Commentary

The role of food supplements in the treatment of the infertile man

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Abstract

Recently, concerns have been raised about the presumptive increased risk of serious undesirable side effects in children born after IVF and intracytoplasmic sperm injection (ICSI). These treatments must, therefore, be reserved as the ultimate option after evidence-based and cause-directed treatment of the male patient with deficient semen has been exhausted. The present authors found that sperm quality and function improved with the intake of complementary food supplementation using a combination of zinc and folic acid, or the antioxidant astaxanthin (Astacarox®), or an energy-providing combination containing (actyl)-carnitine (Proxeed®). Also, double blind trials showed that the latter two substances increase spontaneous or intrauterine insemination- (IUI-) assisted conception rates. Extracts of *Pinus maritima* bark (Pycnogenol®), which inhibits the cyclo-oxygenase enzyme, reducing prostaglandin production and inflammatory reaction, and extracts of the Peruvian plant *Lepidium meyenii* were shown to improve sperm morphology and concentration, respectively, in uncontrolled trials. Linseed (flaxseed) oil contains alfa-linolenic acid and lignans. The former corrects the deficient intake of omega-3 essential fatty acids, which is correlated with impaired sperm motility among subfertile men. Lignans are precursors of enterolacton, which inhibits aromatase and reduces the ratio of 16-OH over 2-OH oestrogen metabolites. The resulting reduction in oestrogen load may favourably influence Sertoli cell function.

Keywords: *antioxidants, food supplementation, male infertility, nutriceuticals*

Historical perspective on the management of male infertility

It has been known for a long time that the ejaculate of some men contains too few, or qualitatively inadequate, spermatozoa. However, it is only since 1940–1950 that reliable scientific data have been available regarding the values of the basic sperm variables needed for optimal fertility (MacLeod, 1942; Hellinga, 1976). Also, the role of antibodies against spermatozoa (Rümke, 1965) and biochemical analysis of seminal plasma (Eliasson, 1968) have been highlighted. Many cases of 'hidden' male infertility were detected, but only few modalities of treatment were available. Sperm freezing, artificial insemination and the use of donor sperm were developed (**Figure 1**).

In the 1970s and 1980s, much attention was given to the alleged causes and associations of male infertility. A specific task force of the World Health Organization (WHO) launched large-scale multi-centre trials. These resulted in a diagnostic approach and standardized classification of male infertility (Comhaire *et al*., 1987) and the publication of manuals for the standardized techniques of semen analysis (WHO, 1980, 1987, 1999).

The best methods for the diagnosis of varicocele were determined (Comhaire *et al*., 1976), and the efficacy of treatment of this disease was established in a prospective randomized trial (Hargreave, 1995). Placebo-controlled trials did not reveal any benefit in terms of improving the spontaneous conception rate using antibiotic treatment of male accessory gland infection (Comhaire *et al*., 1986) and of idiopathic oligozoospermia with clomiphene citrate (WHO, 1992) or with mesterolone (Gerris *et al*., 1991). By contrast, treatment of the latter condition with Tamoxifen has been shown to be effective (Comhaire, 1976, 2000).

The introduction of assisted reproductive technology, namely IVF and intracytoplasmic sperm injection (ICSI) (Palermo *et al*., 1992), caused a true revolution in reproductive medicine, while also revealing the magnitude of the male factor contributing to couple infertility. Conventional treatment of the infertile male was considered outdated by some, but others have continued unravelling the mechanisms involved in male defective reproductive capacity.

In recent years, concerns have been raised about the economical and ethical aspects (Comhaire, 2000; Katz *et al*., 2002), and side effects, of assisted reproduction techniques. IVF and ICSI were found to be associated with an increased prevalence of major congenital malformations (Kent-First *et al*., 1996; van der Ven *et al*., 1998; Sutcliffe *et al*., 1999; Koudstaal *et al*., 2000; Wennerholm *et al*., 2000; Hansen *et al*., 2002), impaired development (Stromberg *et al*., 2002), and increased risk of retinoblastoma (Moll *et al*., 2003) in the offspring. It seems, therefore, that the wheel has turned full circle, and that clinical andrology will recapture its well-deserved place in the armamentarium for the treatment of couple infertility.

