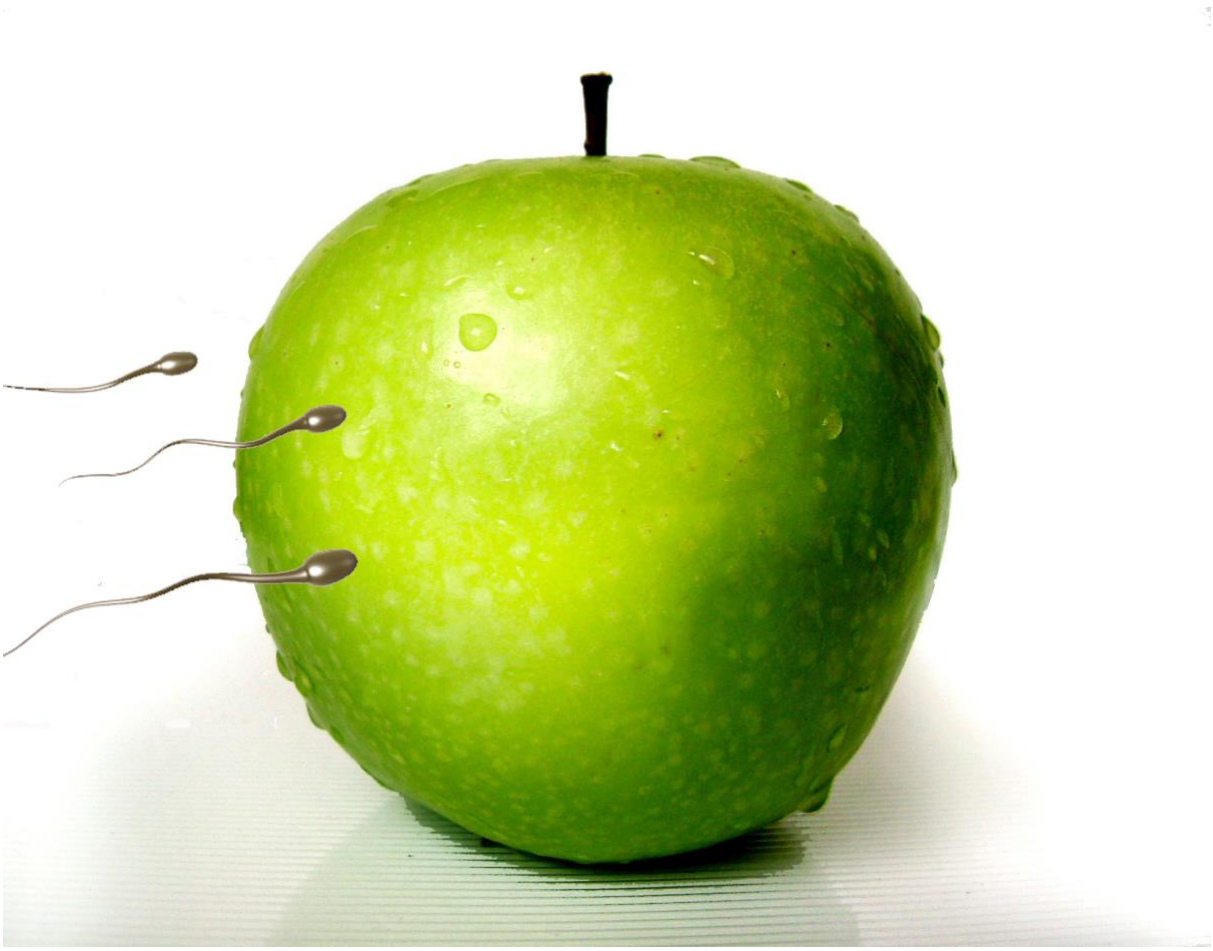
Diet and Semen Quality

A Review of Therapeutic and Detrimental Dietary Agents and Their Relationship with Human Semen Quality



Abstract

Despite a decline in sperm quality in the USA and Europe over the last 50 years, little research has been done investigating its possible causes and therapeutic options. With very few orthodox medical remedies available, apart from assisted reproduction technologies, men with idiopathic poor sperm quality are often left to treat themselves with a mixture of anecdotes, health-store food supplements and more or less useful advises. Considering that the food habits in the West have changed drastically over the last century, this may well be one of the factors needing investigation. Up to today, there is no scientific consensus regarding dietary factors to avoid or to increase for these patients, and this field of research has only recently started to expand.

