

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

Frank H Comhaire<sup>1</sup> and Wim Decleer<sup>2</sup>

<sup>1</sup>Professor emeritus, Ghent University, Brakelmeersstraat, 18, B 9830 Sint Martens-Latem, Belgium <sup>2</sup>Director of Fertility-Belgium Clinic, Weststraat, 16-18, B 9880 Aalter, Belgium

#### 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 particularly useful for the clinical management of couple infertility, and it can be employed for the calculation of the cost/benefit ratio of different treatments.

## Definition, Testicular Physiology and Laboratory Investigation

#### Definition: The infertile couple

According to the definition of the World Health Organization [3] a couple is considered infertile if no spontaneous pregnancy has 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 the infertile couples,

in 65% a problem is present in the female partner, and in approximately 10% no obvious abnormalities are found. Hence, in one out of every 4 couples both the male and the female partner simultaneously present fertility-impairing pathology.

#### **Testicular Function and regulation**

Spermatogenesis takes place in the protected environment of the seminiferous tubules, where the haploid gametes are isolated from the rest of the body by the blood-testis barrier, created mostly by the cells of Sertoli. The optimal functioning of these cells depends on the adequate stimulation by follicle stimulating hormone (FSH) secreted by the pituitary, which is controlled through feedback by Inhibin B. The hormone secretion by the interstitial cells of Leydig submerges the seminiferous tubules in an extremely high concentration of testosterone and of its 5-alfa-reduced metabolite:  $5-\alpha$ -dihydrotestosterone. 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 the hypothalamic cells [4].

#### 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<sup>®</sup>, SCA<sup>®</sup>, or of a semiautomatic system [5] (Autosperm<sup>®</sup>). These permit the reproducible and precise measurement of sperm concentration, motility characteristics

\*Corresponding author: Frank H Comhaire, Professor emeritus, Ghent University, Brakelmeersstraat, 18, B 9830 Sint Martens-Latem, Belgium, E-mail: frank@comhaire.com

Received November 21, 2011; Accepted March 20, 2012; Published March 24, 2012

**Citation:** Comhaire FH, Decleer W (2012) The Benefit of Nutraceutical Food Supplementation and Antioxidants for the Treatment of the Infertile Couple and in Assisted Reproduction. Reproductive Sys Sexual Disord S1:004. doi:10.4172/2161-038X.S1-004

**Copyright:** © 2012 Comhaire FH, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

including curvelinear 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 oneway 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]. Hypoxemia 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.

Page 2 of 6

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 manmade 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 2:** Effects of Xeno-estrogens (XE) on the hypothalamo-pituitarytesticular axis of patients with idiopathic oligozoospermia. LHRH: Luteinizing Hormone Releasing Hormone; LH: Luteinizing Hormone; FSH: Follicle Stimulating Hormone.

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- $\kappa$ B) [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;  $22:6\omega3$ ; 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

Reproductive Sys Sexual Disord

[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 PUFA's Eicosapentaenoid acid (EPA) and DHA, but positively correlated with the ingestion of the short-chain omega-3 PUFA alfa-linolenic acid (ALA; 18:3 $\omega$ 3). 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 shortchain 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.

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<sup>\*</sup>, Korphag, Geneva, Switzerland) reduce the damage caused by IL-6 to the oocytes and to the Fallopian tubes, through the inhibition of the NF- $\kappa$ B [37], of the cycloxygenases 1 and 2 (COX 1&2), and of the 5-lipoxygenase [38]. Both EPA, given as fish oil [39], and Pycnogenol<sup>\*</sup> 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.

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 midpiece of the spermatozoa and generate adenosine triphosphate (ATP). ATP is transported via micro-channels to the flagellum where the protein called Dynein 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 and PUFA. 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-OH-2dG). 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 hypothalamopituitary deregulation. Also, stress exerts direct mutagenic effect on gametes, while the efficiency of the repair protein p53 is inhibited

Page 3 of 6

Citation: Comhaire FH, Decleer W (2012) The Benefit of Nutraceutical Food Supplementation and Antioxidants for the Treatment of the Infertile Couple and in Assisted Reproduction. Reproductive Sys Sexual Disord S1:004. doi:10.4172/2161-038X.S1-004

Page 4 of 6

[43]. The phyto-adaptogen extracted from Lepidium meyenii (MACA) activates the production of the stress suppressing heat-shock protein P70 [44], and can improve fertility in experimental animals [45]. Also, in men Lepidium meyenii was reported to improve sperm quality [46].