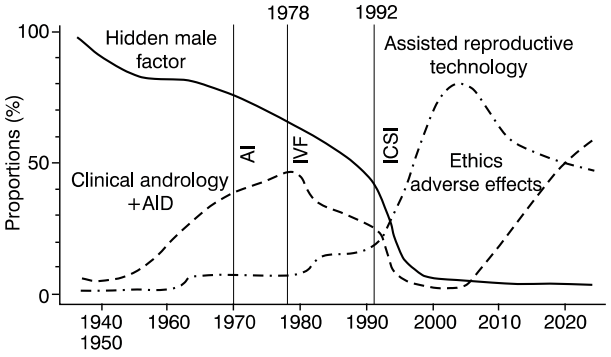

Figure 1. Evolution over time of the proportion of subfertile men treated by the clinical andrologist (dashed line), and those in whom techniques of assisted reproductive technology were applied (dotted and dashed line). The solid line represents the proportion of infertile couples in which the 'male factor' has remained undetected.

Male infertility: a multifactorial disease

Similar to other diseases, male infertility comes to expression as a result of the synergistic coincidence of four major factors: genetic defects or constitution; life style factors; professional and/or environmental exposure; and diseases of the urethro-genital region or endocrine system (**Figure 2**). The latter include the diseases that constitute the traditional interest of the andrologist, such as varicocele, male accessory gland infection, congenital or acquired testicular damage, hypoandrogenism, immunological factors, etc.

The field of genetics is rapidly expanding and includes numerical and structural abnormalities of the chromosomal make-up, as well as microdeletions of the Y chromosome (Tiepolo and Zuffardi, 1976; Ma *et al*., 1992). Whether or not certain of these minor deletions will cause infertility may depend on the coincidental presence of unfavourable life-style factors or exposure to toxic substances or hormone disrupters. These, and the genital diseases, have been shown to increase the load of reactive oxygen species to the ejaculate and the spermatozoa, resulting in increased chromosome fractionation (Hughes *et al*., 1998; Irvine *et al*., 2000) and excessive production of oxidized DNA (8-hydroxy 2-deoxy guanosine) (Fraga *et al*., 1991).

Oxidative overload also changes the phospholipid composition of the sperm membrane (Alvarez and Storey, 1995; Zalata *et al*., 1998), reducing its fluidity and fusogenic capacity as well as the induced acrosome reactivity.

Among life style factors, nutrition, abuse of alcohol, tobacco or recreational drugs, tight clothing and hot baths have been incriminated. Also, men with infertile semen were found to consume less omega-3 fatty acids than fertile men, and a significant correlation was established between the consumption of alfa-linolenic acid (18:3 omega-3) on the one hand and sperm concentration and type a motility on the other hand (Christophe *et al*., 1998).

Exposure to professional toxicants was proven to impair sperm quality, including heavy metals such as lead (Bonde *et al*., 2002) and carbon disulphide (Vanhoorne *et al*., 1994). But it is the exposure to environmental agents with hormone disrupting effects, mainly pseudo- or xeno-oestrogens and anti-androgens, that has caused most concerns recently. The obvious, though regional, deterioration of both sperm variables and fertility, and the parallel increase in the prevalence of testicular cancer, have been linked to an increased internal exposure to artificial chemical substances that mimic or enhance the effects of oestrogens by binding on the human oestrogen receptor or by influencing oestrogen metabolism (for review see Sharpe, 2003).

Inhibin B

Inhibin B is a secretory product of the Sertoli cells that plays an important role in both endocrine feedback, inhibiting the pituitary secretion of FSH, and local regulation of spermatogenesis. Whereas serum inhibin B concentration is significantly related to sperm concentration (for review see Meachem *et al*., 2001), there is evidence of a direct suppressive effect of inhibin B on spermatogenesis (van Dissel-Emiliani *et al*., 1989). Both in-vitro tests (Depuydt *et al*., 1999) and in-vivo data (Mahmoud *et al*., 1998, 2000) suggest that oestrogens and certain heavy metals, such as lead, may inappropriately stimulate the secretion of inhibin B by the Sertoli cells. This results in decreased sperm production, in the presence of normal serum concentrations of inhibin B and FSH.

During treatment with the strong antioxidant astaxanthin (see below), the serum concentration of inhibin was reduced, in spite of unchanged sperm concentration, suggesting that reactive oxygen species stimulate inhibin secretion by the Sertoli cells, similar to the effect of oestrogens (**Figure 3**).

