Improvement of Seminal Parameters with Prelox®: A Randomized, Double-blind, Placebo-controlled, Cross-over Trial

R. Stanislavov¹, V. Nikolova¹ and P. Rohdewald²*
¹Medical University Sofia, Faculty of Medicine, Department of Obstetrics and Gynecology, 2 Zdrave Street, 1413 Sofia, Bulgaria
²Institute of Pharmaceutical Chemistry, Westfälische Wilhelms-Universität Münster, Hittorfstr. 58-62, 48149 Münster, Germany

INTRODUCTION

Male infertility seems to be an increasing problem at least in the industrialized countries. Poor quality of sperm is a frequent finding in patients attending subfertile clinics.

Previous investigations pointed to the possibility of improving the quality of sperm by intake of a food supplement. Treatment of 19 subfertile patients with Pycnogenol (Horphag Research Ltd, UK) for 90 days resulted in a significant improvement in the morphological quality of spermatozoa (Rohdewald, 2005). Pycnogenol consists of a concentrate of phenolic compounds, extracted from the bark of the French maritime pine. The standardized extract contains mainly procyanidins and a variety of phenolic acids (Rohdewald, 2005). One of these phenolic acids, ferulic acid, has been shown to increase the motility of spermatozoa (Zheng and Zhang, 1997).

In an open study with 50 men, a combination of Pycnogenol with l-arginine aspartate and testosterone enhanced significantly the ejaculate volume, concentration and mobility of spermatozoa and increased greatly the percentage of spermatozoa with normal morphology compared with placebo. The placebo had no influence on the parameters of seminalogical analysis. Intake of Pycnogenol for 1 month improved the fertility index to normal values. After treatment, the fertility index decreased again to infertile status. No unwanted effects were reported. Prelox seems to be a promising alternative to treat patients with mild infertility. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: fertility; spermatozoa; Prelox®, l-arginine; Pycnogenol®.

MATERIALS AND METHODS

Patients. From 210 patients visiting the Seminological Laboratory of the Medical University of Sofia, Bulgaria, 50 men were recruited as meeting the criteria for inclusion. Patients gave written informed consent and were informed that they could leave the study at any time. A sample size of 50 patients was chosen on the basis of a previous study (Stanislavov and Nikolova, 2005) with 50 patients showing significant improvement of fertility. Patients were examined for medical history and were undergoing physical and seminalogical examination.

Physical examination. Patients were examined at the first visit for involvement of infertility, signs of hypogonadism,
normal development of musculature, decrease of testicular volume, anatomical changes of penis, fat distribution, voice, height, weight, vital signs: blood pressure, heart rate.

After 18 weeks the patients were examined again for vital signs.

**Medical history.** The patients were asked about impairment of general performance, diminution of beard growth, decrease in erection frequency, lessening of sexual desire and fantasies, recurrent bronchitis or sinusitis in childhood, infections with/without orchitis or epididymitis, harmful occupational or environmental influences, drug or narcotics abuse and chronic diseases.

**Inclusion criteria.** The patients were aged between 30 and 50 years. They had been in a stable sexual partnership for the past 6 months persisting over the whole period. The duration of infertility was more than 2 years and had different deviations in sperm concentration and motility and morphology in terms of seminological criteria. They had different deviations in sperm concentration and motility and morphology in terms of seminological criteria.

**Exclusion criteria.** Testicular maldescent, varicoceles, orchitis, globozoospermia, infections, disturbances of semen deposition (hypospadias), severe cardiovascular disease, severe hypertension ≥ 150 mm/Hg; s ≥ 90 mm/Hg; s ≥ 150 mm/Hg, renal failure, hepatic insufficiency, endocrine hypogonadism abnormality, psychiatric disorders, testicular tumors, treatment of ED with any drugs during the past 4 weeks.

**Random allocation.** Each patient received a number in the order of their enrolment. Patients with even numbers started the first treatment period with medication B, and those with odd numbers received medication A.