## 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, cytokenesis, and perhaps implantation.

# Formulation of the nutraceutical Qualisperm<sup>\*</sup> (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: 25mg,

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



**Figure 3:** The concentration of 8-hydroxy-2-deoxyguanosine (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 [42] (Courtesy Asian J Androl).



Figure 4: 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 5:** Percentage of ongoing pregnancies per transfer after IVF in the time period from the initiation of the ART programme in 1994 to 2010. The dotted line represents the average percentage of ongoing pregnancies in all Belgian ART centres. (1) Indicates the start of systematic investigation and treatment of all male partners of infertile couples before IVF. (2) Indicates the start of the complementary treatment with NFS of the male and the female partner before IVF.

with Qualisperm<sup>®</sup> 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

Page 5 of 6

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.

#### Aknowledgement

The authors thank Gabriël Devriendt M.Sci. (Nutriphyt Ltd, Belgium) for providing the material for the double-blind trials.

#### References

- Palermo G, Joris H, Devroey P, Van Steirteghem AC (1992) Pregnancies after Intracytoplasmic injection of single spermatozoon into an oocyte. Lancet 340: 17-18.
- Laupacis A, Sackett DL, Roberts RS (1988) An assessment of clinically useful measures of the consequences of treatment. N. Engl. J. Med. 318:1726-1733.
- Rowe PJ, Comhaire FH, Hargreave TB, Mahmoud AMA (2000) WHO manual for the standardized investigation, diagnosis and management of the infertile male. Cambridge University Press, Cambridge.
- Schill W-B, Comhaire F, Hargreave T (2006) Andrology for the Clinician. Publ. Springer-Verlag, Berlin, Germany (ISBN 3-540-23171-4): 288-291.
- Hinting A, Schoonjans F, Comhaire F (1988) Validation of a single-step procedure for the objective assessment of sperm motility characteristics. Int J Androl 11: 277-287.
- Schoonjans F, Depuydt C, Comhaire F (1996) Presentation of receiveroperating characteristic (ROC) plots. Clin Chem 42: 986-987.
- Gat Y, Zuckerman Z, Chakraborty J, Gorish M (2005) Varicocele, hypoxia and male infertility. Fluid Mechanics analysis of the impaired testicular venous drainage system. Hum Reprod 20:2614-2619.
- Comhaire F, Vermeulen A (1974) Varicocele sterility: cortisol and cathecholamines. Fertil Steril 25: 88-95.
- Comhaire F, Vermeulen A (1975) Plasma testosterone in patients with varicocele and sexual inadequacy. J Clin Endocrinol Metab 40: 824-829.
- Aitkin RJ, De Iuliis GN (2007) Origins and consequences of DNA damage in male germ cells. Reprod Biomed Online 14: 727-733.
- Everaert K, Mahmoud A, Depuydt C, Maeyaert M, Comhaire F (2003) Chronic prostatitis and male accessory gland infection - is there an impact on male infertility (diagnosis and therapy)? Andrologia 35: 325-330.
- 12. Comhaire F, Monteyne R, Kunnen M (1976) The value of scrotal thermography

as compared with selective retrograde venography of the internal spermatic vein for the diagnosis of "subclinical" varicocele. Fertil Steril 27: 694-698.