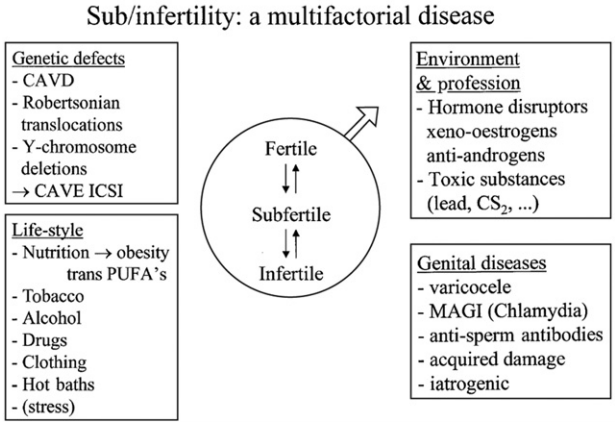

Figure 2. The four major contributing factors that act in synergism to cause subfertility or infertility in men – a multifactorial disease. CAVD, congenital absence of the deferential ducts; CAVE ICSI, be cautious about the transmission of genetic defects to the offspring generated by intracytoplasmic sperm injection; PUFA, poly-unsaturated fatty acids; CS_2, carbon disulphide; MAGI, male accessory gland infection.

Decreasing the secretion of inhibin by reducing the oestrogen effect on the Sertoli cells and the exposure to reactive oxygen species may be a target of medical treatment. Indeed, reducing inhibin secretion may counteract the feedback suppression of FSH secretion by the pituitary and thus may directly improve spermatogenesis.

Food supplementation

Fatty acids

Since there is a positive correlation between the intake of alfa-linolenic acid and sperm concentration and motility, and since the food intake of essential fatty acids of the omega-3 group was found to be sub-optimal among subfertile men (Christophe *et al*., 1998), it seems logical to supplement these patients with a source of 18:3 omega-3, namely linseed oil (also called flaxseed oil). When given in association with the co-factors zinc and vitamin B6, which enhance the elongase and desaturase enzymes, the alfa-linolenic acid will be converted into the long-chain, highly unsaturated omega fatty acids, namely ecosapantaenoic acid and docosahexaenoic acid. The latter increase the fluidity of the sperm membrane, improving the induced acrosome reaction and fusogenic capacity of the spermatozoa (Comhaire *et al*., 2000).

Alternatively, fish oil supplements can be a source of ecosapantaenoic acid and docosahexaenoic acid. These fatty acids are, however, highly susceptible to oxidative damage, which initiates an undesirable chain reaction of lipo-oxidation. If fish oil is given for food supplementation, it is mandatory to ascertain a favourable antioxidant internal environment at the same time.

Antioxidants

The resistance of LDL-cholesterol in serum to an in-vitro oxidative challenge reflects the oxidant–antioxidant balance of a particular person. The time lag before the initiation of LDL-cholesterol oxidation and the occurrence of conjugated dienes is a measure of oxidative stress, being higher if the time lag is shorter and vice versa. Subfertile patients were found to present a significantly shorter time lag than fertile men, indicating an imbalance between excessive oxidative stress and a reduced antioxidant capacity (Christophe *et al*., 1998). The present authors have demonstrated that food supplementation with an antioxidant preparation can significantly and persistently increase the time lag (Bernard *et al*., 2003). Also, it has been shown that treatment with either acetylcysteine (600 mg per day orally) or an antioxidant mixture of β-carotene (30 mg per day orally) and α-tocopherol (180 mg per day orally) significantly reduces the level of reactive oxygen species in semen (Comhaire *et al*., 2000). In combination with fish oil intake, providing 1 g of docosahexaenoic acid per day, antioxidant treatment increases sperm concentration and significantly reduces the concentration of oxidized DNA (8-hydroxy-2-deoxy-guanosine) in spermatozoa of subfertile men. At the same time, the fatty acid composition of the phospholipids of the sperm membrane shift toward the long-chain highly polyunsaturated ecosapantaenoic acid and docosahexaenoic acid, increasing membrane fluidity. This then results in an increased calcium ionophore-induced, but not spontaneous, acrosome reactivity.