**Medication.** Prelox and placebo tablets were prepared by the same producer (Manhattan Drug Company Inc, New York, USA). Tablets were indistinguishable in color, size, shape and weight. Tablets were dispensed by the American producer in identical plastic boxes marked A and B. Investigators were not informed about the identity of A and B tablets until after evaluation of the study results. Patients were instructed to take two tablets between 7 and 9 a.m. and two tablets between 7 and 9 p.m. with 200 mL water. The daily dose of Prelox corresponded to an intake of 80 mg Pycnogenol and 3 g l-arginine aspartate.

**Study design.** The study was designed as a randomly allocated, double-blind, placebo-controlled, cross-over study. The study was approved by the Committee for Approval for Clinical Trials of the Ministry of Health, Republic of Bulgaria and was monitored by an authorized clinical research officer, V. Damianova. The study was conducted at the Seminological Laboratory of the University Hospital Maichin Dom, Sofia, Bulgaria. The study was performed between spring 2005 and summer 2006 and was in accordance with the declaration of Helsinki.

Patients were thoroughly investigated by physical examination and clinical chemistry at the start and at the end of the study (Stanislavov et al., 2007).

The study design is shown in Fig. 1.

After the 4 week run-in period, the patients received blinded medication, group A placebo, group B Prelox, for the first treatment period of 4 weeks. At the end of the first treatment period, the tablet boxes were assessed for the number of tablets taken.

Following the wash-out period, the patients received blinded medication, group A Prelox, group B placebo. After the second treatment period, the tablet boxes were assessed for the remaining tablets.

Seminological analysis was performed with semen samples collected at the start of the run-in period, and after 4, 8, 12, 16 weeks.

At each visit, the patients were asked for concomitant medication and for unwanted effects. All data were collected on case report forms.

**Sperm preparation.** Twelve hours before attendance each subject had to refrain from caffeine- or nicotine-containing agents. After 3–7 days of sexual abstinence, ejaculates were obtained by masturbation in sterile plastic containers between 7 and 9:30 a.m.

The sperm volume was determined gravimetrically. Immediately after liquefaction (30 min at room temperature), the samples were examined according to the guidelines of the World Health Organization (1999) by light microscopy. Two investigators examined each of the semen samples taken, independently of each other. Analyses were carried out in a fertility clinical with access to 4000 patients per year. Semen samples were obtained at screening and after 4, 8, 12 and 16 weeks.

The following seminal parameters were determined.

**Motility of spermatozoa.** The motility of each spermatozoon was graded as a, b, c and d, according to whether it showed: a, % rapid progressive motility (≥25 µm/s at 37 °C); b, % slow or sluggish progressive motility; c, % nonprogressive motility (≤5 µm/s); d, immotility.

**Vitality of spermatozoa.** These tests provide a good internal control of the estimate of motility.
Eosin-nigrosin (a modification of Blom’s technique). One drop of semen was mixed with two drops of 1% Eosin Y. After 30 seconds three drops of 10% nigrosin solution was added and mixed. A drop of the semen-eosin-nigrosin mixture was placed on a microscope slide and a thin smear within 30 seconds of adding the nigrosin was made, air dried and examined under oil immersion (1000×) with a light microscope. The live spermatozoa are white and the dead are stained red.

Hypo-osmotic swelling test. The sperms were suspended in 0.735% sodium citrate and 1.351% fructose aqueous solution. The percentage of swollen cells was determined from 200 spermatozoa.

Morphology of spermatozoa. Semen samples were stained with Spermac stain and checked for normal and abnormal morphology, according to WHO (1999). The following categories of defects should be noted: head defects (%), neck defects (%), cytoplasmatic droplets (%) and tail defects (%).

Optional tests. The following tests were recommended for routine semen analysis. Teratozoospermic index (total number of defects/number of spermatozoa with defects); Fertility index (FI, based on Botella-Casares) is defined by the formula:

$$FI = \frac{MNV}{A} \times 10^9$$

where $M$ is the motility of spermatozoa, given by the percentage of spermatozoa with rapid progression, $N$ is the concentration of spermatozoa in semen (millions/mL), $V$ is the progression speed, $A$ is the % of abnormal spermatozoa.

For measurement of the progression speed, a flat capillary glass tube, 1.2 mm deep and 10 cm long, filled with an isotonic glucose solution, was placed vertically over a drop of semen in a reagent tube (Fig. 2). After 30 min in a thermostat at 37 °C, the tube was placed under a microscope and the distance traveled by the most advanced spermatozoa was measured in mm.

