An Update on Treatments and Interventions for Male Infertility, and the Role of Nutriceutical Food Supplementation

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Abstract: Congenital factors have been thoroughly explored in recent years revealing the role of genetic mutations and polymorphisms, and highlighting the contribution of epigenetics in the pathogenesis of certain forms of infertility. Acquired male infertility is commonly due to varicocele, male accessory gland infection, immunological infertility, and idiopathic oligozoospermia. The mechanisms by which these causes interfere with male reproduction are endocrine deregulation, inflammation through prostaglandins and cytokines, and oxidative overload damaging the cell membrane, inducing mutagenesis of the DNA, and impairing mitochondrial energy production. Causal treatment includes (non-surgical) interruption of spermatic venous reflux in varicocele, adequate antibiotic treatment with third generation Quinolones in accessory gland infection, assisted reproduction techniques in immunological infertility, and the anti-estrogen Tamoxifen for idiopathic oligozoospermia. In addition, a novel nutriceutical food supplement (NFS) has been formulated that aims at correcting the pathological mechanisms and at reducing the influence of detrimental environmental factors. Complementary NFS-treatment also may improve the fertilizing capacity of spermatozoa in some patients with a congenital cause of sperm deficiency. The efficiency of adding this NFS to causal therapy, or in assisted reproduction is expressed as numbers of couples needed to treat (NNT) to obtain one additional pregnancy.

Keywords: Male infertility, causes, treatment, antioxidants, food supplements.

INTRODUCTION

In-vitro fertilisation (IVF) was introduced in 1978, but intra-cytoplasmic sperm injection (ICSI) [1] has been of pivotal importance for the treatment of infertile couples with extremely poor semen quality. These techniques have helped many thousands of couples in attaining pregnancy using their own genetic material. However, recent meta-analyses and retrospective controlled trials have confirmed that children born after IVF, and more particularly after ICSI, more commonly present congenital abnormalities [2-4]. It is suspected that genetic defects of the spermatozoa may be involved. In that case, the implementation of advanced genetic tests, that assess the whole genome of the embryos, may improve the success rate of these techniques and reduce the rate of congenital abnormalities. However, the techniques of IVF and ICSI themselves may be responsible through the induction of epigenetic alterations [5].

Assisted reproduction techniques do not address the essence of the problem, namely why gametes are deficient, and which are the mechanisms affecting their fertilizing potential. The unveiling of these two basic aspects of couple infertility has resulted in the development of rational treatment strategies that have been proven effective and successful.

In order to better understand the logic of treatments and interventions, the physiology of spermatogenesis and the pathology of male reproduction failure will be summarized, with emphasis on the mechanisms that are involved. The composition and effect of a novel nutriceutical food supplementation (NFS) will be highlighted for the clinical management of the infertile couple.

DEFINITIONS, MATERIALS, AND METHODS

The views expressed in the present opinion paper are grounded on the principles of consensus based medicine. This approach involves expert judgement integrating data of epidemiology, of physiopathology, and of different types of clinical trials. The latter include case reports, retrospective and prospective open-label observational cohort studies, case-control trials, meta-analyses and controlled randomized trials (CRTs). Consensus based medicine focusses on the agreement between these sources of information, and on exploring the reasons of possible discordances. The materials included the results of published controlled trials retrieved from PubMed search, and personal data, some of which may not have been published in peer reviewed journals.

The therapeutic outcomes are expressed in terms of numbers needed to treat (NNT) [6]. In addition, the odds ratios (OR) and level of statistical significance is given of the difference in pregnancy rates between

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treated cases and untreated controls. The NNT expresses an epidemiological measure of the effectiveness of particular interventions in treating a particular disease using a particular mode of treatment. The NNT is the average number of persons who need to be treated to obtain one additional good outcome [7]. The NNT takes into account the absolute difference in frequency of successful outcome, rather than the relative change. For example, doubling of the success rate (Relative Risk, RR=2.0) thanks to a particular treatment will result in a completely different NNT in case the doubling is from 1% to 2%, with NNT that equals 100 (OR=2.02), than if the doubling is from 20% to 40% when the NNT is 5 (OR=2.67). Knowing the NNT of different treatments is particularly useful for clinical practice and in managing couple infertility. The NNT can also be employed to calculate the cost/benefit ratio of different modes of treatment.

To assess the effect of treatment of the infertile male is a difficult task. Changes in sperm characteristics are commonly used as surrogate markers of the effect of interventions, but the cumulative rate of on-going clinical pregnancies, the "take home healthy baby" rate, and the time-to-pregnancy are the ultimate measures of intervention outcomes. However, man's potential fertility can only come to expression through attaining pregnancy in his female partner.

According to the definition of the World Health Organization [8] a couple is considered infertile if spontaneous (natural) pregnancy has not been achieved in spite of "exposure to the risk of pregnancy" during at least 12 months. A male factor is detected in nearly half of infertile couples, and in 65% a problem is present in the female partner. In approximately 10% no obvious abnormalities are found, but the latter percentage is lowered to only 1% if both partners are thoroughly investigated, including e.g. laparoscopy, immunological and genetic testing. Both the male and the female partner simultaneously present fertility-reducing pathology in at least one out of every 4 couples [9]. In these couples treatment that optimizes the fertility status of the female partner will render it impossible to assess the effect of simultaneous treatment of the male on the pregnancy rate.

Many meta-analyses come to the conclusions that "there is not sufficient evidence" and that "larger well-designed trials are needed" [10]. These conclusions are drawn each time when the 5 % limit of statistical significance ($P<0.05$) has not been reached. The 5 % limit is purely conventional. Whereas it may be valid for experimental research, it might be preferable to apply the 10% limit of significance in clinical trials, indicating that the probability that a particular result is due to chance is less than 1 out of 10. In addition, a substantial proportion of infertile couples may benefit from a particular treatment and this treatment may be highly effective for a well-defined subgroup of patients, in spite of the fact that meta-analysis does not reach statistical significance.

Furthermore, the adagio "absence of proof is not the proof of absence" should always be kept in mind.

Major ethical issues have been raised regarding controlled randomized trials (CRTs) [11]. The following dilemma's must be solved: "does clinical equipoise apply to CRTs?", and "how do we determine if the benefits outweigh the risks of CRTs?". Regarding infertility treatment these questions can be translated into: "is it ethically acceptable to enrol large numbers of couples in (double- blind) randomized trials if there is a plausible indication that the favourable outcome of a particular treatment surpasses that of placebo or non-treatment". In addition, many couples of the non-treatment group withdraw from the trial and turn to e.g. assisted reproduction technology, if they do not attain pregnancy after a limited period of time. In general, no more than two-thirds of couples complete the clinical trials.

Testicular Physiology

During spermatogenesis, the diploid spermatogonia divide and pass through reduction division, called meiosis, in order to develop into haploid spermatids and spermatozoa. These are the only haploid cells in the body. Spermatogenesis starts at puberty, and the haploid cells are not present at the time when the immune system has developed. Therefore, spermatogenesis takes place in the protected environment of the seminiferous tubules, where the haploid gametes are hermetically isolated from the rest of the body by the blood-testis barrier. The cells of Sertoli, with their tight junctions and complex enzymatic activity, play a pivotal role in creating the "milieu interne" that is required for adequate spermatogenesis and for the survival of the haploid gametes. The optimal functioning of these cells depends on the adequate stimulation by follicle stimulating hormone (FSH) secreted by the pituitary, which is controlled through a negative feedback by Inhibin B. Inhibin B concentration in serum is a well-established marker of Sertoli cell function and spermatogenesis [12-14].

