The Benefit of Nutraceutical Food Supplementation and Antioxidants for the Treatment of the Infertile Couple and in Assisted Reproduction

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Abstract
Varicocele, male accessory gland infection, immunological infertility, and idiopathic oligozoospermia are the main causes of male infertility. Disturbances of ovulation, pelvic inflammatory disease, and endometriosis are common causes of female reproductive failure. The mechanisms by which these causes interfere with male and female reproduction are inflammation through prostaglandins and cytokines, and oxidative overload damaging the cell membrane, inducing mutagenesis of the DNA, and impairing mitochondrial energy production. A unique nutraceutical food supplement (NFS) has been created that aims at correcting these mechanisms and at reducing the influence of detrimental environmental factors. 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. When the NFS is added to the treatment of varicocele the NNT is 3. Treating both partners with the NFS resulted in one additional ongoing pregnancy for every 4 IVF treatments.

Introduction

Since the introduction of in vitro fertilisation (IVF) in 1978, and subsequently of intracytoplasmic sperm injection (ICSI) in 1990 [1], there has been little progress in techniques that aim at achieving pregnancy using deficient gametes. The implementation of advanced genetic tests, that assess the whole genome of the embryos, may improve the success rate of these techniques, but it does 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.

The present paper aims at reviewing the definitions, the physiology and pathology of male and female infertility with emphasis on the mechanisms that are involved, and at highlighting the role of nutraceutical food supplementation (NFS) for the clinical management of infertility. Emphasis is placed on the therapeutic outcome in terms of numbers needed to treat (NNT) which are calculated on the basis of controlled trials. The NNT expresses an epidemiological measure of the effectiveness of particular health care interventions in either preventing or treating a particular disease using a particular mode of treatment. The NNT is the average number of persons who need to be treated to either prevent one additional bad outcome or to obtain one additional good outcome [2]. The NNT takes into account the absolute difference in frequency of successful outcome, rather than the relative change. For instance, doubling of the success rate (relative risk, RR) thanks to a particular treatment will result in a completely different NNT in case the doubling is from 1% to 2% (RR: 2.00, NNT = 100) than if the doubling is from 20% to 40% (RR: 2.00, NNT = 5). Knowing the NNT of different treatments is from 20% to 40% (RR: 2.00, NNT = 5). The role of semen analysis

Semen analysis is of pivotal importance for the systematic investigation of every infertile couple. Computer aided semen analysis (CASA) can be performed by means of fully computerized equipments such as the IVOS HamiltonThorne®, SCA®, or of a semi-automatic system [5] (Antosperm®). These permit the reproducible and precise measurement of sperm concentration, motility characteristics

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including curvilinear and linear velocity as well as angularity, and the adequate classification into motility classes a, b, c, and d. Receiver Operating Characteristic (ROC) curve analysis [6] has revealed the concentration of grade a motile spermatozoa to have acceptable accuracy in discriminating between semen that has the potential for natural conception, and infertile semen (sensitivity: 84%, specificity: 81%).

Causes and Treatment of Male and Female Infertility

Male infertility

Four causes are responsible for approximately 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 infertility in the Western world. This disease results from the destruction or the bypass of the one-way valves in the internal spermatic veins, with increased hydrostatic pressure in the testicular venules [7], as well as reflux of vaso-active catecholamines [8]. Arterial blood supply to the testes is reduced, which affects the function of the cells of Sertoli and, later in life, of the Leydig cells leading to premature andropause [9]. Hypoxia and secondary inflammation increase the production of reactive oxygen species and cytokines, the concentration of which is enhanced in the ejaculate [10].

The detrimental effect of varicocele on sperm production is synergistically augmented by life style factors, in particular the abuse of tobacco, and by the presence of infection of the accessory sex glands with increased number of white blood cells in the ejaculate [11].

The diagnosis of varicocele is made by palpation, and by thermography, either tele- or contact thermography using a strip that contains temperature-sensitive liquid crystals and that visualises increased temperature of the scrotal skin overlying the varicocele [12]. In addition, reflux to the pampiniform plexus is commonly evidenced by means of duplex Doppler examination.

Varicocele treatment is performed with different modalities of surgery, either microsurgical or through laparoscopy, but the least invasive treatment is by means of retrograde venography using the Seldinger technique, and bilateral embolisation of the internal spermatic veins with a tissue adhesive [13].