Statistics. Differences between groups were evaluated statistically using the two-sided t-test. As the primary and secondary outcome variables were obtained by comparison of four pairs of visits, significance is given for $p$ values of 0.05/8 = 0.00625.

RESULTS

The two groups enrolled in the cross-over study did not differ in mean age (36.8/37.2), BMI (26.2/25.9) or mean fertility index (values 0.53 ± 0.20 vs 0.60 ± 0.19) before treatment. All patients were normogonadotropic and were diagnosed as idiopathic infertile.

All enrolled patients completed the study, compliance to intake of medication was excellent, all tablets had been taken. The treatment with Prelox improved significantly ($p < 0.001$) the ejaculate volume, concentration and number of spermatozoa and percentage of vital spermatozoa (Table 1). The percentage of spermatozoa with good progressing motility increased significantly, while the percentage of immotile spermatozoa decreased significantly ($p < 0.001$) following treatment (Table 2).

Also the morphology of spermatozoa and teratozoospermic index (TZI) (Table 3) was improved as a higher percentage of spermatozoa with normal morphology and the percentage of defective spermatozoa decreased accordingly. Also these effects were highly significant ($p < 0.001$).

Table 1. Effect of Prelox on semen analysis parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ejaculate volume (mL)</td>
<td>A</td>
<td>2.3 [0.6]</td>
<td>3.2 [0.5]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>2.9 [0.4]</td>
<td>3.4 [0.4]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Concentration of spermatozoa ($\times 10^6$/mL)</td>
<td>A</td>
<td>34.6 [4.1]</td>
<td>63.4 [8.7]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>43.5 [3.2]</td>
<td>67.2 [3.4]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total number of spermatozoa ($\times 10^9$)</td>
<td>A</td>
<td>81.3 [26.8]</td>
<td>202.6 [46.8]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>125.9 [19.6]</td>
<td>232.2 [36.2]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Percentage of vital spermatozoa (eosin test)</td>
<td>A</td>
<td>61.4 [5.2]</td>
<td>76.1 [6.2]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>65.5 [3.1]</td>
<td>78.2 [3.5]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intact membrane %</td>
<td>A</td>
<td>48.3 [5.7]</td>
<td>67.4 [6.8]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>54.4 [3.0]</td>
<td>69.2 [4.1]</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means and standard deviations SD.
Treatment with placebo did not change any parameter significantly (data not shown).

The fertility index (FI) shows very clearly the effect of treatment because it brings together several sperm parameters (Table 4). Over the whole period of investigation there was a slight increase noted. However, the fertility index increased to normal values after treatment. The results of the group starting with Prelox treatment showed that the increase of the fertility index was restricted to the period of treatment as in the following weeks the FI fell back to the infertile values.

The improvement of sexual wellness (increased morning erection, sexual dreams, sexual fantasy, easier to initiate erection, sustain an erection, partner-noted interest and partner-noted performance) were reported in another paper (Stanislavov et al., 2007).

The patients reported no unwanted effects during treatment.

The data for clinical chemistry indicated a slight increase (below 10%) of values for albumin, hemoglobin, erythrocytes and testosterone (data not shown) and small, but significantly lower values for total cholesterol $-4.81$ (0.41) at the start and $4.66$ (0.39) mmol/L at the end of treatment. Vital signs remained unchanged.

**DISCUSSION**

The improvement in the quality of spermatozoa following a treatment period of 4 weeks points to a positive influence of Prelox on the maturation of spermatozoa in the male reproductive system. The effect of Pycnogenol (a component of Prelox) is dose-dependent within the range $25–200$ mg and persisted for about 6 days according to a controlled nutritional study (Rohdewald and published data).

