

Improvement in Sperm Quality and Function with French Maritime Pine Tree Bark Extract

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OBJECTIVE: To determine the effects of Pycnogenol[®] (French maritime pine tree bark extract) on sperm parameters and function in subfertile men.

STUDY DESIGN: Prospective, nonrandomized, clinical study in a private infertility practice. Nineteen subfertile men were given 200 mg Pycnogenol[®] daily orally for 90 days. Semen samples were analyzed before and after treatment for sperm count, motility score and strict morphology before and after capacitation, and mannose receptor binding.

RESULTS: The mean sperm morphology following Ham's F-10 capacitation increased by 38% following Pycnogenol[®] treatment, and the mannose receptor binding assay scores improved by 19%.

CONCLUSION: Pycnogenol[®] therapy resulted in improved capacitated sperm morphology and mannose receptor binding. The increase in morphologically and functionally normal sperm may allow couples diagnosed with teratozoospermia to forgo in vitro fertilization and either experience improved natural fertility or undergo less invasive and less expensive fertility-promoting procedures, such as intrauterine insemination. (*J Reprod Med* 2002;47:821-824)

Keywords: infertility, male; antioxidants; sperm

count; sperm motility; sperm capacitation; Pycnogenol[®].

Up to 60% of infertile couples have difficulty conceiving due to "male factor" subfertility, meaning that one or more sperm parameters are abnormal. The production of abnormal quantities of reactive oxygen species (ROS) is thought to be involved in many facets of human male infertility.¹ Sperm exposed to superoxide an-

ions are apparently rendered dysfunctional by lipid peroxidation and altered membrane function, along with impaired metabolism, morphology and motility.²⁻⁴ The formation of reactive oxygen species has been associated with decreased sperm-egg interaction and reduced fertility.⁵

Vitamin C has been given to infertile men for years, as it has anecdotally proven to be efficacious in improving sperm parameters. More recently, studies have documented the efficacy of antioxidant treatment on human spermatozoa and fertilization rates, especially in the setting of *in vitro* fertilization (IVF). Indeed, improvements in IVF rates have been demonstrated after vitamin E therapy.^{6,7}

One of the richest natural sources of bioavailable

The increase in morphologically and functionally normal sperm may allow infertile couples diagnosed with teratozoospermia to forgo IVF and donor sperm insemination....

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and bioactive antioxidant compounds known is found in the bark of the *Pinus maritima* tree; the proprietary name of the extract is Pycnogenol® (Horphag Research, Ltd., Geneva, Switzerland). The biologic precursors of the oligomeric procyanidins, such as catechin and taxifoline, are effective and well-known free-radical scavengers. Pycnogenol® counteracts redox-sensitive NFκB-regulated gene expression of various inflammatory mediators, such as endothelial adhesion molecules and cytokines TNF-α and IL-1β.⁸

Pycnogenol® has been found to be many times more potent than other known antioxidants, like vitamin C and E.⁹ The objective of this prospective study was to evaluate the possible influence of Pycnogenol®, one of the most potent known antioxidants, on human sperm parameters, including the ability of sperm to bind to α-D-mannose receptors *in vitro*.

Materials and Methods

Following institutional review board approval, 19 subfertile men with abnormal baseline sperm testing were enrolled. Each patient received Pycnogenol® tablets, 200 mg orally per day, for 90 days. Supplementation with vitamins, minerals and other antioxidants was prohibited.

Men eligible for participation in the study were required to have one or more abnormalities previously demonstrated in their semen analysis (precapacitation or postcapacitation) and/or sperm capacity to bind to mannose receptors. All patients signed an informed consent form after the nature of the study had been fully explained.

Patients were not eligible for the study if they exhibited one or more of the following: (1) sperm antibodies; (2) current drug, tobacco, or alcohol abuse; (3) exposure to antioxidants or medication containing hormones within 90 days prior to the study; and (4) therapy to improve sperm parameters within 90 days prior to admission into this study.

After two to seven days of sexual abstinence, a semen sample was collected by masturbation into a sterile container. Semen analysis, sperm capacitation, sperm antibody testing and the mannose receptor binding assay were carried out per usual protocols. Briefly, the semen analysis consisted of sperm count, motility score and strict morphology. The sperm count (normal ≥20 million/mL) was manually done via a Makler Counting Chamber (Sefi-Medical Instruments, Haifa, Israel). The motility score (normal ≥150) and strict sperm morpholo-

gy (normal ≥14) were calculated according to previously described methods.¹⁰ Direct sperm antibodies were tested via a commercially available immunobead kit (ImmunoSpheres®, Bioscreen Inc., New York, New York). Capacitation was performed by the standard swim-up method utilizing Ham's F-10 solution. Finally, the mannose receptor binding assay (normal ≥36%), which measures sperm's potential to bind to glycoproteins similar to those found on the zona pellucida of human oocytes, was completed using a standardized test kit (Mannose Binding Assay™ [MBA], Embryotech Laboratories, Inc., Wilmington, Massachusetts).

A 90-day supply of Pycnogenol® was given to each patient who entered the study, and each participant took 200 mg/d by mouth for 90 days. A "target" date was set for termination of the study, at which time the patient produced a second masturbated specimen in order to repeat the sperm testing.