Serum levels of inhibin B were found to be positively correlated with sperm concentration, testicular volume and testicular biopsy score count [13,15,16] and negatively with FSH levels in serum [12,13].

The hormone secretion by the interstitial cells of Leydig submerges the seminiferous tubules in an extremely high concentration of testosterone, that is metabolised into 5-α-dihydrotestosterone by the 5-alfa-reductase of the cells of Sertoli. The androgenic activity of the latter is up to 10 times higher than that of testosterone. The Leydig cells are stimulated by the gonadotropin luteinising hormone (LH), the secretion of which depends on pulsatile stimulation by the hypothalamic neuro-secrete: Luteinising Hormone Releasing Hormone (LHRH). Feedback of LHRH secretion by testosterone requires the latter to be aromatised to estradiol-17-β by the hypothalamic cells [17].

Causes and Diagnosis of Male Infertility

Genetic and Congenital Causes

The most common genetic causes of male infertility are Klinefelter syndrome, chromosomal translocations, and Y chromosome micro-deletions. ICSI and testicular sperm extraction made it possible for men with genetic abnormalities and severely impaired sperm quality to father children, by this possibly transmitting infertility and genetic defects to the offspring. Therefore, it is recommended to screen men with sperm concentrations below 10 million/ml for numerical and structural chromosome defects. Screening for Y chromosome micro-deletions is recommended in cases with sperm concentration below 5 million/ml [8]. Congenital bilateral absence of the deferential ducts is present in about 2% of men with azoospermia. This condition is associated with a small ejaculate volume, acidic pH of the seminal plasma, and azoospermia. The cause is a mutation in the cystic fibrosis trans-membrane conductance regulator gene (CFTR) [18]. It is mandatory to screen both partners for mutations of this gene. In cases with the genetic abnormality, genetic counselling and pre-implantation genetic diagnosis (PGD) of the embryos are essential.

Primary ciliary dyskinesia (previously known as immotile cilia syndrome), is usually inherited as an autosomal recessive trait. The condition is characterized by immotile spermatozoa due to absence of outer dynein arms in the sperm tail. The association of this condition with situs inversus and recurring sino-pulmonary infections is known as the Kartagener syndrome [19].

In recent years much attention has been devoted to epigenetic changes that may occur in spermatozoa of infertile men [20].

The most common congenital cause of male infertility is testicular maldescent (cryptorchidism). The fertility in patients with this disease is variable, and it depends on the possible presence of other signs of the testicular dysgenesis syndrome [21], and the age at which surgical correction has been performed. Patients with testicular maldescent, have a higher risk of testicular cancer.

Other less common congenital causes include androgen receptor defects, Sertoli cells only syndrome, spermatogenic arrest, and hypogonadotropic hypogonadism in Kallmann syndrome [22]. Only the latter can be cured by hormonal treatment.

ACQUIRED CAUSES

Four causes are responsible for up to 90% of all cases of male infertility, namely: varicocele, male accessory gland infection, immunological infertility, and idiopathic oligozoospermia.

Varicocele is the most common cause of male infertility in the Western world. This disease is unique to the human species, and it results from the destruction or the bypass of the one-way valves in the internal spermatic veins (Figure **1**). The pathogenic mechanisms include increased hydrostatic pressure in the testicular venules [23], reflux of vaso-active catecholamines [24], and elevated scrotal temperature due to the filling of the pampiniform plexus with blood at body temperature [25]. Because the back pressure in the venules may exceed the pressure of intra-testicular capillaries, the arterial blood supply to the testes is reduced [26]. This can be documented by rapid sequence scanning after injection of the radio-isotope Tc-pertechnetate. The resulting hypoxia affects the function of the cells of Sertoli and, later in life, of the Leydig cells, leading to premature andropause [27]. The high concentration of norepinephrine in the refluxing blood induces arteriolar vasoconstriction which also decreases blood supply to the testis. Hypoxemia induces secondary inflammation and increases the production of reactive oxygen species [28] and of cytokines, more particularly of Interleukin-6 [29], the concentrations of which are enhanced in the ejaculate. Reactive oxygen species damage the DNA of the spermatogenic cells which is evidenced by the increased concentration of the oxidised nucleotide guanosin, namely 8-hydroxy-2'-deoxyguanosine (8-OH-

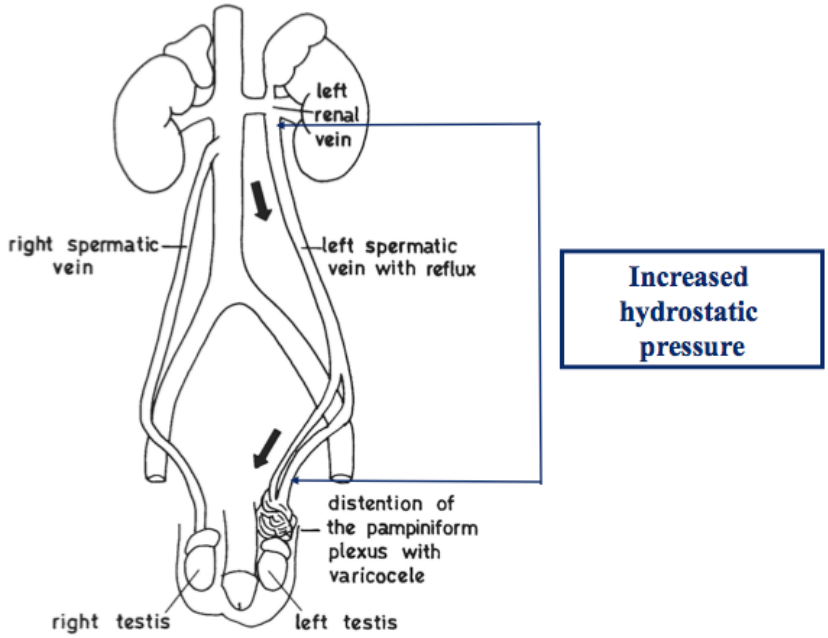


Figure 1: Anatomy of the internal spermatic veins.
In patients with varicocele, the one-way valves are destroyed and the hydrostatic pressure in the intra-testicular venules is increased when the person stands.

2dG), in ejaculated spermatozoa (Figure **2**) [30]. After cell division the 8-OH-2dG induces transition mutagenesis in the resulting embryonic cells by binding to thymine instead of cytosine.

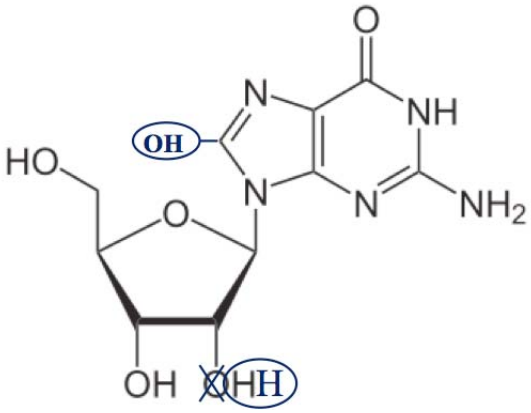


Figure 2: Mechanism of DNA oxidation.
This figure shows the formula of the oxidised nucleotide 8-hydroxy-2-deoxy Guanoside, that is derived from the "normal" nucleotide Guanine. During DNA replication, Guanine normally binds to Cytosine, but 8-OH- 2- d Guanosin will bind to Thymine, causing transition mutagenesis in the daughter cells.