The complex process of division and differentiation of germ cells follows a precise pattern. The number of stages of spermatogenesis differs depending on the species. Spermatogenesis in man develops through six

---

**Table 2. Motility of spermatozoa before and after treatment with Prelox**

<table>
<thead>
<tr>
<th>Percentage of spermatozoa</th>
<th>Group</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A - rapid progressive motility %</td>
<td>A</td>
<td>35.5 [10.7]</td>
<td>55.0 [6.9]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>46.0 [4.3]</td>
<td>56.8 [4.1]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>b - slow or sluggish progressive motility %</td>
<td>A</td>
<td>6.6 [2.8]</td>
<td>5.4 [1.4]</td>
<td>&lt;0.099</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>6.4 [3.0]</td>
<td>5.0 [0.0]</td>
<td>&lt;0.038</td>
</tr>
<tr>
<td>C - nonprogressive motility %</td>
<td>A</td>
<td>7.4 [3.3]</td>
<td>5.4 [1.4]</td>
<td>&lt;0.013</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>5.2 [1.0]</td>
<td>5.0 [0.0]</td>
<td>n.s.</td>
</tr>
<tr>
<td>d - immotile %</td>
<td>A</td>
<td>50.1 [8.6]</td>
<td>34.2 [6.4]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>42.4 [4.1]</td>
<td>33.2 [4.1]</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means and standard deviations SD.

**Table 3. Morphology of spermatozoa before and after treatment with Prelox**

<table>
<thead>
<tr>
<th>Morphology</th>
<th>Group</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal %</td>
<td>A</td>
<td>31.6 [8.6]</td>
<td>60.9 [6.0]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>42.0 [5.0]</td>
<td>66.0 [4.2]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Head defects %</td>
<td>A</td>
<td>25.7 [10.3]</td>
<td>14.6 [5.4]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>20.2 [7.5]</td>
<td>9.8 [4.4]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Neck defects %</td>
<td>A</td>
<td>13.3 [3.8]</td>
<td>9.4 [4.3]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>12.0 [6.3]</td>
<td>7.4 [3.1]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cytoplasmatic droplets %</td>
<td>A</td>
<td>13.6 [5.6]</td>
<td>5.0 [3.8]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>15.2 [4.2]</td>
<td>7.5 [2.0]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tail defects %</td>
<td>A</td>
<td>13.1 [6.8]</td>
<td>8.4 [4.9]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>8.8 [5.0]</td>
<td>6.3 [2.8]</td>
<td>&lt;0.016</td>
</tr>
<tr>
<td>TZI</td>
<td>A</td>
<td>1.88 [0.25]</td>
<td>1.27 [0.22]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1.90 [0.23]</td>
<td>1.19 [0.14]</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means and standard deviations SD.

**Table 4. Fertility index of patients during period of investigation**

<table>
<thead>
<tr>
<th>Group</th>
<th>FI SD</th>
<th>Group</th>
<th>FI SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (n = 25)</td>
<td></td>
<td>B (n = 25)</td>
<td></td>
</tr>
<tr>
<td>At start</td>
<td>0.53 0.20</td>
<td>0.60 0.19</td>
<td></td>
</tr>
<tr>
<td>End of run-in period</td>
<td>0.60 0.18</td>
<td>0.62 0.16</td>
<td></td>
</tr>
<tr>
<td>End of 1st treatment period</td>
<td>1.24 0.31 Prelox</td>
<td>0.70 0.13 Placebo</td>
<td></td>
</tr>
<tr>
<td>End of wash-out period</td>
<td>0.74 0.15</td>
<td>0.80 0.09</td>
<td></td>
</tr>
<tr>
<td>End of 2nd treatment period</td>
<td>0.70 0.14 Placebo</td>
<td>1.20 0.14 Prelox</td>
<td></td>
</tr>
</tbody>
</table>
stages (I–VI). Human spermatogenesis confirmed the principle of helical patterns but not the complete succession of all stages (Johnson, 1994; Johnson et al., 1996).

The enhanced quality of spermatozoa from an infertile status to a normal fertility index could be caused by two mechanisms: The first possibility is that the antioxidant constituent, Pycnogenol, of Prelox inhibited the peroxidation of the lipid membrane of spermatozoa, thus improving the shape and motility of spermatozoa.

The second action could be that Prelox enhanced the sperm motility and function by stimulating the activity of endothelial nitric oxide synthase.

Both mechanisms, maybe acting in concert, have been shown to influence positively the quality of spermatozoa.

