

## IMPROVEMENT OF SPERM QUALITY BY *PYCNOGENOL*<sup>®</sup>

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### SUMMARY

*Published and anecdotal reports have claimed various sperm parameters improved after antioxidant treatment for male infertility. Accordingly, we evaluated the effects of Pycnogenol<sup>®</sup>, a potent antioxidant, on abnormal sperm parameters in a small group of subfertile men. Four subfertile male patients received Pycnogenol<sup>®</sup> tablets, 200 mg orally per day, for 90 days, after which all sperm tests were repeated. Pre- and post-Pycnogenol<sup>®</sup> treatment sperm tests included: count, motility score and strict (Kruger) morphology, both before and after capacitation with Ham's F-10 solution (swim-up); direct sperm antibodies; and marmose receptor binding for sperm function.*

*The potent antioxidant Pycnogenol<sup>®</sup> significantly improved sperm morphology in a small group of subfertile men. This increase in morphologically normal sperm may allow couples diagnosed with teratozoospermia to forgo in vitro fertilization and undergo less invasive and less expensive fertility-promoting procedures such as intra-uterine insemination. None of the other sperm indices revealed considerable changes following Pycnogenol<sup>®</sup> treatment.*

### INTRODUCTION

Up to 60% of infertile couples have difficulty conceiving due to "male factor" subfertility, meaning one or more of the 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 (Lewis *et al.*, 1998). Sperm exposed to superoxide anions are apparently rendered dysfunctional by lipid peroxidation and altered membrane function, along with impaired metabolism, morphology, and motility (Aitken and Fisher, 1994; Alvarez *et al.*, 1987; De Lamirande and Gagnon, 1992). The formation of reactive oxygen species has been associated with decreased sperm-egg interaction and reduced fertility (Aitken *et al.*, 1989).

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 in vitro fertilization rates have been demonstrated after vitamin E therapy (Geva *et al.*, 1996; Kessopoulou *et al.*, 1995).

One of the richest natural sources of bioavailable and bioactive antioxidant compounds known is found in the bark of the "*Pinus maritima*" tree, the extract of which is called *Pycnogenol*<sup>®</sup> (Horphag Research Geneva, Switzerland). The biological precursors of the oligomeric procyanidins such as catechin and taxifoline are effective and well-known free-radical scavengers. *Pycnogenol*<sup>®</sup>'s components inhibit the cyclooxygenases that produce inflammatory prostaglandins (Baumann *et al.*, 1980).

This prospective pilot study was aimed to evaluate the possible influence of *Pycnogenol*<sup>®</sup>, one of the most potent known antioxidants, on human sperm parameters, including the ability of sperm to bind to oocyte receptors *in vitro*.

## MATERIALS AND METHODS

Following IRB approval, four (4) subfertile men with abnormal baseline sperm testing were enrolled. Each patient received *Pycnogenol*<sup>®</sup> tablets, 200 mg orally per day, for 90 days. Ingestion of other vitamins, minerals, and antioxidant supplements were prohibited.

**Inclusion Criteria.** Men eligible for participation in this study were required to have one or more abnormalities previously demonstrated in their semen analysis (pre- or post-capacitation), sperm binding to mannose receptors, or sperm antibody testing. Patients all signed the informed consent after the nature of the study had been fully explained.

**Exclusion Criteria.** Patients were not eligible for study if they exhibited one or more of the following:

1. Current drug, tobacco, or alcohol abuse;
2. Exposure to antioxidants or medication containing hormones within 90 days prior to the study; and

3. Having undergone therapy to improve sperm parameters within 90 days prior to admission into this study.

After 2-7 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 the sperm count, motility score, and morphology. The sperm count (normal  $\geq 60$  million/cc) was manually done via a Makler Counting Chamber (Sefi-Medical Instruments; Haifa, Israel). The motility score (normal  $\geq 150$ ) and strict sperm morphology (normal  $\geq 14$ ) were calculated according to previously described methods (Acosta and Kruger, 1996). Direct sperm antibodies were tested via a commercially available immunobead kit (*Immuno-Spheres*<sup>®</sup>; Bioscreen Inc., NY). Capacitation was performed by the standard "swim-up" method utilizing Ham's F-10 solution. Finally, the mannose receptor binding assay (normal  $\geq 36\%$ ), which measured the sperm's potential to bind to glycoproteins similar to those found on the zona pellucida of the human oocytes, was completed using a standardized test kit (Mannose Binding Assay<sup>™</sup>; Embryotech Laboratories, Inc., MA).

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

## RESULTS

None of the patients tested positive for direct sperm antibodies. Compared to pretreatment, the mean change from baseline sperm morphology increased significantly by 99% (*Figure 1*).

None of the other sperm indices, including the Ham's F-10 capacitated count, motility score, sperm count, and mannose receptor binding assay, revealed considerable changes following *Pycnogenol*<sup>®</sup> treatment. There were no adverse effects reported by the men during the test period.

Baseline sperm morphology. Percent change after *Pycnogenol*<sup>®</sup> treatment

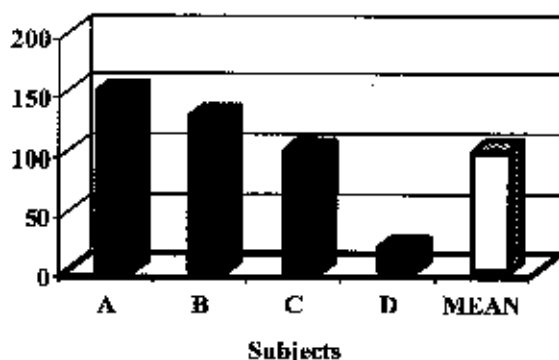


Figure 1. Percent change from baseline in sperm morphology following 90 days of *Pycnogenol*<sup>®</sup> treatment in four subjects.

## 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) (Kim and Parthasarathy, 1998). This high PUFA content of sperm membranes has drawn attention to their susceptibility to peroxidative changes. Most of the sperm membrane's polyunsaturated fatty acids contain five or 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 (Kim and Parthasarathy, 1998).

When sperm membrane proteins are damaged, the membranes become "leaky", and eventually the

membrane breaks down completely, leading to the functional impairment of sperm (Jeyendran *et al.*, 1984). Altered sperm structure and function due to ROS may be evidenced by loss of sperm motility (Alvarez and Storey, 1982), midpiece abnormalities (Rao *et al.*, 1989), decreased sperm and oocytes fusion (binding) (Aitken *et al.*, 1989), and abnormal morphology (Aitken *et al.*, 1994).

Normally, the seminal fluid surrounding sperm contains antioxidant factors (such as glutathione, urate, ascorbate,  $\alpha$ -tocopherol, taurine, *etc*) protecting them from oxidative damage (Kim and Parthasarathy, 1998). 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 leucocytes in the seminal plasma (Tamura *et al.*, 1988). 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, we all take daily antioxidant supplements.

Iwasaki and Gagnon (1992) detected ROS formation in 40% of semen specimens of men attending an infertility clinic. Mazilli *et al.*, (1994) 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. Infertile couples may therefore need to resort to donor sperm inseminations or costly advanced assisted reproductive techniques such as in-vitro fertilization (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.

This study demonstrated a 99% mean improvement in baseline sperm morphology following three months of *Pycnogenol*<sup>®</sup> therapy in a small group of subfertile men. This increase in morphologically normal sperm may allow infertile couples diagnosed with teratozoospermia to forgo in-vitro fertilization or donor sperm insemination, and thereby undergo less stressful, less invasive, and less expensive fertility enhancing procedures such as intrauterine insemination with their partner's sperm.

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