

# Improvement of seminal quality and sexual function of men with oligoasthenoteratozoospermia syndrome following supplementation with L-arginine and Pycnogenol®

Yoshitomo Kobori <sup>1</sup>, Keisuke Suzuki <sup>1</sup>, Toshiyuki Iwahata <sup>1</sup>, Takeshi Shin <sup>1</sup>, Yuko Sadaoka <sup>1</sup>, Ryo Sato <sup>1</sup>, Kojiro Nishio <sup>1</sup>, Hiroshi Yagi <sup>1</sup>, Gaku Arai <sup>1</sup>, Shigehiro Soh <sup>1</sup>, Hiroshi Okada <sup>1</sup>, Jeffrey Michael Strong <sup>2</sup>, Peter Rohdewald <sup>3</sup>

<sup>1</sup> Department of Urology, Dokkyo Medical University Koshigaya Hospital, Saitama, Japan;

<sup>2</sup> Horphag Research, Cointrin/Geneva, Switzerland;

<sup>3</sup> Institute of Pharmaceutical Chemistry, University of Munster, Munster, Germany.

**Summary** We evaluated the effectiveness of antioxidant co-supplementation therapy using L-arginine and Pycnogenol® in Japanese men with oligoasthenoteratozoospermia and mild erectile dysfunction (ED). A total of forty-seven adult males with oligoasthenoteratozoospermia syndrome (OAT) were eligible for enrollment. The effectiveness of supplementation with a combination of L-arginine 690 mg and French maritime pine bark extract (Pycnogenol®) 60mg for OAT and ED was investigated. The sperm concentration was enhanced significantly after treatment 2 and 4 months ( $11.79 \pm 9.86$  to  $21.22 \pm 28.17$  and  $20.15 \pm 23.99 \times 10^6/\text{ml}$ ). Significant improvements in the International Index of Erectile Function (IIEF) were observed in the total score of IIEF ( $57.69 \pm 11.04$  to  $59.43 \pm 12.57$ ) and domain of Orgasmic Function ( $9.01 \pm 1.92$  to  $9.34 \pm 1.66$ ) after 4 months of treatment. L-arginine acts to increase the production of nitric oxide and Pycnogenol® activates the endothelial nitric oxide synthase and it is a potent antioxidant and inhibitor of inducible nitric oxide synthase. This study suggests that the combination of Pycnogenol® and L-arginine (Edicare®) is helpful for infertile men to ameliorate simultaneously quality of sperms as well as erectile functions.

**KEY WORDS:** Male infertility; Spermatogenesis, erectile dysfunction, L-arginine, Pycnogenol.

Submitted 1 May 2015; Accepted 30 June 2015

## INTRODUCTION

World-wide about 1 in 7 couples have problems in conceiving. Male infertility is involved in 30-50% of involuntarily childless couples according to *European Guidelines on Male Infertility* (1).

A specific investigation on male fertility was performed in Japan. Semen quality of 1559 young Japanese men was evaluated in a large multi-center, cross-sectional study (2). Within this group of men between 18-24 years, 9% had a lower sperm concentration than 15

mill/ml and 31.9% produced a total sperm number below 40 mill/ml. These numbers are below the limits given in the WHO manual for *Examination and Processing of Human Semen* (3). So a significant proportion of men will have a lower chance to achieve fertilization because of their sub-optimal semen quality. There is doubtless a need to enhance male fertility for those men diagnosed to have the Oligoasthenoteratozoospermia syndrome (OAT) (4).

Clinical studies conducted in Europe and USA have confirmed that the proprietary, patented combination of L-arginine aspartate and Pycnogenol® French maritime pine bark extract marketed as *Prelox®* (both trademarks of Horphag Research Ltd.,) can improve mild to moderate erectile dysfunction (ED) and increase quantity and quality of sperms (5).

Pycnogenol is a specific pine bark extract consisting of a concentrate of polyphenols, mainly procyanidins, and is listed in the *United States Pharmacopoeia 34* as *Maritime Pine Extract* (6).

Pycnogenol demonstrates its action to improve ED by activating endothelial nitric oxide synthase (e-NOS), thereby increasing nitric oxide production and promoting vasodilation (7).

A synergistic effect in nitric oxide production is achieved when Pycnogenol is administered in combination with L-arginine, the substrate for e-NOS (8). It has been reported that the combination of Pycnogenol and L-arginine may increase testosterone concentration, which is suggested to be a physiologic response secondary to increased sexual activity (9).