In the majority of patients the arterial blood supply is restored within hours after the blockage of the internal spermatic veins. In some patients, however, the impaired perfusion persists, probably because of endothelial hyperplasia of the testicular arterioles that has developed as a result of the long-term hypoxia [31].

The detrimental effect of varicocele on sperm production is synergistically augmented by life style factors, in particular smoking, and by the presence of infection of the accessory sex glands with increased number of white blood cells in the ejaculate [32]. This can be evidenced by calculating the number of ejaculated spermatozoa per cubic centimetre of testicular volume (sperm concentration x ejaculate volume/total testicular volume). In fertile men this value is 5 million/cc or more. The frequency distribution of this "sperm production index" in infertile man with varicocele who do or do not smoke is shown in Figure **3**, sustaining the synergistic suppression of sperm production by smoking. The concentration of white blood cells in the ejaculate needed to decrease the sperm production index is 1.7 million/ml in men without varicocele, but 0.4 million/ml in men with varicocele, again evidencing the synergistic negative effect of both factors [32]. It seems logical to accept that this synergism is related to the amplification of oxidative overload and inflammation.

The diagnosis of varicocele is made by palpation and by thermography, either tele- or contact-thermography. The latter uses a strip containing temperature-sensitive liquid crystals (Varicoscreen®, Fertipro, Beernem, Belgium) that visualise the elevated temperature of the scrotal skin overlying the varicocele [33]. The patient has to stand upright for this test. In addition, reflux to the pampiniform plexus can be

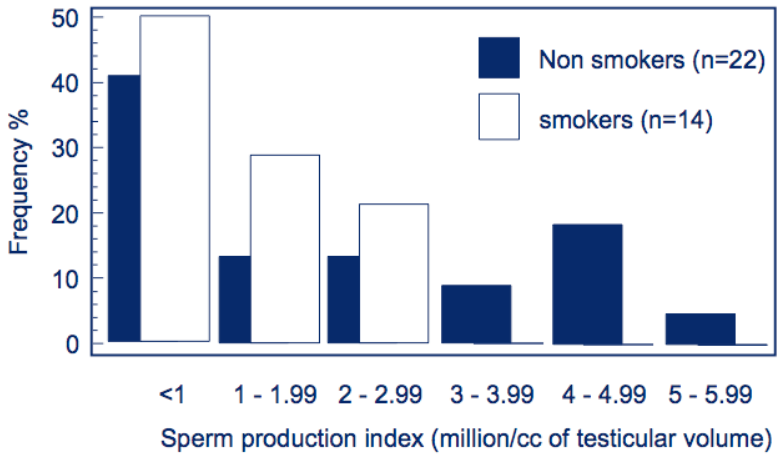


Figure 3: Varicocele and smoking.
The sperm production index was calculated by dividing the total number of spermatozoa in the ejaculate by the testicular volume (left and right testes added up).
There is a different frequency distribution between patients with varicocele who smoke or do not smoke. The sperm production index of the former is significantly lower than among the latter. The distribution of the sperm production index in the non-smokers appears to be bimodal.

revealed by means of (duplex) Doppler ultrasonography. For this test the patient is examined in recumbent position and the blood flow in the pampiniform plexus is inverted during the Valsalva manoeuvre when varicocele is present.

The clinical degree of distension of the intra-scrotal veins is related to the extent of reduction of the testicular volume, with larger varicoceles causing lower testicular volume. However, the pathogenic mechanisms involved in testicular dysfunction are identical in cases with large, small, or subclinical varicoceles. The main difference between large or small varicoceles resides in the capacity of collateral venous systems, *via* the cremasteric and the differential veins, to compensate for the hydrostatic pressure caused by the reflux in the internal spermatic veins.

Male accessory gland infection (MAGI) is four times more common among smokers than in non-smokers, and it can affect the prostate, and/or the seminal vesicles, and/or the epididymides. Common urinary pathogens, mostly E. Coli or Enterococcus species, rarely Klebsiella or Proteus species, are usually detected in men consulting for infertility.

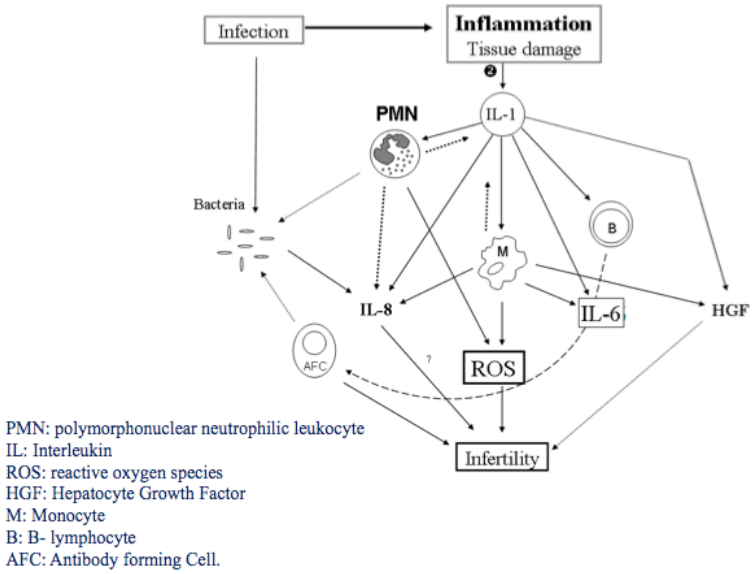


Figure 4: Mechanisms involved in the interaction of infection of the accessory sex glands with male fertility (figure from the paper by De Puydt *et al*.) [34].

Ascending infection due to sexually transmitted diseases, particularly Chlamydia trachomatis, has become less common.

Infection of the prostate can propagate through the vas deferens to reach the seminal vesicles and the epididymides at their caudal region, whereas the inflammatory process is often located at the epididymal head in case of Chlamydia infection.

The mechanisms by which MAGI interferes with fertility include the production of reactive oxygen species, inflammatory cytokines and growth factors (Figure **4**) [34]. There is a positive correlation between the concentration of peroxidase-positive white blood cells (neutrophile polymorphonuclear leukocytes) and the amount of Interleukin-6 in the ejaculate [35].

The diagnosis of accessory gland infection is based on the combination of information from history taking, palpation of the scrotal content, scrotal thermography, digital rectal examination, trans-abdominal or trans-rectal ultrasound imaging, and urine analysis after prostatic massage [36]. Semen analysis is of pivotal importance for the diagnosis, including the differentiation of so-called round cells into peroxidase-positive white blood cells and peroxidase-negative spermatogenic cells, bacterial culture, and biochemical analysis [37].