Reactive oxygen species (ROS) have been detected in 40% of semen of infertile men, whereas ROS could not be found in the semen of normal, fertile men (Iwasaki and Gagnon, 1992). High doses of vitamin E as an antioxidant therapy had been reported to reduce peroxidative damage of spermatozoa in a placebo-controlled clinical trial (Suleiman et al., 1996). As lipid peroxides are cytotoxic to human spermatozoa (Jones et al., 1979), it is to be expected that a potent scavenger of free oxygen species would prevent malformations of spermatozoa during the capacitation process.

An overview of the positive effects of antioxidants on sperm quality was reported by Agarwal et al. (2005), however, the authors listed several controlled studies in which antioxidants had no effect on the quality of sperm.

Pycnogenol, one of the components of Prelox, has been shown in clinical trials to increase the antioxidative activity of blood (Devaraj et al., 2002; Durackova et al., 2003).

In vitro, Pycnogenol also increased the intracellular activity of antioxidant enzymes such as superoxide dismutase or catalase (Wei et al., 1997; Bayeta et al., 2000). These findings support the hypothesis that Prelox may improve the quality of sperms by antioxidative effects.

This increase of intracellular antioxidative defense, observed in cell cultures, could increase the concentration of antioxidative substances in the epididymis, as the epididymis contains high concentrations of superoxide dismutase and glutathione peroxidase (Perry et al., 1992, 1993).

The other possibility is linked to the stimulation of e-NOS activity in sperms by Prelox (Stanislavov and Nikolova, 2005; Stanislavov et al., 2007). Following the intake of Prelox, the activity of e-NOS in sperms increases very significantly in patients with erectile dysfunction. In the same patients, the quality of sperms improved in terms of concentration, morphology and motility (Stanislavov and Nikolova, 2005), simultaneously with the increase of e-NOS activity. Prelox provides the e-NOS with its natural substrate, l-arginine and its other component, Pycnogenol, stimulates the activity of the enzyme (Fitzpatrick et al., 1998). An increased NO production leads to the activation of cGMP.

Several investigations demonstrated the positive effect of an enhanced e-NOS activity and NO production on the motility of spermatozoa.

In our investigation, Prelox increased sperm motility. This has to be seen in the context of the findings of our previous investigation, that Prelox enhances very significantly e-NOS activity in spermatozoa in a double-blind, placebo-controlled investigation (Stanislavov et al., 2007). Spermatozoa possess cyclic nucleotide-gated (CNG) calcium channels in the sperm flagellum (Wiesner et al., 1998). These CNG channels respond to cGMP and to cAMP (Wiesner et al., 1998). The cyclic nucleotides regulate calcium influx and subsequent phosphorylation of the flagellum protein (Tash and Means, 1982, 1983; San Agustin and Witman, 1994). This CNG channel pathway represents one mechanism for increasing sperm motility by NO (Turner, 2003). The addition of ferulic acid, a component of Pycnogenol, which is detectable in the plasma of human volunteers after consumption of Pycnogenol, increases intracellular cGMP as well as cAMP in spermatozoa. Simultaneously, the motility of spermatozoa was increased (Zheng and Zhang, 1997). Further evidence for the importance of the NO-cyclic nucleotide messenger system for the motility of sperms comes from experiments with phosphodiesterase inhibitors.

Taking the results obtained by the stimulation of NO production, it is reasonable to conclude that increased motility of sperms in both series of experiments is based on the enhanced level of cyclic nucleotides inside the spermatozoa. An excellent review by Revelli et al. (2002) discusses the detailed mechanism of cGMP interactions with ion channels, protein kinases C and G, tyrosine kinase, leading to an increase in the motility and maturation of spermatozoa.

The study by Roseff demonstrated a 99% mean improvement in baseline sperm morphology following Pycnogenol therapy in a small group of subfertile men.

The results from our controlled study suggest that administration of Prelox to subfertile men improves significantly the quality of sperms in terms of morphology and motility. As no unwanted effects were reported, pretreatment with Prelox offers a comfortable alternative for men to achieve fertilization in a normal way.

REFERENCES


Durackova Z, Trebaticky B, Novotny V, Zitanova A, Breza J.


Herrero MB, de Lamirande E, Gagnon C. 1999. Nitric oxide...