A Pycnogenol and L-arginine containing supplement formulated for the Japanese population (Edicare®) for ED has been tested in Japanese patients specifically and improved after 8 weeks of ingestion the erectile function significantly (10). In the present pilot study the influence of Edicare® on quality of sperms was evaluated. Simultaneously, the influence of the preparation on ED on men with confirmed OAT was investigated.

Conflicts of interest:

Partly supported by a grant from Kobayashi Pharmaceutical.

## MATERIALS AND METHODS

### Subjects

This study was conducted in compliance with the *Helsinki Declaration* after obtaining the approval of the *Ethics Committee of Dokkyo Medical University Koshigaya Hospital*. Subjects were selected from 47 outpatients with OAT and mild to moderate ED who had given written informed consent. All presented with infertility after at least 2 years of unprotected intercourse. Male infertility was diagnosed if one or more standard semen parameters were below cutoff levels (sperm concentration  $< 20 \times 10^6/\text{ml}$ , sperm motility  $< 50\%$ , normal morphology  $< 30\%$ , and/or semen volume based  $< 2 \text{ ml}$ ) based on at least two semen analyses performed 3 months apart to eliminate accidental and possible adverse effects of exogenous factors on spermatogenesis. After providing a complete medical and reproductive history exploring all aspects that might be related to fertility, patients underwent physical examination and serum chemical and hematological laboratory tests. Testicular volume was measured using a punched orchidometer.

Serum follicle stimulating hormone, luteinizing hormone, and testosterone levels were measured in all patients (Table 1).

Subjects who did not meet the following 12 exclusion criteria were selected: Habitual consumption of medication and/or a food supplement intended for ED improvement; habitual consumption of medication and/or food supplement that contained either L-arginine, aspartic acid or maritime pine bark extract; allergy to the substances investigated in this study; participation in another clinical study at the start of the study; existence of diabetes mellitus, heart disease, renal disease, hepatic disease, moderate or severe hypertension; habitual consumption of any food supplement to improve the circulation; body mass index (BMI)  $\geq 35 \text{ kg/m}^2$ ; receiving concurrent dental treatment; reported smoking 20 or more cigarettes a day; morning erection achieved most days. Those judged by the investigator (on the basis of laboratory test value or other reasons) as inappropriate for participation in this study were also excluded.

### Sperm preparation

Semen samples were obtained at start of the study and after 2 and 4 months. After at least 2 days of sexual abstinence, ejaculates were obtained by masturbation in sterile plastic containers. After liquefaction at room temperature, semen volume was determined and samples were immediately examined microscopically according to the guidelines of the WHO (3). Motility of spermatozoa was investigated at  $37^\circ\text{C}$  and given as average motility rate. Each specimen was investigated by two laboratory technicians.

### Erectile function

To evaluate the influence of *Edicare*<sup>®</sup> on erectile function, participants answered to the questions contained in the International Index of Erectile Function (IIEF) in the extended version with 15 questions (11). The questionnaire of IIEF, translated into Japanese, was provided to the subjects for assessment before the start of the study, at 4 weeks and again at 8 weeks. We examined the total score of IIEF, IIEF5, and each domain: Erectile Function (EF); Orgasmic Function (OF); Sexual Desire (SD); Intercourse Satisfaction (IS); and Overall Satisfaction (OS).

### Supplement use

The commercialized food supplement *Edicare*<sup>®</sup> (manufactured by *Kobayashi Pharmaceutical Co., Ltd.*) containing 10 mg of *Pycnogenol*, 115 mg of L-arginine and 92 mg of aspartic acid per tablet was used in the present study. The subjects were asked to take six tablets each day and to record their supplement intake in a diary.

### Statistical analysis

The student's t-test was employed for inter-group comparison of the change in IIEF and each domain scores using the Statcel 3 program (*OMS Publishing, 2011, Japan*). For seminal parameters Wilcoxon's signed rank sum test was used for intra-group comparisons, and differences in mean values were assayed. In each case, the level of statistical significance was set at  $p < 0.05$ .

## RESULTS

Baseline values of the participants are given in Table 1. Testosterone levels are partly below, LH and FSH are

**Table 1.**  
Characteristics of the patients.

Characteristics	Range	Mean $\pm$ SD
Age	26-48	36 $\pm$ 9
Age of wife	22-44	34 $\pm$ 8
Serum hormones:		
Testosterone (ng/dl)	169-988	464 $\pm$ 151
LH (IU/l)	1.1-11.5	4.9 $\pm$ 2.8
FSH (IU/l)	1.6-26.8	1.6-26.8
Testicular volume (ml):		
Right	8-26	18 $\pm$ 5
Left	4-26	16 $\pm$ 5

*n* = 47  
LH, luteinizing hormone; FSH, follicle-stimulating hormone.  
Normal reference range LH: 2.2-8.4 mIU/ml; FSH: 1.8-12.0 mIU/ml;  
Testosterone: 225-1040 ng/dl.