Immunological Infertility

Accessory gland infection, and epididymitis in particular, testicular traumatism, or (partial) obstruction of the vas deferens may cause rupture of the blood-testis barrier, provoking the production of antisperm-antibodies of the IgG and/or the IgA classes. This is a physiological immune response to the contact of the immune competent lymphocytes with the haploid spermatocytes, spermatids and spermatozoa. The large antibodies of the IgM class are seldom detected in the ejaculate, and they cause massive agglutination of spermatozoa. IgG antibodies are present in semen and in serum, whereas the secretory immunoglobulin of the IgA class is exclusively found in the semen.

The immunoglobulins attach to the spermatozoa and hinder their transit through the cervical mucus. Also, the binding of the complex of IgG antibodies with Complement C3 causes cytotoxic damage to the sperm membrane, and generates an important amount of oxygen radicals. Both these factors reduce the fertilizing capacity of spermatozoa, also during *in vitro* fertilisation.

Immunological infertility can easily be detected by means of the direct mixed antiglobulin reaction (SperMAR®- test, Fertipro Inc., Beernem, Belgium) for anti-sperm antibodies attached to spermatozoa [38], or by the indirect MAR-test for IgG- antibodies in serum. In this test, based on the principle of the indirect Coombs test, motile spermatozoa of the patient are mixed with latex particles covered with IgG, and with an excess amount of anti-IgG. In the presence of anti-sperm antibodies attached to the spermatozoa, the particles will bind to motile spermatozoa.

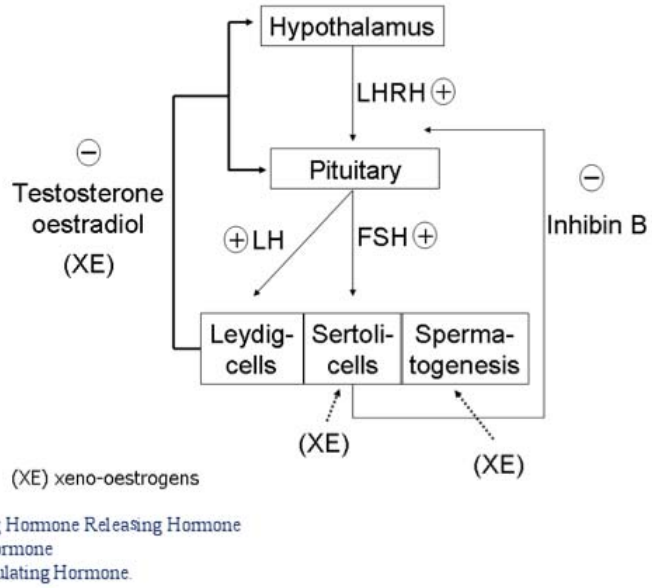


Figure 5: Suppressive effects of xeno-estrogens (XE) on the hypothalamo-pituitary-testicular axis in patients with idiopathic oligozoospermia.

The diagnosis of ***idiopathic male infertility*** is given when sperm quality is abnormal, and no other diagnosis is applicable. Life style factors and environmental influences may be involved in the pathogenesis of idiopathic oligo- and/or astheno- and/or terato-zoospermia. These include overweight, unbalanced nutrition with insufficient intake of omega-3 poly-unsaturated fatty acids [39], smoking, abuse of alcohol or of recreational drugs, and exposure to high temperature, professional toxic agents, or hormone disrupting substances.

The latter are absorbed from the contaminated environment through air, water and food. Some agents have an anti-androgenic or anti-thyroid action, but man-made chemicals with oestrogen-like effect, called pseudo- or xeno-oestrogens, are particularly harmful [40]. Xeno-oestrogens are accumulated through the biological food-chain, and by long-term storage in human fat tissue, and they may suppress the hypothalamo-pituitary function resulting in oligozoospermia [41]. Typically, the oligozoospermia is not compensated by increased secretion of gonadotropins (Figure **5**).

These patients often present with a low or low-normal testosterone concentration in blood, and LH concentration that is below the median value of fertile men.

Examples of xeno-oestrogens are: polychlorinated bisphenols (PCBs), phtalates, methyl-parabene, alkylphenols, ethinyloestradiol, Bisfenol-A, and dioxins. The level of exposure to xeno-oestrogens is variable between regions [42] and depends on whether the person lives in an urban or in a rural environment, in the neighbourhood of industrial activity, or is exposed to pesticides, etc.

The detection of individual xeno-oestrogenic agents in the environment and in blood of patients is technically feasible, but it is the total oestrogen load that should be assessed. This can be done by means of *in vitro* cell tests, such as the human breast cancer cell test or the test using modified yeast. These tests may, however, yield incorrect, false negative results because of direct toxicity of the sample to be tested on the cultured cells. In contrast, the oestrogen-receptor bioassay (ERBA) using a cell-free system of competitive binding, is more robust [43]. When applying the latter assay we were able to demonstrate extremely high concentrations of chemical xeno-estrogens in some surface waters in Flanders (Figure **6**), which would have remained undetected by the cell-based tests.

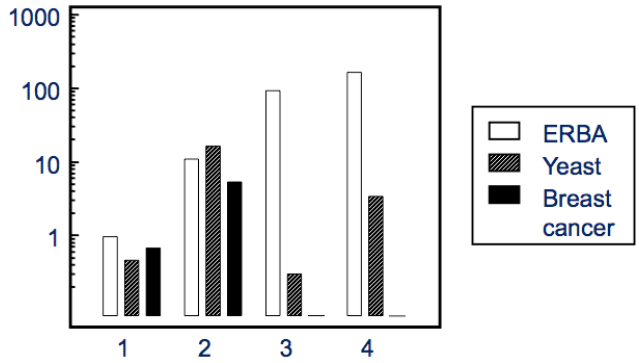


Figure 6: Total estrogenic activity measured in samples of surface water.

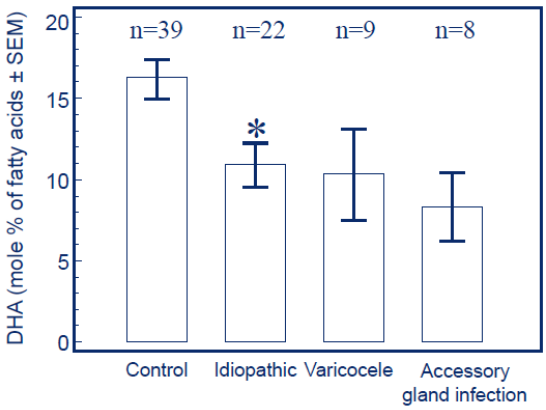
This bar histogram represents the total oestrogen activity measured in 4 samples of surface water collected from different locations along the upper-Scheldt in Flanders. The oestrogen activity is expressed in oestradiol-equivalents (pg/ml) on the y-axis (Logarithmic scale). The total oestrogen activity was estimated using 3 different methods, namely: "ERBA": Oestrogen receptor binding assay; [43] "Yeast": *in vitro* bioassay using genetically modified yeast; and "Breast cancer": *in vitro* bioassay using human breast cancer cell. The 3 tests gave similar results in the samples 1 and 2, with relatively low oestrogen activity. The receptor assay revealed very high oestrogen activity in the samples 3 and 4, whereas the yeast test suggested a low oestrogen content, and the breast cancer test gave a negative outcome. Based on the yeast and the breast cancer cell tests samples 3 and 4 would erroneously have been considered only slightly contaminated (unpublished results).

Clearly, the elimination or tapering-down of environmental hormone disrupting agents will decrease the prevalence of poor semen quality among community dwelling men, as we could indeed observe in Flanders [44].