From the evidence available, there are a few hopeful candidates that have been shown to improve sperm quality, but the majority of studies have been of less than ideal designs. This paper aims to provide an overview of the connection between human sperm quality and therapeutic agents from the plethora of food, herbs and dietary supplements. Detrimental factors are also briefly discussed, as are potential substances to conduct further research on. As research into the complex world of food micronutrients evolves, it may provide us with information that proves crucial for the future of human fertility.

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Tabea Guhl for the fantastic illustrations, having put my scattered thoughts into organised graphs.

Signed Declaration

I declare that this paper is my own production. The people stated above have helped with inputs and suggestions, but the main ideas and body of text is nothing but my own. I would also like to stress that English is not my mother tongue, and excuse myself in advance for any incorrect uses or incomprehensive sentences.

Table of Contents **Page Number**

List of abbreviations

AA	Amino Acids
AP-1	Activator Protein 1
ART	Assisted Reproductive Technology
BHA	Butylated hydroxyanisole
BMI	Body Mass Index
BTB	Blood-testis barrier
COX-2	Cyclooxygenase enzyme 2
DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic acid
FSH	Follicle stimulating hormone
HPA	Hypothalamic-Pituitary-Adrenal
HPG	Hypothalamic-Pituitary-Gonadal
ICSI	Intracytoplasmic sperm injection
iNOS	Inducible nitric oxide synthase
IVF	*In vitro* fertilisation
LH	Luteinizing hormone
MDA	Malondialdehyde
MRP1	Multidrug-resistance-associated protein 1
PG	Prostaglandin
PGE2	Prostaglandin E 2
PUFA	Polyunsaturated fatty acids
RCT	Randomized controlled trial
RNA	Ribonucleic acid
ROS	Reactive oxygen species
Se	Selenium
StAR	Steroid acute regulator protein
TCM	Traditional Chinese medicine
Zn	Zinc

List of Figures

Diet and semen quality

1. Background and Objectives

Despite being a common pathology, there have been relatively few investigations into the dietary associations with semen quality, or scientific assessments of the dietary supplements and herbal preparations available on the market. This paper aims to provide a critical overview of the evidence available for both dietary and herbal agents taken orally, and add some hints to substances that may prove valuable to do further research on.

2. Methodology

Material: Research papers and reviews from Medline, PubMed, Embase, Ovid, Web of Science and outsourced books and journals (see Appendix I) were searched for relevant data (for key words, see Appendix II). The individual papers dated between 1952-2010 in English, German and French were then scrutinized and included or excluded on their basis of content, research design and relevance.

Research inclusion criteria: The majority of studies included investigated human adult exposure to different agents, either from epidemiological data, clinical trials, *in vitro* studies or tissue examinations, with an emphasis on *in vivo*, human studies.
Due to the paucity of material available on the subject, certain animal studies have been included and denoted as such. Several agents had only one, poorly designed study conducted on them, and these were largely omitted.

3. Introduction

Male factor infertility is thought to be responsible for roughly half of conjugal infertilities, and ca 39 % of these men present with an abnormal semen analysis (Hassun Filho *et al* 2005). Carlsen and her team (1992) concluded that sperm quality has declined drastically the last 50 years, in line with several older studies (Nelson and Bunge 1974, Leto and Frensilli 1981, Bostofte *et al* 1983, Bendvold 1989) and meta-analyses (Swan *et al* 1997, Swan *et al* 2000). Several critical papers have refuted Carlsens statements, at least partly (Merzenich *et al* 2010, Fisch and Goluboff 1996), pointing to selection and measurement bias and natural variation. Considering that there will always be mistakes and natural variation in sperm quality and its assessments, what we can deduct from the possible trend is quite alarming. Worth mentioning is also that the lower reference point for “normal” sperm has been adjusted from 60 * 106/ml in 1945 (MacLeod and Heim 1945), to 20 *106/ml today (World Health Organisation 1999), and there have been undisputed increases in testicular cancer and cryptorchidism (Merzenich *et al* 2010, Menecksha and Fitzpatrick 2009), opening up for discussions of a common aetiology. Comhaire and Mahmoud (2006) and Joffe (2010) suggest that gene-environment interactions with intergenerational acceleration of genetic damage involving small duplications and deletions (D & D) to the germ cells may be one of the causes of deterioration in semen quality, especially if paired with exposure to certain toxins or hormonal disruptors - also found in our daily food.