**Table 2.**  
Sperm variables throughout the study.

	Mean $\pm$ SD		
	Baseline	2 months	4 months
Semen volume (ml)	3.02 $\pm$ 1.55	2.69 $\pm$ 1.38	2.63 $\pm$ 1.42
p-value		0.12	0.09
Sperm concentration ( $\times 10^6/\text{ml}$ )	11.79 $\pm$ 9.86	21.22 $\pm$ 28.17	20.15 $\pm$ 23.99
p-value		* 0.02	* 0.01
Sperm motility (%)	32.31 $\pm$ 24.47	35.42 $\pm$ 23.71	33.81 $\pm$ 21.46
p-value		0.15	0.08

\*:  $p < 0.05$ .  
Wilcoxon signed-ranks test

**Table 3.**  
Scores for Erectile Function – IIEF.

	Mean ± SD		p-values
	Baseline	After treatment	
OS	7.36 ± 1.55	7.63 ± 1.23	0.12
IS	9.02 ± 3.18	9.13 ± 3.94	0.11
SD	6.86 ± 1.46	6.95 ± 1.83	0.69
OF	9.01 ± 1.92	9.34 ± 1.66	* 0.03
EF	25.43 ± 5.90	26.36 ± 6.14	0.08
Total	57.69 ± 11.04	59.43 ± 12.57	* 0.02
IIEF5	21.26 ± 4.75	21.47 ± 5.67	0.25

Wilcoxon signed-ranks test

\*:  $p < 0.05$ .

OS: Overall Satisfaction, IS: Intercourse Satisfaction, SD: Sexual Desire, OF: Orgasmic Function, EF: Erectile Function

partly high but the mean is in the normal range. Supplementation with the combination of L-arginine and French maritime pine bark extract effectively increased the average sperm concentration significantly after 2 and 4 months (Table 2). Furthermore, there was a slight, but not significant improvement in the motility rate and the number of functioning sperms after supplementation for 4 months. During the initial screening, volunteers showed a mild erectile dysfunction according to IIEF. Erectile function was significantly ameliorated after 4 months according to the answers of the participants in the total score of IIEF and domain of OF (Table 3).

## DISCUSSION

Several approaches have been proposed for the management of infertility and ED caused by oxidative stress. Once an individual has been identified as having oxidative stress-related reproduction and sexual dysfunction, treatment should be aimed at identification and amelioration of the underlying cause before considering antioxidant treatment.

Lifestyle behaviors such as smoking, poor diet, alcohol abuse, pollution and environmental toxins, obesity, and psychological stress have all been linked to oxidative stress. While the effectiveness of eliminating these lifestyle triggers on oxidative stress has not been formally tested, it is likely that making positive lifestyle changes such as changing to a diet high in fruit and vegetables, maintaining normal weight, and reducing smoking or alcohol intake would have at least some beneficial effects on sperm health.

Several studies have reported that levels of reactive oxygen species within semen can be reduced by augmenting the scavenging capacity of seminal plasma using oral antioxidant supplements (7).

The obtained results show the positive effect of the combination of L-arginine and *Pycnogenol*<sup>®</sup> in *Edicare*<sup>®</sup> on sperm function and erectile function. The dosage of L-arginine 690 mg and *Pycnogenol*<sup>®</sup> 60 mg used in this investigation was considerably lower than the amounts of L-arginine and *Pycnogenol*<sup>®</sup> given in previous studies of Stanislavov and Nikolova with 1.43 g L-arginine and 120 mg *Pycnogenol*<sup>®</sup> (4, 5).

A comparison of the results of the present study with the studies with the higher dosage indicate that the lower dosage is less effective.

The assumption, that the lower body weight of Japanese men compared to Bulgarian men will compensate the lower dosage is not fully justified.

In principle, the combination of *Pycnogenol*<sup>®</sup> and L-arginine acts by increasing the production of NO from L-arginine. As has been demonstrated with healthy Japanese volunteers that *Pycnogenol*<sup>®</sup> activates the endothelial nitric oxide synthase (e-NOS) (12). An enhanced activity of e-NOS plus a high concentration of its substrate L-arginine results in a greater production of NO. NO is activating guanylate cyclase to produce cGMP. The cGMP-protein kinase G signaling pathway leads to an increase of sperm motility (13).