Cellular Mechanisms of Sperm Dysfunction and their Implication for the Treatment with Nutriceutical Food Supplementation

The membrane of normal spermatozoa contains a high concentration of the long-chain poly-unsaturated fatty acid (PUFA) of the omega-3 group, namely docosahexaenoid acid (DHA; 22: 6ω3; also called cervonic acid) [39], which procures a high fluidity to the membrane's phospholipids. When semen is processed over a density-gradient column, the high quality spermatozoa are concentrated into the high density fraction. The cell membrane of spermatozoa from the high density fraction of patients with varicocele, male accessory gland infection, or idiopathic infertility contains less DHA, and displays lower fluidity (Figure **7**) [45]. This is related to inadequate nutritional intake of omega-3 fatty acids, and to the higher oxidative load

that was registered among infertile men as compared to fertile controls. As a result of the lowered membrane fluidity, the capacity of spermatozoa to undergo induced acrosome reaction [46] and to fuse with the oocytes is diminished, which explains their reduced fertilizing potential.



DHA: docosahexaenoid acid, SEM: standard error of the mean

Figure 7: Proportion of DHA of the sperm membrane.
This bar histogram represents the proportion (in %, mean values with Standard Error of the Mean) of docohexaenoic acid (DHA) of the phospholipids of the cell membrane of ejaculated spermatozoa obtained from fertile controls, patients with idiopathic oligozoospermia, or with varicocele, or with male accessory gland infection [45].

Quite remarkably though, sperm concentration and sperm-motility were inversely related to the nutritional intake of the long-chain PUFAs Eicosapentaenoid acid (EPA) and DHA (n=24, r:-0.62, P<0.02), but positively correlated with the ingestion of the short-chain omega-3 PUFA Alfa-linolenic acid (ALA; 18:3ω3)(r:0.59, P<0.02). At the other hand, testicular tissue contains an exceptionally high concentration of the enzymes elongase and desaturase [47]. Also, mice that were genetically "knocked out" for delta-6-desaturase were infertile and presented with arrest of the maturation during spermatogenesis [48].

It seems logical to assume that the long-chain PUFAs DHA and EPA cannot pass through the blood-testis barrier because of their large molecular size, whereas the short-chain ALA can do so. Thanks to the abundant presence of elongase and desaturase within the seminiferous tubules, ALA is metabolised into the long-chain PUFAs by the cells of Sertoli [49].

Providing an extra nutritional amount of the long-chain fatty acids was reported not to exert any favourable effect on sperm characteristics. In contrast, giving ALA to infertile men seems indicated, with linseed-oil serving as a suitable source. Since vitamin B6 and zinc act as co-factors for the elongase and desaturase enzymes, these substances should also be added.

The oxidative/anti-oxidant balance can be estimated in blood by measuring the lag-time before extracted LDL-cholesterol is oxidised when exposed to copper *in vitro*. The lag-time was, on an average, 25% shorter in blood of infertile men as compared to that of fertile controls. This indicates oxidative overload in infertile men.

Oxidative stress not only damages the phospholipid composition of the cell membrane containing a high concentration of PUFAs, but it also inhibits the function of the mitochondria. These are situated at the mid-piece of the spermatozoa and generate adenosine triphosphate (ATP). ATP is transported *via* the micro-channels inside the flagellum where the protein called Dynein contracts, inducing sperm movement. The mitochondria produce ATP through the Krebs cycle using the long-chain PUFAs (EPA and DHA) for a substrate. The latter are transported from the cell cytoplasm into the mitochondrial matrix through binding to acetyl-carnitine-CoA.

Oxidative overload impairs mitochondrial function and reduces sperm motility [50]. It may also disturb cell division during spermatogenesis, and embryogenesis [51]. Complementary treatment with the strong anti-oxidant Astaxanthin, the carotenoid from the algae Hematococcus pluvialis, improves sperm motility [52, 53], and so does the supplementation with L-(acetyl)-carnitine and PUFA. Since ATP-production in the Krebs cycle generates relatively large quantities of reactive oxygen species, the mitochondrial anti-oxidant co-enzyme Ubiquinone Q10 should also be supplemented.

Oxidative overload affects DNA-integrity by oxidising Guanine into 8-hydroxy-2-deoxy Guanosin (8-OH-2dG) (Figure **2**). During cell replication, the latter does not bind to Cytosine, which Guanine normally should do, but to Thymine, causing transition mutagenesis in the daughter cells. Sperm of infertile men commonly contains a high concentration of 8-OH-2dG, which is dramatically reduced by oral anti-oxidant treatment [53]. Also, oxidative stress results in an elevated rate of DNA-fragmentation of spermatozoa, which may be related with the increased rates of miscarriage and enhanced risk of diseases that is observed in the offspring after IVF/ICSI [54]. Epigenetic changes, such as abnormal methylation or (de)-acetylation have been reported to occur in spermatozoa of infertile men, and may be related to nutritional factors [20].

Psychological stress is a common phenomenon among infertile couples. It reflects on their intimate relationship [55], and it is well-known to induce ovulation disturbances through hypothalamo-pituitary deregulation. Also, stress exerts direct mutagenic effect on gametes, while the efficiency of the repair protein P 53 is inhibited [56]. Experimental animals exposed to stress produce a specific protein called heat-shock protein P70, that exerts a protective effect. The phyto-adaptogen extracted from Lepidium meyenii (MACA) activates the production of this heat-shock protein P70 [57], and thus reduces the negative impact of stress. MACA was found to enhance fertility in experimental animals [58]. Also, Lepidium meyenii was reported to improve sperm quality in humans [59].

Treatment of Causal Factors and Correction of Cellular Mechanisms

Treatment of varicocele aims at completely interrupting reflux of blood in the internal spermatic veins and is performed with different modalities of surgery. Retrograde venography using the Seldinger-technique and inguinal approach through the saphenous vein is the least invasive treatment. Bilateral embolization of the internal spermatic veins using a tissue adhesive [60] is performed under local anaesthesia, on an out-patient basis. A control thermography and (duplex) Doppler evaluation should be performed 3 months after treatment to certify the completeness of the interruption of reflux.

The effect of varicocele treatment on couple fertility is being debated. The Cochrane meta-analysis repeatedly published and updated by Evers *et al*. (2008) [61] concludes that there is not sufficient evidence for accepting a positive effect on fertility. This conclusion was contradicted by two other meta-analyses [62, 63]. French *et al*. (2008) [62] have suggested that the conclusion of the Cochrane meta-analysis may be misleading due to "improper criteria for selecting the papers to be included". Indeed, scepticism comes up for discussion regarding the trials which may, or may not be included into a particular meta-analysis. It has been amply documented that the spontaneous, treatment-independent pregnancy rate among infertile couples with a male factor is approximately 12 to 16 % within twelve months of follow-up (Figure **8**). In the randomised single centre trial published by Nilsson *et al*. (1979) [64] the pregnancy rate of untreated controls was of the expected order of magnitude. In the couples where the man was surgically treated for varicocele the pregnancy rate was identical to that of the controls, which is less than half the pregnancy rate observed in treated couples of all other trials. Also, no changes in sperm quality occurred among the treated cases in Nilsson's trial [64], which equally contrasts with all other published results as well. These findings suggest that varicocele surgery may not have been performed correctly.

