamin E were assessed in vitro (15). Sperm samples (n=123 from 73 volunteers) were collected from normal volunteers. Chemicals known to induce oxidative bursts were added to these samples, and varying levels of vitamin E were added to the samples to assess its protective effects. Several variables were then monitored including the levels of malonaldehyde (malonaldehyde is a

toxic and reactive aldehyde byproduct of lipid peroxidation), sperm motility, and the ability of the spermatozoa to fuse with oocytes. The addition of vitamin E to these samples lowered the levels of malonaldehyde and increased the ability of spermatozoa to fuse with oocytes. No protective effects on motility were observed. Although the researchers conclude that the experiments demonstrated the protective effects of vitamin E in semen, this author believes the results of this experiment to be inconclusive, especially in light of the concentrations of vitamin E used; the researchers had used mM levels of vitamin E in their studies which is not representative of the uM levels present in vivo.

In a recent clinical study (12) designed to determine the in vivo effects of oral vitamin E supplementation in infertile men (n=30) with elevated levels of ROS, patients were administered 600 mg of viatmin E daily for 3 months, followed by a one month washout period, which was then followed by three months of placebo administration. Before and after each treatment arm, semen was collected and the quality determined by measuring the levels of ROS, the sperm motility, and the ability of the spermatozoa to bind to oocytes. Following vitamin E supplementation, oocyte binding improved, but other parameters were not affected. Although the authors conclude that vitamin E supplementation aids in fertilization, the study is fraught with weaknesses. For one, the number of samples measured was not enough to confidently determine the effects of vitamin E supplementation on oocyte binding (their power calculations indicate that they needed at least 88 people). Furthermore, although their protocol resulted in elevated levels of vitamin E in blood plasma, it had no effect on the vitamin E levels in seminal plasma. Therefore, this study is inconclusive because the levels of vitamin E were not affected in the seminal plasma. Other studies (16) have shown that oral administration of vitamin E can result in elevated levels of seminal plasma vitamin E.

Many studies are also being conducted on ascorbate and its roles in spermatozoa quality. The experiments conducted by Thiele et. al (14) indicate that the infertile men they studied (n=28) generally had lower levels of ascorbate and elevated levels of ROS with respect to the controls. They also established correlations and showed that low levels of vitamin C were correlated with high percentages of sperm with abnormal morphology and motility ($n=46$, $r=0.4$, $p<0.006$). Low levels of vitamin C were also correlated with higher levels of ROS ($n=46$, $r=-0.5$, $p<0.0006$).

No well supported study observing the protective effects of ascorbate supplementation on seminal quality has been published as of the date of submission of the paper by Thiele, et al. However, well supported studies do show that ascorbate does protect spermatozoa from oxidative damage. In a study by Fraga et. al (11), the biomarker, 8-hydroxy-2'-deoxyguanosine (a biomarker indicative of DNA damage mediated by ROS), was measured in DNA isolated from sperm from healthy men (n=24) and compared to their ascorbate levels. The study shows that higher levels of this lesion are present in men with lower seminal plasma concentrations of ascorbate. In the same study, a group of men (n=24) were maintained on diets which differed only with respect to the levels of ascorbate. The daily intake of ascorbate was lowered from 250 mg/day to 5 mg/day. This was accompanied by a concomitant decrease in seminal plasma ascorbate levels by one-half and an increase in the biomarker by 91%. When the dietary levels of ascorbate were replenished, the seminal plasma ascorbate levels doubled and the levels of the biomarker dropped 36% to roughly original levels. Although this study did not directly study the effects of ascorbate levels on sperm quality, it shows strong evidence suggesting that ascorbate supplementation can prevent biomolecular damage mediated by ROS in spermatozoa. Given that ROS damage affects sperm quality, the experiment indirectly suggests that ascorbate can have beneficial effects on fertility. However, direct clinical studies supporting this claim have yet to be conducted.

V. Remarks

It has been established from in vitro studies that infertile men have higher levels of ROS, that ROS damage sperm and that certain enzymatic antioxidants confer protection from the damaging ROS. However, more studies need to be performed to establish the effects of the antioxidants ascorbate and tocopherol on sperm qualilty and on fertililty.

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