There is strong evidence that varicocele treatment increases the probability of spontaneous pregnancy as compared to that of untreated controls. The numbers needed to treat (NNT) calculated on the basis of results of multi-centre prospective randomised trials [14,15] was 6, and in single-centre trials [16-18] that number was 7. The simultaneous correction of complementary life style factors and pathology, such as tobacco smoking and male accessory gland infection, is mandatory. Addition of a specific NFS reduced the NNT within 3 months after embolisation to 3 in comparison with treated patients not taking NFS, and to 2 compared to untreated controls.

Male accessory gland infection (MAGI) is four times more common among smokers than non-smokers, and it can affect the prostate, and/or the seminal vesicles, and/or the epididymides. The latter may be accompanied by immunological infertility due to the rupture of the blood-testis barrier, provoking the production of antisperm-antibodies of the IgG and/or the IgM classes.

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, echography, urine analysis, and semen analysis including differentiation between peroxidase-positive white blood cells and peroxidase-negative spermatogenic cells. Immunological infertility is detected by means of the direct mixed antiglobulin reaction (MAR) test for anti-sperm antibodies attached to spermatozoa [19], or the indirect MAR test for antibodies in serum.

Antibiotic treatment with third-generation quinolones [20] may eradicate infection, but functional deficiency of the affected glands may persist, as well as inflammation with elevated concentration of cytokines [21], oxidative overload (Figure 1), and impairment of the production of anti-oxidants by the epididymides. There are no randomized prospective therapeutic trials available, but it seems possible to improve fertility by means of oral oxidants [22] with a NNT of 8.

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 probably are involved in the pathogenesis of idiopathic oligo- and/or astheno- and/or teratozoospermia. These include overweight, unbalanced nutrition with insufficient intake of omega-3 poly-unsaturated fatty acids [23], abuse of tobacco, alcohol or drugs, and exposure to professional toxic agents or hormone disrupting substances. The latter are absorbed from the contaminated environment through air and food, and man-made chemicals with estrogen-like effect, called xeno-estrogens, are particularly harmful [24]. Xeno-estrogens are accumulated through the biological food-chain, and by long-term storage in human fat tissue, and they suppress the hypothalamo-pituitary function resulting in oligozoospermia that is not compensated by increased secretion of gonadotropins (Figure 2).

Patients with idiopathic normogonadotropic oligozoospermia commonly benefit from treatment with the selective anti-estrogen Tamoxifen [25], but not Clomiphene citrate, that blocks the inhibitory effect of the xeno-estrogens at the hypothalamic level. The NNT in a double-blind trial by Adamopoulos et al. [26], combining Tamoxifen with testosterone-undecanoate, was 4.

Female infertility

The main causes of female infertility are anomalies of ovulation, endometriosis, and pelvic inflammatory disease (PID).

![Figure 1: Mechanisms involved in the interaction of infection of the accessory sex glands with male fertility](image-url)
Among infertile men as compared to fertile controls. Consequently, intake of omega-3 fatty acids, and to higher oxidative load registered DHA, and displays lower fluidity. This is due to inadequate nutritional male accessory gland infection, or idiopathic infertility, contains less group, docosahexaenoid acid (DHA; 22:6\_3; also called cervonic acid) of the longchain poly-unsaturated fatty acid (PUFA) of the omega-3 κB) [29].

However, persisting inflammation exerts a deleterious effect on the oocyte membrane, among other factors by activating the rapid reaction as elevated concentrations of IL-1 and IL-6. The diagnosis is made by randomised trial [30] equals 9, but no effect was recorded in another cases of mild to moderate endometriosis observed in the Canadian pathological foci, but inflammation may persist and tissue adhesions diagnosis requires laparoscopy during which surgery can remove the latter are transported from the cytoplasm into the mitochondrial togenesis Spermatogenesis. Therefore, providing an extra amount of ALA to infertile men seems indicated, which is given as linseed- or flaxseed-oil. Also, vitamin B6 and zinc should be added, since these are co-factors for the elongase and the desaturase enzymes. Eicosapentaenoid acid and the extract of the bark of the Mediterranean pine tree (Pycnogenol®, Korphag, Geneva, Switzerland) reduce the damage caused by IL-6 to the oocytes and to the Fallopian tubes, through the inhibition of the NF-κB [37], of the cyclooxygenases 1 and 2 (COX 1&2), and of the 5-lipoxygenase [38]. Both EPA, given as fish oil [39], and Pycnogenol® were shown to be beneficial for infertile female patients.

The oxidative/anti-oxidant balance can be estimated in blood by measuring the lagtime before extracted LDL-cholesterol is oxidised when exposed to copper. The lag-time was on an average 25% shorter in blood infertile men than in fertile controls, indicating that there was oxidative overload in the former.