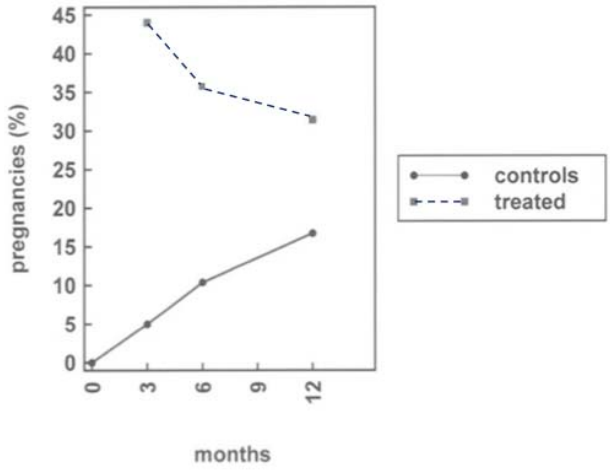


Figure 8: Rate of on-going pregnancies (on the y-axis, in %) in untreated and treated couples of different controlled trials in relation to the duration of treatment or follow-up (on the x-axis, in months).

After 3 months, 5% of untreated couples, where the male partner suffered from varicocele attained pregnancy, as compared to 44% of couples in whom varicocele was treated and the men were given the nutriceutical food supplement (NNT = 2.6). After 6 months 10.4% of couples with untreated idiopathic oligozoospermia conceived compared to 35.8% when the men were treated with Tamoxifen plus testosterone undecanoate (NNT = 3.9). After 12 months of observation 16.3% of untreated patients with varicocele achieved pregnancy compared to 31.4% who were treated for their varicocele without taking complementary food supplementation (single- and multicentre CRTs taken together)(NNT = 6.6). The pregnancy rate among untreated couples corresponds to a constant probability of conception of approximately 1.5% per month. A higher number of cases is needed to treat (NNT is higher) when the difference between treated and untreated cases decreases.

During a site visit, the first author (FC) has established that surgical failure to block reflux in the internal spermatic veins explained the negative outcome of another controlled trial [65]. In general, recurrence or persistence of varicocele after surgery could be documented by means of retrograde venography [66].

In a another randomised single-centre trial there was no significant difference in pregnancy rate between patients who were either treated for their varicocele, or were "counselled" [67]. The pregnancy rate in the treated group (29.0%) was similar to that reported in other controlled trials and cohort studies,

but the pregnancy rate in the controls (25.4%) was significantly higher than expected. It may be assumed that the so-called counselling, which was applied to both partners of the control group, could have resulted in the effective correction of subtle pathology in the female partner. Clearly, it would be impossible to assess the effect of treatment of the male partner whenever the fertility potential of the female partner is enhanced.

A large multi-centre, prospective controlled trial by WHO [68, 69] was excluded from the Cochrane meta-analysis [59] because the complete results have not been published in a peer-reviewed journal. This trial included 283 couples, and the NNT was 5.4 (OR: 2.94, CI: 1.64-5.29; P=0.0003). Figure **9** shows the life-table calculation (Kaplan-Meyer plot) of the cumulative probability of conception in the controls and the treated cases of this trial.

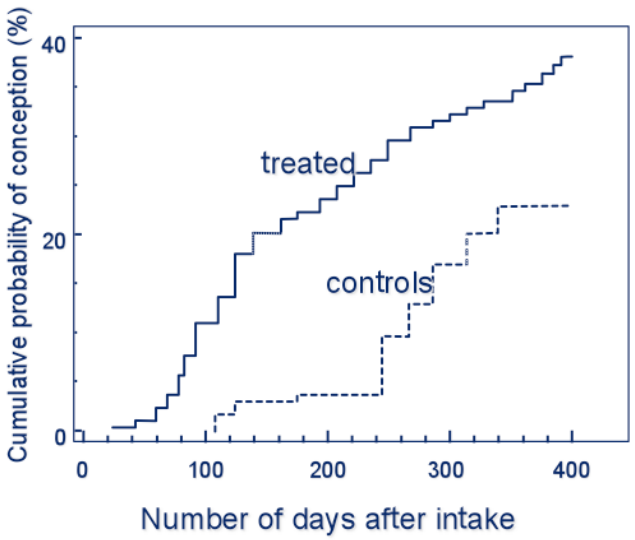


Figure 9: The cumulative *probability* of conception (on the y-axis, in %) was calculated with the Kaplan-Meier life table statistics (MedCalc®, MedCalc Inc, Mariakerke-Ostend, Belgium), and is depicted in relation to the number of days after inclusion (x-axis) in the randomised prospective multicentre trial coordinated by WHO (unpublished data). The curve "treated" shows the cumulative probability of pregnancy in couples where the man had abnormal semen quality, and varicocele and was immediately corrected by surgery. The curve "controls" represents the probability of conception among couples where the man had abnormal semen quality and varicocele, but surgery was postponed for 12 months.

The figure illustrates a major problem with controlled trials of male infertility, namely that couples not receiving treatment tend to withdraw from the trial and turn to other treatments after a few months, when not attaining conception. As a result, the number of couples completing the trial is commonly between 60 and 65%, with numbers remaining in the control group after 6 months of observation being relatively small. Therefore, every single pregnancy occurring among this residual control population will be represented as a relatively important increase of the cumulative probability of conception. It can also be deduced from this figure that the time needed to attain pregnancy by 20% of the couples is decreased by half in the treated group, in whom surgery was performed immediately.

We conclude that there is strong evidence that varicocele treatment improves semen quality and increases the probability of natural conception as compared to that of untreated controls. The numbers needed to treat (NNT) calculated on the basis of results of 2 large multi-centre prospective randomised trials [68, 70] was 6.3 (total number of cases included n= 350, OR: 3.84, 95% CI: 2.19-6.72, P<0.0001), and the NNT in single-centre controlled trials [67, 69, 71] was 6.8 (n= 308, OR: 2.20, 95%CI: 1.30-3.74, P=0.0036).

It is mandatory to simultaneously correct complementary factors and pathology that synergistically enhance the unfavourable influence of varicocele, such as tobacco smoking and male accessory gland infection. Complementary treatment of the male with the specific NFS during 3 months after embolization reduced the NNT to 2.6 in a case-control study (n=64, OR: 16.65, 95%CI: 4.73-58.55, P=0.0008) (Figure **8**).

Male Accessory Gland Infection

The majority of urinary pathogens are sensitive to the third-generation quinolones [72] that penetrate into the diseased prostate, seminal vesicles and epididymis. Thanks to their characteristic of a Zwitter-ion these quinolones are active in both an alkaline and an acidic environment. Tetracyclins are concentrated in acidic tissues, and have been recommended for the treatment of prostatitis in the past. However, the secretion of citric acid by the diseased prostate is commonly decreased, by which its tissue pH becomes alkaline. Hence treatment with tetracyclins will be ineffective. The concentration of quinolones in the ejaculate is generally insufficient to eradicate Streptococcus species. In case the latter pathogen is cultured from the ejaculate of infertile men, amino-penicillin should be prescribed. Treatment must always use an adequate dose of the antibiotic, and should be given during at least two weeks.

