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Beneficial effect of food supplementation with the nutriceutical Improve® for the treatment of infertile couple

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ABSTRACT

Objective: To assess the possible benefit of food supplementation with the nutriceutical Improve® for the treatment of the infertile couple. **Methods:** The treatment of diseases causing male or female infertility should be completed by the prescription of a judiciously formulated composite nutriceutical (Improve® Nutriphyt Inc, Oostkamp, Belgium) which counteracts the pathogenic mechanisms involved in sperm and oocyte dysfunction, enhances cellular energy production, corrects oxidation-induced damage to the cell membrane and to DNA, and repairs mitochondrial insufficiency. The efficiency of this nutraceutical was tested in controlled trials and in assisted reproduction, including 1 888 infertile couples. **Results:** Complementary food supplementation with the nutriceutical Improve® plus linseed oil improved the quantity and functional quality of spermatozoa, significantly increasing their fertilizing potential. This supplement with added fish oil enhanced female fertility with higher probablity of natural conception and ongoing pregnancy using techniques of assisted reproduction. **Conclusions:** Complementary food supplementation with the nutriceutical Improve® has significant beneficial effects for the treatment of the infertile couple and reduces the cost per delivery.

1. Introduction

Since *in vitro* fertilisation (IVF), and later Intracytoplasmic Sperm Injection (ICSI), were introduced, these treatments have been offered to very many couples who are confronted with infertility. Techniques of assisted reproduction (ART) have long been publicised as offering rapid success, without any side effects. However, follow-up studies have shown the children born after IVF/ICSI to more commonly present poor health and congenital abnormalities than children born after natural conception[1-3]. The latter is not surprising, because ART may use spermatozoa of mediocre quality or "old" oocytes[4], and the technique itself exerts enormous stress on the embryos[5]. Furthermore, the cost per delivery after conception by IVF is extremely high[6], whereas many couples with failed IVF will spontaneously conceive in the years after[7].

Assisted reproduction techniques do not address the core of the problem, namely why gametes are deficient and which are the mechanisms affecting the gametes. The recent understanding of these two basic aspects of couple infertility has resulted in the development of rational treatment strategies that have been proven both effective and efficient.

1.1. The role of external factors in infertility

Infertility results from multiple pathogenic influences. Four groups of factors seem to act in synergy: genetic defects or constitution, life style factors, professional and/or environmental exposure to toxic substances, and diseases of the reproductive organs.

Among infertile males genetic defects include numerical and structural abnormalities of the chromosomal make-up, as well as micro-deletions of the Y chromosome, and in infertile women chromosomal abnormalities result in disturbed ovulation or repeated abortions. Whether or not genetic defects will cause infertility may depend on the severity of the defect and the coincidental presence of external factors. These, and diseases of the reproductive organs, such as varicocele and male accessory gland

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infection (MAGI), have been shown to increase the load of reactive oxygen species (ROS) to the ejaculate and to the spermatozoa[8,9]. Among other things, increased chromosome fractionation[10] and excessive production of oxidized DNA (8-hydroxy 2-deoxy guanosin)[11] have been reported.

Endometriosis, pelvic inflammatory disease, and probably PCO cause oxidative stress to the oocytes and to the early embryo's[12].

The membrane of spermatozoa of fertile men contains a high concentration of docosahexaenoic acid (DHA, also called: cervonic acid, 22:6ω3) that increases fluidity[13]. Fluidity is necessary for the induced acrosome reaction to occur and for the sperm membrane to fuse with the membrane of the oocyte. Evidently, the fatty acid composition of the oocyte membrane is equally important for the latter event[14]. The sperm membrane of infertile men contains less DHA, reducing its fluidity and fusogenic capacity. However, DHA holds a strong oxidative potential because of the high number of double bonds. The serum of infertile men, and the peritoneal fluid, the follicular fluid and the tissues of women with endometriosis or pelvic inflammatory disease, present imbalance between oxidative overload and decreased antioxidant capacity[15,16]. Oxidative overload changes the phospholipid composition of the membrane of the spermatozoa[13] and of the oocytes, reducing its fluidity and the capacity of fertilization.