Individual percent changes (from baseline) in sperm parameters were calculated, and the mean of the percent changes was utilized for data analysis. Data were statistically analyzed by paired *t* testing; *P* < .05 was considered significant.

Results

None of the patients tested positive for direct sperm antibodies. The pretreatment and posttreatment mean sperm parameters analyzed in the 19 subjects are listed in Table I. As compared to pretreatment, the mean percent change from baseline sperm count after Pycnogenol® therapy decreased nonsignificantly by 10%. There was also a nonsignificant decline in the mean Ham's F-10 capacitated motility score (4%). The mean change from baseline morphology increased by 33%, but this improvement was not statistically significant.

After Ham's F-10 capacitation, the mean percent changes in strict morphology and the mannose receptor binding assay revealed significant improvements following Pycnogenol® treatment ($38 \pm 0.6\%$, *P* < .001, and $19 \pm 1.5\%$, *P* < .005, respectively). There were no adverse effects reported by the men during the test period.

Discussion

We have known for over 30 years that the human sperm plasma membrane has a high content of phospholipid-bound polyunsaturated fatty acids (PUFA).¹¹ This high PUFA content of sperm membranes has drawn attention to their susceptibility to peroxidative changes. Most of the sperm mem-

Table 1 Sperm Parameters in 19 Subfertile Men Before and After 90 Days of Treatment with Pycnogenol®

Pycnogenol® treatment	Baseline count	Baseline motility score	Baseline morphology	Capacitated count	Capacitated motility	Capacitated morphology	Mannose binding
Before	118.0 ± 17.2	145.8 ± 11.5	4.3 ± 0.6	87.8 ± 11.0	248.3 ± 5.9	8.9 ± 0.8	32.9 ± 1.8
After	92.0 ± 10.5	152.6 ± 14.3	4.9 ± 0.6	89.7 ± 9.7	238.4 ± 15.7	11.4* ± 0.9	37.8** ± 1.1

Values are mean ± SEM.

Normal ranges: count 20 million/mL, motility score ≥ 150, morphology ≥ 14%, mannose binding ≥ 36%.

**P* = .001.

***P* < .005.

brane's polyunsaturated fatty acids contain carbon atoms with five and six double bonds. PUFA containing two or more double bonds are readily attacked by oxygen radicals, so sperm lipids that are very enriched in fatty acids possessing five or six double bonds are particularly vulnerable to peroxidation.¹¹

When sperm membrane proteins are damaged, the membranes become "leaky," and eventually the membrane breaks down completely, leading to the functional impairment of sperm.¹² Altered sperm structure and function due to ROS may be evidenced by loss of sperm motility,¹³ midpiece abnormalities,¹⁴ decreased sperm and oocyte fusion (binding),⁵ and abnormal morphology.²

Normally, the seminal fluid surrounding sperm contains antioxidant factors (glutathione, urate, ascorbate, α -tocopherol, taurine, etc.), protecting them from oxidative damage.¹¹ In many subfertile men, however, for poorly understood reasons, the seminal fluid may either lack sufficient protective elements or the man's body may be so overloaded with ROS so as to overwhelm the normal inherent antioxidative mechanisms. Increased levels of ROS may be generated internally from damaged or defective sperm as well as from leukocytes in seminal plasma.¹⁵ High levels of circulating ROS may result from external sources, such as air/water pollution and common environmental toxin exposures, for which it has been widely suggested that we all take daily antioxidant supplements.

Iwasaki¹⁶ detected ROS formation in 40% of semen specimens from men attending an infertility clinic. Mazilli and coworkers¹⁷ found significantly elevated levels of superoxide anion in 87% of infertile patients.

It is common knowledge that severe defects in sperm morphology render sperm dysfunctional and greatly reduce a couple's chances of pregnancy with either coitus or intrauterine insemination. In-

fertile couples may therefore need to resort to donor sperm inseminations or costly advanced assisted reproductive techniques, such as IVF. Many couples reject the notion of donor sperm insemination, as they prefer to pass the male partner's genes on to their offspring. Other patients are unable to undergo IVF due to either religious beliefs or cost restrictions.

A number of glycoproteins on the oocyte's zona pellucida play a role in the binding of human sperm to the egg. The predominant sugar residue in this glycoprotein is mannose. Mannose residues are hypothesized to interact with a sperm surface enzyme as part of the recognition mechanism leading to sperm/oocyte binding. This sperm surface enzyme is expressed on a percentage of normal sperm following capacitation.^{18,19}

The MBA allows measurement of the ability of sperm to bind to α -D-mannose. Although a normal MBA does not guarantee fertilization or fertility, as it tests only the first step in the complex of sperm/egg interactions, data show that normal MBA levels correlate with normal fertilization *in vitro*.^{20,21}

This study demonstrated a 38% mean improvement in capacitated sperm morphology following three months of Pycnogenol® therapy in a group of subfertile men. Additionally, a significant mean increase, 19%, in the MBA after Pycnogenol® treatment was found. The increase in morphologically and functionally normal sperm may allow infertile couples diagnosed with teratozoospermia to forgo IVF and donor sperm insemination and thereby undergo less stressful, invasive and expensive fertility-enhancing procedures, such as intrauterine insemination with the male partner's sperm.

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