Antibiotic treatment usually does eradicate infection, but the functional deficiency of the affected glands, as well as inflammation with elevated concentration of cytokines [34] and oxidative overload may persist (Figure **4**). Also, the secretion of anti-oxidants by the epididymides commonly remains impaired. Though there are no randomised prospective therapeutic trials available, it seems that oral antioxidant

supplementation may improve fertility after antibiotic treatment [73, 74].

Immunological Infertility

Anti-sperm antibodies (ASA) continue to be produced as long as spermatogenesis takes place. It may be possible to temporarily inhibit the production of ASA by high dose corticosteroids, but this treatment has a poor success rate, and it carries a risk of serious side effects such as stomach ulcers and aseptic necrosis of the hip. Since the ASA impair the passage of spermatozoa through the cervical mucus, intra-uterine insemination (IUI) has been advocated as a mode of treatment. In our retrospective cohort study, including 106 sequential couples with male immunological infertility, treatment with 6 cycles of IUI was indeed of significant benefit [75], with a NNT of 5.3 (n=106, OR: 3.00, 95%CI: 1.14-7.86, P=0.025). Oxidative and cytotoxic damage to the sperm membrane may hinder sperm-oocyte fusion limiting the success rate of IUI and IVF. Also, oxidative damage to sperm-DNA may cause an increased risk for the offspring if ICSI is used. Therefore it is recommended to provide nutriceutical food supplementation with antioxidants to both partners before IVF/ICSI is applied.

Idiopathic Oligozoospermia

Patients with idiopathic normo-gonadotropic oligozoospermia commonly benefit from treatment with the selective anti-estrogen Tamoxifen [76], that blocks the oestrogen receptor at the hypothalamic level. Xeno-oestrogens bind to the oestrogen receptor. Blocking this receptor by means of an anti-oestrogen should increase the secretion of LHRH and the gonadotropins LH and FSH, enhancing testosterone production as well as spermatogenesis. This indeed occurred during Tamoxifen treatment [77]. In order to assess the response to Tamoxifen in an individual patient it is recommended to measure the increment of serum testosterone concentration after one month of treatment. If the hypothalamo-pituitary-Leydig cell axis reacts correctly, the testosterone concentration will increase by 50 to 100%, and Tamoxifen treatment should be continued for at least 6 months, or until the occurrence of pregnancy.

The relation between the probability of conception per month and sperm motility as well as with sperm morphology was found to be linear, but the relation with sperm concentration is parabolic [78]. In our own prospective cohort study six months of Tamoxifen treatment resulted in the increase of the mean sperm concentration from 5.5 million/ml to 14.4 million/ml. The corresponding increase of the probability of conception per month, as deduced from the parabolic regression line, would be 210%. In other published trials the sperm concentration doubled from average 14.0 million/ml before treatment, to 28 million/ml during Tamoxifen

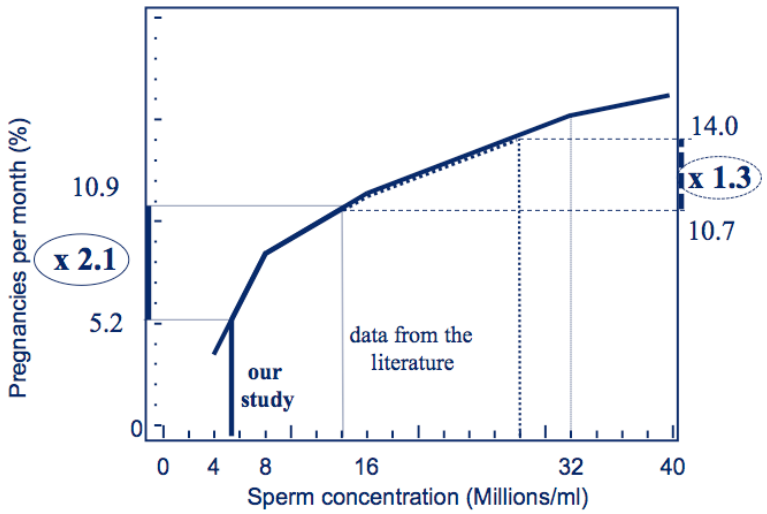


Figure 10: Relation between sperm concentration and fecundity.

This graph depicts the relation between sperm concentration (on the x-axis, in millions/ml), and the fecundity (probability of conception per month of exposure to the risk of pregnancy in %) on the y-axis. The solid lines show the change in sperm concentration, and corresponding increase of fecundity in the group of couples reported in our Tamoxifen trial. Sperm concentration increased from average 5.5 million/ml to 14.4 million/ml after 6 months treatment. The dotted line shows the mean change in sperm concentration and corresponding increase in fecundity reported in several other published studies. The average initial sperm concentration was 14.0 million/ml doubling to 28 million/ml after treatment. Due to the fact that the relation between sperm concentration and fecundity is not linear, but parabolic [78], the relative increment of fecundity in our trial (from 5.2% to 10.9% per month, corresponding to a multiplication by 2.1) is much more important than in the other published studies (from 10.7% per month to 14% per month, corresponding to a multiplication by 1.3).

intake. The corresponding increase of the probability of conception would be only 130% (Figure **10**). Thus, treating men with idiopathic oligozoospermia will be more efficacious in cases with lower sperm concentration, than in patients with so-called mild sperm deficiency.

The NNT in a double-blind trial by Adamopoulos *et al*., [79] combining Tamoxifen with testosterone-undecanoate, was 3.9 (n=128, OR: 4.57, 95%CI:1.79-11.65, P=0.0015).

Treatment of the male with Clomiphene citrate does not improve fertility [80]. Clomiphene is a racemic mixture of two isomers, one of which displays rather strong intrinsic xeno-oestrogenic activity. The latter has a deleterious effect on sperm quality, morphology in particular. Therefore the Cochrane meta-analysis, whereby trials with Clomiphene citrate and with Tamoxifen were added-up [81], and no therapeutic effect could be certified, is senseless and the conclusion is incorrect (withdrawn in 2007).

Cytokinesis and Embryo Development

Cytokinesis taking place during the process of cell division and embryo development, requires the contraction of non-muscular Myosin II to fold the cell membrane [82]. This contractile protein uses ATP as its source of energy, which is produced by the mitochondria. Excess reactive oxygen species in female infertile patients with polycystic ovary syndrome [83], endometriosis [84] or pelvic inflammatory disease (PID), may reduce the production of ATP during embryogenesis, causing inadequate function of the Myosin II, and impaired embryo development [85]. Food supplementation of the female partner with the appropriate NFS together with long-chain PUFA before oocyte pick-up, and during the first weeks after conception, may improve embryogenesis, cytokinesis, and possibly implantation.

Formulation of the Nutriceutical Food Supplement "Qualisperm®" (Nutriphyt Inc, Oostkamp, Belgium)

Based on the rationale mentioned above, a novel NFS has been created that is composed of the following constituents:

Lepidium meyennii (MACA): 125 mg, Pine bark extract (Pycnogenol®, Horphag, Geneva, Switserland): 50 mg, Acetyl-carnitine: 50 mg, oxido-reductase Ubiquinone Q10: 12.5 mg, Astaxanthin (AstaReal, Fuji Chemical Industries, Japan): 4 mg, Zinc bisglycinate: 3.75 mg, Pyridoxine (Vit B6): 1.5 mg, Folic acid (Vit B9): 0.1mg, and Cobalamine (Vit B12): 0.75 µg.