Inappropriate nutrition[17], abuse of alcohol, of tobacco [18,19] and of recreational drugs[20,21] in both sexes, as well as tight clothing and hot baths in men[22] have been demonstrated to be life style factors unfavorably influencing fertility. Also, a higher proportion of infertile men and women were found to have a body mass index in excess of 25, being overweight or obese. Overweight also is a certified cause of poor outcome of ART[23]. Infertile couples consume less omega-3 fatty acids, and the ratio of omega-3 over omega-6 fatty acids in their nutrition commonly is sub-optimal[24]. In our studies a significant positive correlation was found between the consumption of alpha linolenic acid (ALA, 18:3ω3) at the one hand, and sperm concentration and progressive motility at the other hand[25]. There was a negative correlation between these sperm characteristics and the consumption of long-chain poly-unsaturated fatty acids (Eicosapentaenoic acid, EPA, 20:5ω3, and DHA).

It has been demonstrated that testicular tissue, Sertoli cells in particular, contain more desaturase[26] and elongase[27] than other body tissues, which enzymes convert alpha-linolenic acid into the long chain polyunsaturated fatty acids[28,29]. This process generates higher concentrations of DHA inside the seminiferous tubules, whereas orally administered DHA was found not to cross the blood-testis barrier[30]. Knocking out the desaturase in male experimental animals induces infertility by maturation arrest[31] that could be restored by a DHA supplement. Reproductive abnormalities were also recoded in female transgenic mice with impaired desaturase activity[32].

Little is known about the nutrition of infertile women, but there is reason to believe that they as well consume too little food containing polyunsaturated omega-3 fatty acids[33].

Exposure to professional toxicants was proven to impair sperm quality, including heavy metals such as lead[34] and carbondisulfide[35]. But it is the exposure to environmental agents with hormone disrupting effects, mainly pseudo- or xeno-estrogens and anti-androgens, which has caused most concerns recently[36]. The obvious, though regional, deterioration of both sperm variables[37] and fertility, and the parallel increase of the prevalence of testicular cancer have been linked to an increased exposure to man-made chemical substances that mimic or enhance the effects of estrogens by binding on the human estrogen receptor or by influencing estrogen metabolism[38,39]. In women, hormone disrupters may interfere with the hypothalamo-pituitary regulation of ovulation, and may facilitate the development of fibroids and endometriosis[40,41] .

Regarding diseases causing female infertility much attention has been given to the role of inflammation, where interleukin-6[42] and Interleukin-1 seem to be involved in the pathogenesis of endometriosis through the Nuclear factor kappaB pathway[43]. Also, the excess of these inflammatory cytokines and of reactive oxygen species[44] among patients with endometriosis or with pelvic inflammatory disease, or who were exposed to environmental toxins, was found to reduce ciliary beat frequency in the human Fallopian tubes[45]. Ovulation disturbances may be due to deregulation of the hypothalamo-pituitary-ovarian axis by stress, or to polycystic ovary syndrome. The latter is often combined with increased inflammatory reaction.

Cytokinesis and cell cleavage occurring during embryogenesis require the activation of the non-muscular myosin II[46] by adenosine triphosphate (ATP) that is produced by the mitochondria. ATP is also needed for ubiquitylation that is important for DNA transcription and for the repair mechanism during cell division[47]. Whenever mitochondrial function is impaired by oxidative overload, the production of ATP decreases resulting in deficient myosin activation, defective cytokinesis, and inadequate DNA transcription.

1.2. Food supplementation

In view of the considerations above, it was envisaged to create a nutriceutical food supplement that would correct these defective mechanisms. The nutriceutical should have no side effects, and it should be used in complement to the causal treatment. Its alleged therapeutic effect should, moreover, be proven empirically and in controlled trials.