Another positive effect of *Pycnogenol*<sup>®</sup> is based on its inhibition of inducible nitric oxide synthase (i-NOS) (14). This enzyme is over-expressed in OAT and causes oxidative damage of the sperms. As *Pycnogenol*<sup>®</sup> is a potent antioxidant (7) and inhibitor of i-NOS, it is reasonable to assume that the supplementation with *Edicare*<sup>®</sup> is able to reduce the damage of sperms of the patients with OAT syndrome.

These effects led the improvement of ED.

As no unwanted effects were observed or reported from patients, *Edicare*<sup>®</sup> may be considered as a safe natural product which helps to improve fertility in men with OAT syndrome.

## CONTRIBUTIONS

All authors participated in the experimental design and method, and the collection, analysis and interpretation of the data.

## REFERENCES

- Jungwirth A, Giwercman A, Tournaye H et al. European Association of Urology Guidelines on Male Infertility: The 2012 Update. *Eur Urol*. 2012; 62:324-332.
- Iwamoto T, Nozawa S, Mieno MN, et al. Semen quality of 1559 young men from four cities in Japan: a cross-sectional population-based study. *BMJ open*. 2013; 3: e 00222 doi: 1136/bmj.open-2012-002222.
- WHO Manual for Examination and Processing of Human Semen. 5th Ed. WHO 2010.
- Stanislavov R, Nikolova V, Rohdewald P. Improvement of erectile function with *Prelox*<sup>®</sup>: a randomized, double-blind, placebo-controlled, cross-over trial. *Int J Impot Res*. 2008; 20:173-180.
- Stanislavov R, Rohdewald P. Sperm quality in men is improved by supplementation with a combination of L-arginine, L-citrullin, roburins and *Pycnogenol*<sup>®</sup>. *Minerva Urol Nefrol*. 2014; 66:217-23.
- Stanislavov R, Nikolova V, Rohdewald P. Improvement of Seminal Parameters with *Prelox*<sup>®</sup>: A randomized, double-blind, placebo-controlled, cross-over trial. *Phytother Res*. 2009; 23:297-302.
- Rohdewald P. A review of the French maritime pine bark extract (*Pycnogenol*<sup>®</sup>), a herbal medication with a diverse pharmacology. *Int J Clin Pharmacol Ther*. 2002; 40:158-168.
- Stanislavov R, Nikolova V. Treatment of erectile dysfunction

with Pycnogenol® and L-arginine. *J Sex Marital Ther.* 2003; 29:207-213.

9. Lamm S. Prelox® for Improvement of Erectile Quality. *Eur J Endocrinol.* 2009; 4:70-74.

10. Aoki H, Nagao J, Ueda T, et al. Clinical assessment of a supplement of Pycnogenol and L-arginine in Japanese patients with mild to moderate erectile dysfunction. *Phytother Res.* 2012; 26:204-207.

11. Rosen RC, Cappelleri JC, Gendrano N 3rd. The International Index of Erectile Function (IIEF): A state of the science review. *Int J Impot Res.* 2002; 14:226-244.

12. Nishioka K, Hidaka T, Nakamura S, et al. Pycnogenol, French Maritime Pine Bark Extract, Augments Endothelium-Dependent Vasodilation in Humans. *Hypertens Res.* 2007; 30:775-780.

13. O'Bryan MK, Zini A, Cheng CY, et al. Human endothelial nitric oxide synthase expression: correlation with sperm motility. *Fertil Steril.* 1998; 70:1143-1147.

14. Uhlenhuth E, Högger P. Facilitated cellular uptake and suppression of inducible nitric oxide synthase by a metabolite of maritime pine bark extract (Pycnogenol®). *Free Radic Biol Med.* 2012; 53:305-313.

### Correspondence

Yoshitomo Kobori, MD (Corresponding Author)  
ykobori@dokkyomed.ac.jp

Department of Urology, Dokkyo Medical University Koshigaya Hospital  
2-1-50 Minami-Koshigaya, Koshigaya, Saitama 343-8555, Japan

Keisuke Suzuki, MD  
Toshiyuki Iwahata, MD  
Takeshi Shin, MD  
Yuko Sadaoka, MD  
Ryo Sato, MD  
Kojiro Nishio, MD  
Hiroshi Yagi, MD  
Gaku Arai, MD  
Shigehiro Soh, MD  
Hiroshi Okada, MD

Department of Urology, Dokkyo Medical University Koshigaya Hospital,  
Saitama, Japan

Jeffrey Michael Strong  
Horphag Research, Cointrin/Geneva, Switzerland

Peter Rohdewald  
Institute of Pharmaceutical Chemistry, University of Munster, Munster, Germany