- Kunnen M (1981) Non-surgical cure of varicocele by embolization with isobutyl-2-cyano-acrylate. Ann Radiol (Paris) 24: 406-409.
- Krause W, Müller HH, Schäfer H, Weidner W (2002) Does treatment of varicocele improve male fertility? results of the "Deutsche Varikozelenstudie", a multicentre study of 14 collaborating centres. Andrologia 34:164- 171.
- Hargreave TB (1995) Debate on the pros and cons of varicocele treatment in favour of varicocele treatment. Hum Reprod 1:151-157.
- Baazeem A, Belzile E, Ciampi A, Dohle G, Jarvi K, et al. (2011) Varicocele and male factor infertility treatment: a new meta-analysis and review of the role of varicocele repair. Eur Urol 60: 796-808.
- Madgar J, Weissenberg R, Lunenfeld B, Karasik A, Goldwasser B (1995) Controlled trial of high spermatic vein ligation for varicocele in infertile men. Fertil Steril 63: 120-124.
- Nieschlag E, Hertle L, Fischedick A, Abshagen K, Behre HM (1998) Update on treatment of varicocele: counseling as effective as occlusion of the vena spermatica. Hum Reprod 13:2147-2150.
- Kremer J, Jager S, van Slochteren-Draaisma T, Kuiken J (1979) A simple laboratory test for the detection of antispermatozoa-antibodies in males and females. Ned Tijdschr Geneeskd 123: 438-441.
- Comhaire F (1987) Concentration of pefloxacine in split ejaculates of patients with chronic male accessory gland infection. J Urol 138: 828-830.
- Depuydt C, Zalata A, Christophe A, Mahmoud A, Comhaire F (1998) Mechanisms of sperm deficiency in male accessory gland infection. Andrologia 1: 29-33.
- Comhaire F, Decleer W (2011) Quantifying the effectiveness and cost-efficiency of food supplementation with antioxidants for male infertility. Reprod Biomed Online 23: 361-362.
- Zalata A, Christophe AB, Depuydt CE, Schoonjans F, Comhaire FH (1998) The fatty acid composition of phospholipids of spermatozoa from infertile patients. Mol Hum Reprod 4: 111-118.
- Eertmans F, Dhooge W, Stuyvaert S, Comhaire F (2003) Endocrine disruptors: effect on male fertility and screening tools for their assessment. Toxicol *In Vitro* 17: 515-524.
- Comhaire F (1976) Treatment of oligozoospermia with Tamoxifen. Int J Fertil 21:232-238.
- Adamopoulos DA, Pappa A, Billa E, Nicopoulos S, Koukkou E, et al. (2003) Effectiveness of combined tamoxifen citrate and testosterone undecanoate treatment in men with idiopathic oligozoospermia. Fertil Steril 80: 914-920.
- Beck JL, Boothroyd C, Proctor M, Farquhar C, Hughes E (2005) Oral anti-oestrogens and medical adjuncts for subfertility associated with anovulation. Cochrane Database Syst Rev CD002249.
- Huang DM, Huang GY, Lu FE, Stefan D, Andreas N, et al. (2011) Acupuncture for infertility: is it effective therapy? Chin J Integr Med 17: 386-395.
- Ponce C, Torres M, Galleguillos C, Sovino H, Boric MA, et al. (2009) Nuclear factor kappaB pathway and Interleukin-6 are affected in eutropic endometrium of women with endometriosis. Reproduction 137: 727-737.
- Marcoux S, Maheux R, Bérubé S (1997) Laparoscopic surgery in infertile women with minimal or mild endometriosis. Canadian Collaborative Group on Endometriosis. N Engl J Med 337: 217-222.
- 31. Parazzini F (1999) Ablation of lesions or no treatment in minimal-mild endometriosis in infertile women: a randomized trial. Gruppo Italiano per lo Studio dell'Endometriosi. Hum Reprod 14: 1332-1334.
- Mehendale SS, Kilari Bams AS, Deshmukh CS, Dhorepatil BS, Nimbargi VN, et al. (2009) Oxidative stress-mediated essential polyunsaturated fatty acid alterations in female infertility. Hum Fertil (Camb) 12: 28-33.
- Zalata A, Ahmed AH, Allamaneni SS, Comhaire F, Agarwal A (2004) Relationship between acrosin activity of human spermatozoa and oxidative stress. Asian J Androl 6:313-318.
- 34. Saether T, Tran TN, Rootwelt H, Christophersen BO, Haugen TB (2003) Expression and regulation of delta5-desaturase, delta6-esaturase, stearoyl-coenzyme A (CoA) desaturase 1, and stearoyl-CoA desaturase 2 in rat testis. Biol Reprod 69: 117-124.