1.2.1. Fatty acids

Since there is a positive correlation between the intake of alpha-linolenic acid (ALA) and the concentration and motility of spermatozoa, and since the nutritional intake of essential fatty acids of the omega-3 group was found to be sub-optimal among infertile men[25], it is indicated

to supplement with ALA given as linseed oil, also called flaxseed-oil. When administered in association with the co-factors Zinc and pyridoxine (Vit. B6), which enhance the activity of the elongase and desaturase enzymes, ALA is converted in situ to the long-chain, highly polyunsaturated omega-3 fatty acids EPA and DHA. The fluidity of the sperm membrane improves, and the induced - but not the spontaneous - acrosome reaction, as well as the fusogenic capacity of the spermatozoa increase[48].

The long-chain fatty acids improve the quality of the oocyte membrane and they also provide energy for cell metabolism while serving as substrate in the Krebs cycle[49]. Also, EPA present in fish oil, have been shown to suppress the activation of the Nuclear Factor kappaB[50]. Therefore, supplementation with fish oil is indicated in female patients with infertility due to endometriosis or pelvic inflammatory disease.

1.2.2. Antioxidants

Subfertile patients were found to present an imbalance between excessive oxidative stress and reduced antioxidant capacity[13]. Food supplementation with antioxidants significantly and persistently improves the balance between oxidative overload and antioxidant defense[51]. Also, the treatment with either acetyl-cysteine (600 mg per day orally) or an antioxidant mixture significantly reduces the level of reactive oxygen species (ROS) in semen[48]. Antioxidant treatment increases sperm concentration, significantly reduces the concentration of oxidized DNA (8-OH-2d-Guanosine), and it improves the overall DNA quality[52] of spermatozoa of subfertile men. Long chain polyunsaturated fatty acids are highly susceptible to oxidative damage, and Vit E supplementation was found to improve the *in vitro* function of spermatozoa as assessed in the zona free hamster oocyte test[53].

Whereas supplementation with Vit C to smokers with abnormal sperm quality was reported to improve semen quality[54], no such effect was seen in another trial using high-dose Vit C[55]. The latter may be due to the well-known pro-oxidative effect of high-dose Vit C[11], particularly in men with the haptoglobin type 1-2 or 2-2[51]. Similarly supplementation with a high dose of the synthetic D-alfa-tocopherol succinate exerts deleterious effects by disrupting the gap-junctions[56] which are of pivotal importance for the maintenance of optimal conditions within the seminiferous tubules.

When added *in vitro*, or given orally[57], the oxido-reductase ubiquinone Q10 increased sperm motility in cases with asthenozoospermia. Also other antioxidants, such as selenium[58] and glutathione[59], were found to improve sperm motility in subgroups of patients.

A recent meta-analysis, completed by our own observations, of 1013 infertile couples[60] has revealed that oral anti-oxidant treatment of the male increases the probability of spontaneous pregnancy from 4.4% to 17.2% (RR: 3.91, CI: 2.49-6.14, $P<0.0001$, Figure 1), reducing the cost per pregnancy by 60%.

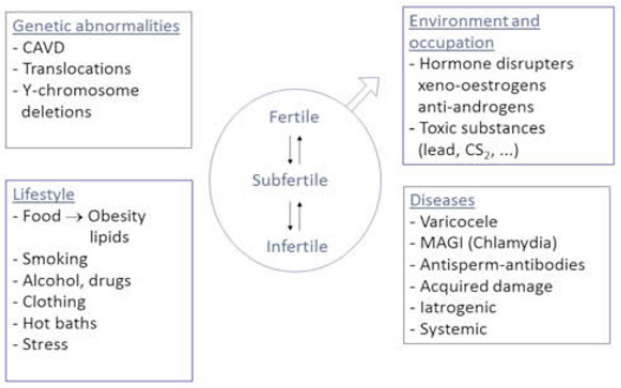

Figure 1. The transition of fertility to impaired male reproductive capacity and infertility results from the synergistic amplification of the genetic predisposition, lifestyle factors, environmental and occupational influences, and diseases affecting the reproductive organs.