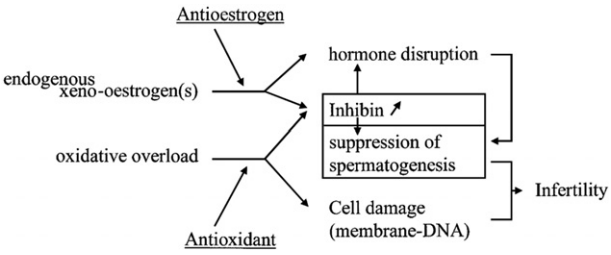

Figure 3. Endogenously produced as well as exogenous xeno-oestrogens act in concert inhibiting the secretion of gonadotrophins by the hypothalamo-pituitary unit, and stimulating the secretion of inhibin B by Sertoli cells. This effect can be counteracted by the administration of a specific anti-oestrogen. Oxidative overload also seems to increase inhibin B production by Sertoli cells, which directly suppresses spermatogenesis and decreases the secretion of FSH by the pituitary. The oxidative overload can be balanced by the administration of anti-oxidant(s).

This agrees with the finding that vitamin E supplementation improves the in-vitro function of spermatozoa in the zona-free hamster oocyte test (Kessopoulou *et al*., 1995). In the present trial, the spontaneous pregnancy rate during the treatment period was 7.2% per month in the partners of (ex)-smokers, but remained at baseline among the partners of non-smokers (1.6%) (OR: 4.57, not significant).

Supplementation with vitamin C among smokers with abnormal sperm quality was reported to improve semen quality (Dawson *et al*., 1992), whereas no such effect was seen in another trial using high-dose vitamin C (Rolf *et al*., 1999). The latter may be related to the known pro-oxidative effect of high-dose vitamin C (Fraga *et al*., 1991), particularly in men with the haptoglobin type 1–2 or 2–2 (Bernard *et al*., 2003).

The oxido-reductase ubiquinone Q10 increased sperm motility in cases of asthenozoospermia, when added *in vitro* or given orally (Lewin and Lavon, 1997). Also other antioxidants such as selenium (Scott *et al*., 1998) and glutathione (Lenzi *et al*., 1993) were found to improve sperm motility in subgroups of patients.

Astaxanthin is a lipophilic carotenoid produced by the alga *Haematococcus pluvialis*, and it has a strong antioxidant capacity (Iwamoto *et al*., 2000; Goto *et al*., 2001). In a pilot double-blind randomized trial, 16 mg per day of the natural astaxanthin (AstaCarox, Astacarotene AB, Gustavsberg, Sweden) was given to the male partners of 20 infertile couples, whose semen characteristics were below the WHO recommended reference values. This food supplementation resulted in a significant reduction of seminal reactive oxygen species and serum inhibin B concentration among treated cases, but not in the placebo controls. Rapid linear progressive motility significantly increased, and sperm morphology presented an insignificant increase in the astaxanthin group, but sperm concentration remained unchanged. In the treated group, the total and monthly pregnancy rates were 54.5% and 23.1%, respectively, compared with 11.1% and 3.6% in the placebo group (OR: 9.6, $P = 0.08$) (Comhaire *et al*., submitted).

Carnitine

L-carnitine plays a pivotal role in the transport mechanisms necessary for the translocation of longer-chain-length fatty acids from the cellular cytosol into the mitochondrial matrix, where these can be oxidized and generate energy (Wildman and Medeiros, 2000) and stimulate respiratory chain complexes (Ruiz-Pesini *et al*., 2001). Free carnitine and acetyl-L-carnitine play an important role in the post-gonadal maturation of mammalian spermatozoa (Jeulin and Lewin, 1996), and the ratio of acetylcarnitine/carnitine was different in extracts of sperm with good or poor motility (Golan *et al*., 1984; Bartellini *et al*., 1987). Acetyl-L-carnitine is the prominent carnitine in spermatozoa, and its concentration is reduced in the semen of infertile men (Kohengkul *et al*., 1977; Soufir *et al*., 1984). The free carnitine concentration in seminal plasma is significantly correlated with sperm concentration and motility ($P < 0.01$) (Menchini-Fabris *et al*., 1984), and sperm motility can be stimulated by the addition of acetylcarnitine *in vitro* (Tanphaichitr, 1977).

Treatment with a food supplement containing a combination of L-carnitine (2 g per day) and acetyl-L-carnitine (1 g per day) together with fructose and citric acid (Proxeed, Sigma-tau Health Science, Rome, Italy), was found to significantly increase sperm concentration and forward progressive motility (by 40% or more, $P < 0.001$) in both open label trials (Moncada *et al*., 1992; Costa *et al*., 1994; Vitali *et al*., 1995) and a double-blind crossover trial ($P < 0.01$) (Lenzi *et al*., 2003). In the open label trial, a total spontaneous pregnancy rate of 6.7% in 3 months was registered (Voliani *et al*., 2001). There are no data on the pregnancy rate in the placebo-controlled trial.