Men should take 2 pills per day together with twice per day 1 g of linseed oil, and women should take 2 pills per day together with 2 g of fish oil, rich in EPA and DHA.

The Role of Food Supplementation and Clinical Trials with the Novel Nutriceutical

When studying the recent literature on food supplements [10, 86-88] it is striking that little attention, if any, is given to "the great different between antiradical and antioxidant activity" [89]. In fact, certain substances act as reducing agents and others as scavengers. Among the former, Vit C (ascorbic acid) exerts a pro-oxidant effect when given in a high dose, particularly in persons with the haptoglobin Hp 2-1 and 2-2 haplotype [90]. Also, high dose supplementation with Vit A (retinol) was found to increase the concentration of DNA-adducts [91]. Natural vitamin E is a scavenger and it is composed of 8 different forms (alpha, beta, delta and gamma tocopherols and tocotriolenes). The synthetic Vit E-analog (tocopherol-succinate) exerts biological effects that are different from those of natural Vit E. High doses of the analog inhibits the function of the gap-junctions [92], which are of pivotal importance for intercellular communication during spermatogenesis. Consequently, supplementing men suffering from oligo- or asthenozoospermia with high-dose Vit C and tocopherol-succinate (more than 50 times the recommended daily dose of natural Vit E) showed no improvement of sperm quality, nor fertility [93]. Regretfully, these critical scientific considerations are not taken into account in published meta-analyses.

Several clinical trials have been undertaken to estimate the effect of treating the causal factors and adding the novel NFS described above.

In an uncontrolled, open-label, dose-finding, prospective cohort-trial of 3 months duration, whereby infertile men were given food supplementation with the NFS plus linseed oil, sperm concentration increased by 60%, rapid linear (grade "a") motility was increased threefold, motile sperm concentration was multiplied by (median) 3.7, and morphology improved by 40% (Figure **11**). The concentration of oxidized DNA of the spermatozoa decreased significantly (Figure **12**).

In a case-control trial comparing men treated for varicocele and receiving the NFS with matched untreated controls, the NNT within 3 months was 2.6

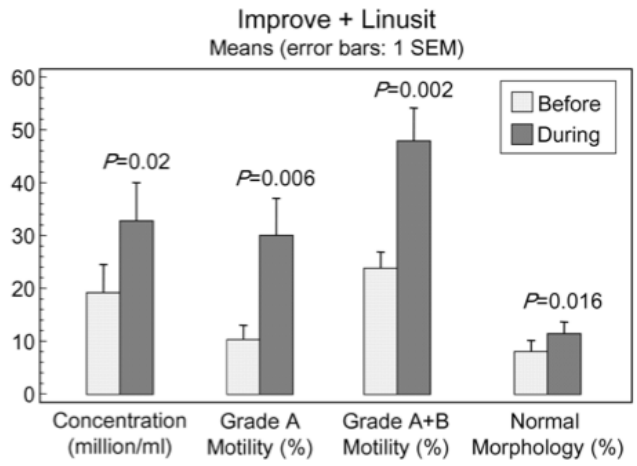


Figure 11: Effect of the nutriceutical food supplement (NFS) and linseed oil on sperm quality in an open-label, prospective, dose-finding cohort trial. This bar histogram shows the results of sperm concentration (in millions per ml of native semen), rapid linear progressive motility (grade a, in %), all progressive motility (grades a and b added, in %), and morphology (% spermatozoa with normal morphological appearance, WHO criteria, Papanicolaou stain), before initiation and after 3 months of intake of the NFS together with linseed oil. The data are presented as Mean, error bars indicate 1 Standard Error of the Mean. *P*- values of statistical significance were calculated using the student's t-test for paired observations.

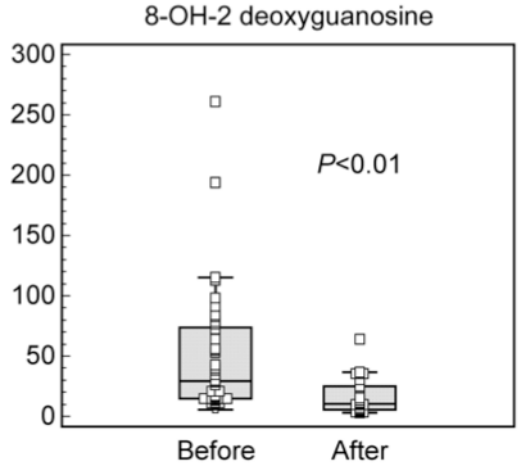


Figure 12: Effect of mixed antioxidant therapy on sperm DNA oxidation.

The concentration of 8-hydroxy-2-deoxyguanosine (on the Y-axis, in fMol) in ejaculates of infertile men is represented in this box-and-whisker plot with individual values in dots. The column "before" shows the values before treatment, and the column "after" represents the values after 3 months of intake of a mixed antioxidant [53] (Courtesy Asian J Androl).

(Figure **8**) (n=64, OR:16.65, 95%CI:4.73-58.55, P=0.0008).

The on-going pregnancy rate per transfer obtained by IVF, complemented with ICSI whenever indicated, recorded by the Fertility-Belgium centre [6] has increased from 23% in 2006 (historical controls) to 35% in 2010, thanks to treating the causal factors and adding the NFS to the male partner. This corresponds with a NNT of 8.3 (n=1741, OR: 1.79, 95%CI: 1.45-2.22, P<0.0001). Over the same time period the average pregnancy rate in all Belgian IVF-centres combined increased from 23.1% in 2006, to 27.8% in 2010.

In a proof of principle double-blind trial against folic acid, both the female and the male partner were given the NFS. The male partner took the NFS plus linseed oil during 8 weeks before pick-up, and the female partner was treated with the NFS plus DHA and EPA-enriched fish oil during the 6 weeks before, and 2 weeks after pick-up. The on-going pregnancy rate was 45% in the treated couples as compared to 20% in the controls treated with folic acid only. In this small trial the NNT for on-going pregnancy was 4 [6]. (n=25, OR:3.33, 95%CI: 0.47-23.47, P=0.23). Due to the small number of couples included, the difference between treated and control couples is not significant. However, the question should be asked whether it is ethically acceptable [11] to enrol a large number of infertile couples in a CRT, knowing that there is a concurrent trend for the on-going pregnancy rate after nutriceutical food supplementation to surpass that in placebo-treated controls.

CONCLUSION

Assessment of the infertile couple must always include the thorough investigation of both partners, the adequate treatment of all causal diseases, and the correction of unfavourable external factors that are associated with, or may cause the impaired reproductive capacity. It seems indicated to simultaneously counteract the mechanisms involved with the malfunctioning of the gametes. This can be done by complementary intake of a judiciously formulated NFS.

There is concurrent evidence that this treatment strategy results in a remarkable increase of the probability of natural conception. This approach may also downgrade the complexity of the required techniques of assisted reproduction, i.e. from IVF to IUI, or from ICSI to simple IVF. In addition, data obtained from preliminary trials suggest a trend for this treatment strategy to enhance the success rate of IVF.

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CONFLICT OF INTEREST

The first author serves as an occasional consultant of the Nutriphyt Inc. that produces the nutriceutical food supplement.

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