In view of the unfavorable influence of oxidative stress on the maturation of oocytes[12], and of the fact that reactive oxygen species were increased in infertile women[61], their fertility may be enhanced by antioxidant treatment[62].

Astaxanthin is a lipophilic carotenoïd produced by the algae Haematococcus pluvialis that has a strong antioxidant capacity[63,64].

Since large variability of the concentration of carotenoids have been observed in follicular fluid of women without reproductive success[65], the supplementation with Astaxanthin is expected to also increase fertility.

The oxydo-reductase ubiquinone Q10 is a highly active antioxidant at the level of the mitochondria[66]. The production of ATP through the Krebs cycle in the mitochondria is enhanced, thanks to increased supply of long chain fatty acids and of carnitines. Ubiquinone Q10 will scavenge the resulting oxygen radicals[67], and maintain optimal cellular metabolic activity.

Anthocyanidines such as the Pine bark extract Pycnogenol® (Horphag Research, Geneva, Switserland) display scavenger effects on intracellular free oxygen radicals[68]. In addition, these substances protect the cell membrane from lipid peroxidation, increase membrane fluidity[69], and function as effective anti-oxidants.

Several recent randomized trials have revealed an increased rate of successful pregnancies by anti-oxidant supplementation during IVF and IVF-ICSI cycles[70,71].

1.2.3. Carnitine and L-carnitine

L-carnitine and acetyl-carnitine play a pivotal role in the transport mechanisms that are involved in the passage of the long-chain fatty acids from the cellular cytosol into the mitochondrial matrix, where these are oxidized to generate energy[72] and to stimulate the respiratory chain complexes[73]. Also, free carnitine and acetyl-carnitine play an important role in the post-gonadal maturation of mammalian spermatozoa[74], and the ratio of acetyl-carnitine over carnitine was different in extracts of sperm with good compared to poor motility[75,76]. Acetyl-L-carnitine is the prominent carnitine in spermatozoa and its concentration

was lower in semen of infertile men[77,78]. The free carnitine concentration in seminal plasma was significantly correlated with sperm concentration and motility[79], and sperm motility could be stimulated *in vitro* by adding acetyl-carnitine[80].

Treatment with a food supplement containing a combination of high dose L-carnitine and acety-L-carnitine together with fructose and citric acid (Proxeed®, Sigma-tau Health Science, Rome, Italy), was reported to increase sperm concentration and forward progressive motility in a double blind cross-over trial[81]. However, we could not confirm these positive results in a double-blind trial (unpublished) where 3 months of treatment with either high dose carnitines or placebo generated pregnancy rates of respectively 4.6 and 5.3%.

Energy provided by ATP is critically important for the metabolism of oocytes, and for the activation of Myosin II during cell cleavage. Supplementing *in vitro* culture media with carnitines was beneficial to the cytokinesis of mouse embryos[82], particularly when these were incubated in the presence of peritoneal fluid of patients with endometriosis[61]. Also, the *in vitro* maturation of human oocytes could be supported by the addition of L-carnitine[83].

1.2.4. Folic Acid, Zinc and Vit B12

Folic acid and zinc have been given orally to both men with normal sperm quality and to patients with moderate oligozoospermia during a placebo controlled trial[84]. This combination significantly increased sperm concentration and morphology in the subfertile men, though the mechanism through which this occurs remains unknown[85]. These changes occurred though the blood levels of folic acid and zinc were not deficient before treatment.