Ovulatory disturbances include the polycystic ovary syndrome (PCOS), stress induced hypothalamo-pituitary deregulation, and obesity. Treatment commonly applies the anti-estrogen Clomiphene citrate and gonadotropins, with NNT of 6 [27]. Methods to correct obesity, to restore normal insulin interaction with its receptor (Metformine chloride), and to reduce stress may also be useful. Among the latter phyto-adaptogens and acupuncture [28] have been claimed successful.

In endometriosis endometrial tissue is present outside of the uterus, which is accompanied by oxidative overload and high concentrations of inflammatory cytokines, in particular Interleukin-6 (IL-6) [29]. The diagnosis requires laparoscopy during which surgery can remove the pathological foci, but inflammation may persist and tissue adhesions may disturb the normal function of the Fallopian tubes. The NNT in cases of mild to moderate endometriosis observed in the Canadian randomised trial [30] equals 9, but no effect was recorded in another trial [31].

Pelvic Inflammatory disease (PID) is another common cause of female infertility. It causes important oxidative overload [32] as well as elevated concentrations of IL-1 and IL-6. The diagnosis is made by laparoscopy and adhesions may be removed by laparoscopic surgery. However, persisting inflammation exerts a deleterious effect on the oocyte membrane, among other factors by activating the rapid reaction mechanism to inflammation, namely the Nuclear Factor kappaB (NF-κB) [29].

**Mechanisms of Sperm Dysfunction and Female Infertility and their Implication for Treatment with Nutraceutical Food Supplementation**

The membrane of normal spermatozoa contains a high concentration of the longchain poly-unsaturated fatty acid (PUFA) of the omega-3 group, docosahexaenoid acid (DHA; 22:6\_3; also called cervonic acid) [33]. This procures a high fluidity to the membrane's phospholipids. The cell membrane of poor-quality spermatozoa of patients with varicocele, male accessory gland infection, or idiopathic infertility, contains less DHA, and displays lower fluidity. This is due to inadequate nutritional intake of omega-3 fatty acids, and to higher oxidative load registered among infertile men as compared to fertile controls. Consequently, the capacity of spermatozoa to undergo induced acrosome reaction [33] and to fuse with the oocyte is diminished, explaining their poor fertilizing potential.

Sperm concentration and motility were inversely related to the nutritional intake of the long-chain PUFAs Eicosapentaenoic acid (EPA) and DHA, but positively correlated with the ingestion of the short-chain omega-3 PUFA alfa-linolenic acid (ALA; 18:3\_6). Testicular tissue contains an exceptionally high concentration of the enzymes elongase and desaturase [34], and mice that were “knocked out” for delta-6-desaturase were infertile with maturation arrest of spermatogenesis [35]. It seems logical to assume that the long-chain PUFAs cannot pass through the blood-testis barrier, whereas the short-chain ALA can do so. The latter is then metabolised into the long-chain PUFAs within the cells of Sertoli [36] thanks to the abundant elongase and desaturase.

The oxidative/anti-oxidant balance can be estimated in blood by measuring the lagtime before extracted LDL-cholesterol is oxidised when exposed to copper. The lag-time was on an average 25% shorter in blood infertile men than in fertile controls, indicating that there was oxidative overload in the former.

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 micro-channels to the flagellum where the protein called Dynemin contracts, inducing sperm movement. Similarly, the mitochondria in the oocytes are necessary for energy production, and during embryogenesis. The mitochondria produce ATP through the Krebs cycle using the long-chain PUFAs (EPA, DHA) as substrate. The latter are transported from the cytoplasm into the mitochondrial matrix through binding to acetyl-carnitine-CoA. Oxidative overload impairs mitochondrial function and reduces sperm motility, and it may disturb cell division during embryogenesis [41]. Complementary treatment with a strong anti-oxidant Astaxanthin, the carotenoid from the algae Hematococcus pluvialis, improves sperm motility [40,42], and so does the supplementation with L- (acetyl)-carnitine andPUFA. Since ATP production in the Krebs cycle generates 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-deoxyguanosine (8-OHdG) [43]. This procures a high fluidity to the membrane's phospholipids. The cell membrane of poor-quality spermatozoa of patients with varicocele, male accessory gland infection, or idiopathic infertility, contains less DHA, and displays lower fluidity. This is due to inadequate nutritional intake of omega-3 fatty acids, and to higher oxidative load registered among infertile men as compared to fertile controls. Consequently, the capacity of spermatozoa to undergo induced acrosome reaction [33] and to fuse with the oocyte is diminished, explaining their poor fertilizing potential.