Folic acid and zinc

Folic acid (5 mg per day) and zinc sulphate (66 mg per day) have been given orally both to men with normal sperm quality and to patients with moderate oligozoospermia in a placebo-controlled trial (Wong *et al*., 2002). This combination was found to significantly increase sperm concentration (by an average of 60%, $P < 0.05$) and morphology in the subfertile men. These changes occurred in spite of the absence of deficient blood levels of folic acid or zinc before supplementation in the subfertile men. It was hypothesized that the supplementation with lower, physiological doses of micronutrients may even have a larger beneficial effect, since these have a stronger influence on absorption, transport and metabolic processes. It remains, however, to be established whether the administration of the combination of folic acid and zinc will result in improvement of fertility.

Seed oil and lignans

Aside from alfa-linolenic acid (see above), linseed or flaxseed oil contains several lignans, which are converted in the intestine into enterodiol and enterolactone. These are phytoestrogens with weak and short-lasting oestrogenic effects. However, enterolacton is a rather strong aromatase inhibitor. Thanks to this inhibitory effect, enterolacton reduces the conversion of androgens (androstenedione and testosterone) into the potent oestrogens oestrone and oestradiol (Adlercreutz *et al*., 1993; Wang *et al*., 1994). Hence, food supplementation with linseed oil will decrease the level of endogenous oestrogens, which were commonly found to be increased in men combining oligozoospermia with normal serum concentrations of FSH and inhibin B (Mahmoud *et al*., 1998).

Plant extracts

Using immune histochemical techniques, Mayerhofer *et al*. (2002) have recently demonstrated that the cyclo-oxygenase iso-enzyme 2, which converts arachidonic acid (20:4 omega-6) into the inflammatory prostaglandin E_2, is present in the testicular interstitial tissue of patients with idiopathic oligozoospermia, but not in men with normal spermatogenesis. Extracts of *Pinus maritima* bark (Pycnogenol; SiberHegner, PO Box 888, CH-8034 Zurich, Switzerland) contains substances that inhibit the cyclo-oxygenase enzyme (Baumann *et al*., 1980; Rohdewald, 2002), reduce the mRNA of the inflammatory cytokine interleukin-1β (Cho *et al*., 2001), and protect the effects of vitamin E on endothelial cells (Virgili *et al*., 1998). In an open label study including four subfertile men, oral administration of 200 mg per day of this extract improved sperm morphology by an average of 99% (Roseff and Gulati, 1999).

Extracts of *Lepidium meyenii* (maca), a plant growing in the central Andean region of Peru between 4000 and 4500 m altitude, increases sexual function of male mice and rats (Zheng *et al*., 2000) and invigorates spermatogenesis at the mitotic stages (Gonzales *et al*., 2001b). When given to eight men with normal spermatogenesis, this extract significantly increased sperm production (+85%, $P < 0.05$) and motility (+15%) without interfering with endocrine regulation (Gonzales *et al*., 2001a).

Though these plant extracts may show promise for the future, complementary studies are needed before they can be recommended for the treatment of male infertility.

Miscellaneous substances

For several years, arginine (De Aloysio *et al*., 1982; Aydin *et al*., 1995) and kallikrein (Schill *et al*., 1979) have been promoted for the treatment of men with oligozoospermia, but the alleged favourable effects of these supplements have been questioned (Pryor *et al*., 1978; Comhaire and Vermeulen, 1983).

Discussion

Several controlled and well-validated trials provide evidence that food supplementation with particular substances can improve semen quality and function in subfertile men. These substances include carnitine, zinc, folic acid, tocopherol and astaxanthin. There is evidence to suggest that certain of these supplements, when given as a complement to the WHO recommended conventional treatment (Rowe *et al*., 2000; Tournaye, 2003), can improve male fertility.

On a deductive basis, but without convincing data from clinical trials, certain other food supplements such as linseed oil and plant extracts may favourably influence sperm quality.

Although the exact mechanisms of action of these supplements on spermatogenesis and sperm function remain to be unravelled, a direct effect on the Sertoli cells (**Figure 3**) and via epididymal function seems conceivable.

It makes sense to further explore the therapeutic potential of food supplementation in the management of couple infertility due to the male factor.

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