Folic acid is a "nutrogenomic" substance, playing a key role in the epigenic regulation of gene expression, and reducing the risk of neural tube defects[86] and of the schisis-syndrome. Folic acid decreases the homocysteïn accumulation and diminishes the risk of pregnancy complications[87]. Preconception folic acid supplementation optimizes the microenvironment for maturing oocytes[88], resulting in better implantation rates and a higher birth weight.

Cobalamine (Vit B12) is transferred from blood into the male reproductive organs and plays an important role in spermatogenesis[89].

1.2.5. Plant extracts

Using immune histochemical techniques Schell *et al.* have demonstrated that the cyclo-oxygenase iso-enzyme 2 (COX-2), that converts arachidonic acid (20:4ω6) into the inflammatory prostaglandin E2, is present in peritubular testicular cells of patients with idiopathic oligozoospermia, but not in men with normal spermatogenesis[90]. The extract of the bark of the Pinus maritima (Pycnogenol®) contains anthocyadines that inhibit the COX enzymes[91], reduce the m-RNA of the inflammatory cytokine Interleukin 1 beta[92], and protect the effects of Vit E of endothelial cells[93]. In an open label trial, oral administration of 200 mg per day of Pycnogenol® improved sperm morphology[94], and this effect on sperm quality was confirmed in a double-blind trial combining Pycnogenol with L-arginine[95].

The anti-inflammatory effects of pine bark extract should benefit patients with endometriosis[96] or with pelvic inflammatory disease in whom the concentration of inflammatory cytokines in peritoneal fluid is increased and the activation of the Nuclear Factor kappaB is enhanced[97]. Equally, Pycnogenol® may increase ciliary beat frequency that is decreased by inflammation.

The extract of the root of Lepidium meyenii (also called Maca), a plant growing in the central Andean region of Peru, increases sexual function in humans[98] and invigorates spermatogenesis at the mitotic stages[99]. When given to men with normal spermatogenesis, this extract significantly increased sperm production and motility without interfering with endocrine regulation[100].

Both infertility and its treatment are certainly accompanied by enhanced psychological stress, provoking ovulation disturbances. Phyto adaptogens such as *Lepidium meyenii* probably exert their stress-protective effect[101] by increasing the production of heat shock protein 70[102].

2. Materials and methods

2.1. The nutraceutical Improve®

Improve® (Nutriphyt Inc., Oostkamp, Belgium) is a formulation-registered nutraceutical containing appropriate doses of Lepidium meyennii, Pycnogenol®, carnitine and L-acetylcarnitine, co-enzyme Q10, Astaxanthin, zinc-chelate, pyridoxin (Vit B6) folic acid (Vit B9), and cyanocobalamine (Vit B12). This nutraceutical, in combination with linseed oil (Linusit®, Nutriphyt Inc.) is given as a complementary treatment to subfertile men with idiopathic sperm deficiency, or in whom causal factors (such as varicocele, male accessory gland infection, or endocrine disturbance) have been corrected[103]. In infertile females Improve® is given as a complementary nutriceutical together with fish oil (Omarin® Nutriphyt Inc.), rich in EPA and DHA.

2.2. Clinical trials

In a double blind placebo controlled randomized trial, Astaxanthin (AstaReal®, Gustavsberg, Sweden) was given for 3 months to the male partners of 20 infertile couples, whose semen characteristics were below the WHO recommended reference values.

3. Results

The food supplement resulted in a significant reduction of seminal ROS, while no changes occurred in the placebo controls. Rapid linear progressive motility significantly increased and sperm morphology presented improvement in the Astaxanthin group[104].

In an open label prospective cohort trial complementary Improve® plus linseed oil was given to the male partners of 20 couples with an average duration of 21 months infertility. Motile sperm concentration more than doubled and spontaneous pregnancy occurred in 8 couples, with 16% probability of conception per month[105].