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Therefore, providing an extra amount of ALA to infertile men seems indicated, which is given as linseed- or flaxseed-oil. Also, vitamin B6 and zinc should be added, since these are co-factors for the elongase and the desaturase enzymes. Eicosapentaenoid acid and the extract of the bark of the Mediterranean pine tree (Pycnogenol®, Korphag, Geneva, Switzerland) reduce the damage caused by IL-6 to the oocytes and to the Fallopian tubes, through the inhibition of the NF-κB [37], of the cyclooxygenases 1 and 2 (COX 1&2), and of the 5-lipoxygenase [38]. Both EPA, given as fish oil [39], and Pycnogenol® were shown to be beneficial for infertile female patients.

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Oxidative overload affects DNA integrity by oxidising guanine into 8-hydroxy-2-deoxyguanosine (8-OHdG) [43]. During cell replication, the latter will not bind to cytosine, which guanine normally should do, but to thymine, causing transition mutagenesis. Sperm of infertile men commonly contains a high concentration of 8-OH-2dG, which is dramatically reduced by oral anti-oxidant treatment (Figure 3) [42].

Stress is a common phenomenon among infertile couples, 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 p53 is inhibited
Fish oil, rich in EPA and DHA. Linseed oil, and women should take 2 pills per day together with 2 g of co-enzyme ubiquinone Q10 may improve embryogenesis, cytokinesis, and perhaps implantation.

Cytokinesis and embryo development

Cytokinesis taking place during the process of cell division and embryo development, requires the contraction of non-muscular Myosin II [47]. This contractile protein uses ATP as its source of energy, which is produced by the mitochondria. Excess reactive oxygen species in the infertile patients with endometriosis or PID, may reduce the production of ATP, causing inadequate function of the Myosin II, and impaired embryo development. Food supplementation during the post-conceptional weeks with the anti-oxidant Astaxanthin, long-chain PUFA as a source of energy to the mitochondria, carnitines, and the co-enzyme ubiquinone Q10 may improve embryogenesis, cytokinesis, and perhaps implantation.

Formulation of the nutraceutical Qualisperm® (Nutriphyt, Oostkamp, Belgium)

Based on the rationale mentioned above, a novel NFS has been created that is formulated [48] as follows (dose per day):

- Lepidium meyenii (MACA): 250 mg,
- Pycnogenol*: 100 mg,
- L-acetylcarnitine: 100 mg,
- Carnitine: 100 mg,
- Co-enzyme ubiquinone Q10: 25 mg,
- Astaxanthin: 8 mg,
- Zinc-chelate: 7.5 mg,
- Vit B6 (pyridoxine): 3 mg,
- Folic acid: 0.2 mg,
- Vit B12 (cyanocobalamine): 15 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.

Clinical trials of the nutraceutical food supplement

In an open-label prospective trial of the effect of 3 months of NFS with Qualisperm® to infertile men, sperm concentration increased by 60%, grade a motility was increased threefold, motile sperm concentration was multiplied by (median) 3.7, and morphology improved by 40% (Figure 4). The NNT calculated on the compiled data of 1053 couples included in a meta-analysis of double-blind trials with different types of antioxidants [49] and from our own clinic [22] was 8.

Since external factors exert synergistic amplification treatment should address both the causes and the mechanisms involved. This was done in a case-control trial comparing men treated for varicocele together with the NFS described above with matched untreated controls. The NNT within 3 months of treatment was 3.

The pregnancy rate per transfer obtained by IVF, complemented...
with ICSI whenever indicated, by the Fertility-Belgium centre has increased from 26% in 2006 (historical controls) to 35% in 2010 (Figure 5), by treating causal factors, and by adding NFS to the male partner (NNT = 8).

In a pilot 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 ongoing pregnancy rate was 45% in the treated couples as compared to 20% in the controls treated with folic acid only. In this trial the NNT for ongoing pregnancy was 4 [50].

Conclusion

Treatment of the infertile couple should start with the thorough investigation of both partners, the adequate treatment of all causal diseases, and the correction of unfavorable external factors that are associated with, or may cause the impaired reproductive capacity. In addition, efforts should be made to counteract the mechanisms involved with the malfunction of the gametes. It is suggested to include the use of a judiciously composed NFS.

By applying this strategy, remarkable results have been obtained in terms of increasing the probability of spontaneous conception, or downgrading the complexity of the required technique of assisted reproduction, e.g. from IVF to IUI, or from ICSI to simple IVF. Preliminary data suggest a trend for this approach to enhance the success rate of IVF.

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