Page 6 of 6

- 35. Stoffel W, Holz B, Jenke B, Binczek E, Günther RH, et al. (2008) Delta6desaturase (FADS2) deficiency unveils the role of omega3- and omega6polyunsaturated fatty acids. EMBO J 27: 2281-2292.
- Retterstöl K, Haugen TB, Tran TN, Christophersen BO (2001) Studies on the metabolism of essential fatty acids in isolated human testicular cells. Reproduction 121: 881-887.
- Peng Q, Wei Z, Lau BH (2000) Pycnogenol inhibits tumor necrosis factoralfa-induced nuclear factor kappa B activation and adhesion molecule expression in human vascular endothelial cells. Cell Mol Life Sci 57: 834-841.
- Canali R, Comitato R, Schonlau F, Virgili F (2009) The anti-inflammatory pharmacology of Pycnogenol in humans involves COX-2 and 5-LOX mRNA expression in leukocytes. Int Immunopharmacol 9: 1145-1149.
- Hammiche F, Vujkovic M, Wijburg W, de Vries JH, Maclon NS, et al. (2011) Increased preconception omega-3 polyunsaturated fatty acid intake improves embryo morphology. Fertil Steril 95: 1820-1823.
- 40. Comhaire FH, Christophe AB, Zalata AA, Dhooge WS, Mahmoud AM, et al. (2000) The effects of combined conventional treatment, oral antioxidants and essential fatty acids on sperm biology in subfertile men. Prostaglandins Leukot Essent Fatty Acids 63: 159-165.
- duPlessis SS, Makker K, Desai NR, Agarwal A (2008) Impact of oxidative stress on IVF. www.Expert Reviews.com ISSN 1747-4108: 539-554.
- 42. Comhaire FH, El Garem Y, Mahmoud A, Eertmans F, Schoonjans F (2005)

Combined conventional/antioxidant "Astaxanthin" treatment for male infertility: a double blind, randomized trial. Asian J Androl 7: 257-262.

- 43. Hara MR, Kovacs JJ, Whalen EJ, Rajagopal S, Strachan RT, et al. (2011) A stress response pathway regulates DNA damage through β2- adrenoreceptors and β-arrestin-1. Nature 477: 349-353.
- 44. Panossian A, Wikman G (2009) Evidence-based efficacy of adaptogens in fatigue, and molecular mechanisms related to their stress-protective activity. Curr Clin Pharmacol 4:198-219.
- 45. Ruiz-Luna AC, Salazar S, Aspajo NJ, Ribio J, Gasco M, et al. (2005) Lepidium meyenii (Maca) increased litter size in normal adult female mice. Reprod Biol Endocrinol 3: 16.
- Gonzales GF, Cordova A, Gonzales C, Chung A, Vega K, et al. (2001) Lepidium meyenii (Maca) improved semen parameters in adult men. Asian J Androl 3: 301-303.
- Murthy K, Wadsworth P (2005) Myosin-II-Dependent Localization and Dynamics of F-Actin during Cytokinesis. Curr Biol 15:724-731.
- Comhaire F (2010) The role of food supplementation in the treatment of the infertile couple and for assisted reproduction. Andrologia 42:331-340.
- Showell MG, Brown J, Yazdabi A, Stankiewicz MT, Hart RJ (2011) Antioxidants for male subfertility. Cochrane Data-base Syst Rev CD007411.
- Comhaire F, Decleer W (in press 2012) Comparing the effectiveness of infertility treatments by Numbers Needed to Treat (NNT). Andrologia.

This article was originally published in a special issue, **Reproduction & Developmental Biology** handled by Editor(s). Dr. Tin-Lap Lee, National Institutes of Health, USA