Complementary treatment with Improve was given during 3

months to 22 patients treated by transcatheter embolization because of varicocele, and the pregnancy rate was compared to that of 40 untreated controls participating in a randomized prospective trial by WHO. The pregnancy rate was 5% in the controls as compared to 44% in the couples where the man was treated for his varicocele and did take the foodsupplement (Numbers needed to treat 2.6; $P<0.01$).

In the year 2006, 23% of the couples treated by IVF, with or without ICSI, attained ongoing pregnancy. In 2010 the pregnancy rate in 952 IVF-treated couples was increased to 35%, thanks to the systematic investigation and conventional treatment of the male partner and food supplementation ($P<0.0001$)

Finally 50 partners of infertile couples, of whom 78% had previously failed to attain pregnancy with IVF, were entered in a "proof of principle" double-blind randomized trial. The men were given either Improve® or identically looking capsules with folic acid, plus linseed oil during 8 weeks before the pick-up. The female partners took either the food supplement or the folic acid capsules, plus fish oil during 6 weeks before, and 2 weeks after pick up. The ongoing pregnancy rate in the couples taking the food supplement was 45% as compared to 20% in the controls (NNT: 4). The ongoing pregnancy rate is a reliable measure of the life birth rate[106].

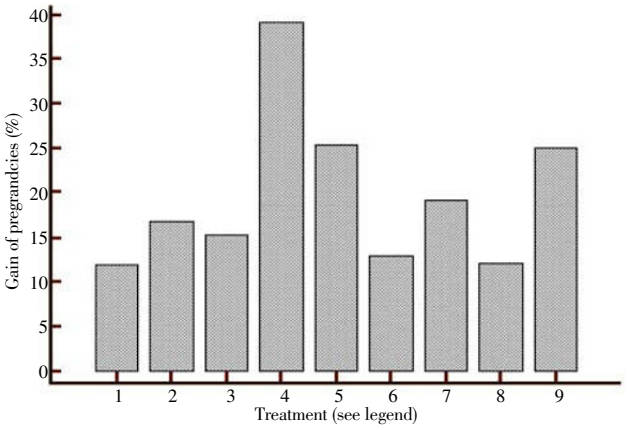

Figure 2. The histogram represents the gain in cumulative pregnancy rate (pregnancy rate in treated couples minus pregnancy rate in untreated controls) obtained by different modes of treatment of the infertile couples, as recorded in controlled trials.

1. Laparoscopic surgery for mild or moderate endometriosis
2. Treatment of disturbed ovulation with clomiphene citrate
3. Varicocele embolization or surgery; 12 months observation
4. Varicocele embolization plus nutriceutical supplementation; 3 months observation
5. Tamoxifen plus testosterone undecanoate; 6 months treatment
6. Meta-analysis of Antioxidant treatment of the male partner
7. Six cycles of Intra Uterine Insemination because of immunological infertility due to anti-sperm antibodies.
8. Treatment of causal factors plus nutriceutical food supplementation of the male partner before IVF (results in 2010) in comparison with historical controls (results of the year 2006)
9. Double-blind trial with Improve® plus Linusit® given to the male partner, and Improve® plus Omarin® given to the female partner undergoing IVF.

4. Conclusions

Several trials provide evidence that complementary food supplementation with the nutriceutical Improve® plus linseed oil does improve the quantity and functional quality of spermatozoa, and their fertilizing potential. Added to causal treatment of the male, this increases the probability of successful pregnancy of infertile couples. Also, this supplement with added fish oil enhances female fertility, increasing the probability of spontaneous conception and of ongoing pregnancy using techniques of assisted reproduction. The nutriceutical contains the antioxidants astaxanthin, anthocyanidines and ubiquinone Q10, zinc, folic acid, and the extract Lepidium meyenii and pine bark extract. Obviously, these findings and the favorable cost/benefit ratio should encourage fertility specialists to systematically prescribe the nutriceutical as part of infertility treatment.

Conflict of interest statement

We declare that we have no conflict of interest.

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