The usefulness of standard sperm analysis has recently been questioned (Isidori *et al* 2005, McLachlan 2004) with the actual fertilizing capacity not necessarily correlating with sperm parameters. Reflecting this knowledge, an increasing amount of research is conducted on sperm DNA integrity rather than just sperm count and motility. Modern assisted reproductive technologies (ART), and especially intracytoplasmic sperm injection (ICSI), has shifted the need for increased sperm concentration to focus more on increasing the fertilisation potential of the sperm available (Isidori *et al* 2005, Liu and Baker 2002). ICSI is, however, linked to increases in genetic defects and congenital malformations (Edwards and Ludwis 2003, Van der Ven *et al* 1998, Koudstall *et al* 2000, Kent-First *et al* 1996, Hansen *et al* 2002, Green 2004, Wennerholm 2000), as well as

malignant tumours (Moll *et al* 2003, Maher *et al* 2003, DeBaun *et al* 2003) and defective development (Stromberg *et al* 2002, Pinborg *et al* 2004) of the offspring. There are also concerns regarding the ethical and economical (Comhaire 2000, Katz *et al* 2002), as well as pathophysiological (Schieve *et al* 2004, Lambert 2002) aspects of ART. Therefore, if sperm quality could be optimised with medical or dietary agents, this may well reduce the need for ART in the first place, or improve its results.

Studies investigating dietary factors in relations to poor semen quality are largely lacking. Due to the meagre choice of treatment options for infertile men, it is not surprising that alternative therapies, including herbal medicine and supplements, are commonly self-administered among the affected (Zini *et al* 2004). Apart from general health suggestions such as to stop smoking, loose weight if overweight, reduce the use of drugs and alcohol and factors increasing testicular temperature (reviewed in Anderson *et al* 2010 and Cabler *et al* 2010), what recommendations can be given to idiopathic subfertile or infertile men?

This paper aims to provide an overview of therapeutic agents, find a possible scientific consensus of the different approaches available, briefly discuss detrimental factors and present hints for future research.

4. Therapeutic Agents

4.1 Vitamins and Minerals

Vitamins and minerals are natural elements required in minute amounts by several physiological processes. Many of these act as antioxidants, a heterogenous group of substances that are able to counteract reactive oxygen species (ROS). Although small amounts of ROS are needed for the normal function of sperm, including capacitation and acrosome reaction (de Lamirande and Gagnon 1993, Zini *et al* 1995), excess ROS, either from pathological conditions, lifestyle or exposure to pollutants, is detrimental to sperm function and fertility (Tremellen 2008, Aitken 1999, Saleh and Agarwal 2002, Agarwal *et al* 2006). ROS may not only damage the sperm membrane leading to defective fertilisation, but also to impair both sperm motility and viability (Aitken 1995, Tremellen 2008) and increase DNA fragmentation (Tremellen 2008), thus reducing the chances of a healthy pregnancy and offspring. ROS may also lead to inappropriate stimulation of inhibin B secretion from the Sertoli cells, and thereby negatively affect spermatogenesis (Comhaire and Mahmoud 2006). Attenuation of detrimental processes or agents is another role of antioxidants - vitamins C and E, zinc and selenium, for example, have all been found to prevent cadmium-induced testicular damage (Hu *et al* 2004, Kara *et al* 2007, Acharya *et al* 2008, Amara *et al* 2008, Burukoglu and Baycu 2008).

Although seminal plasma contains several antioxidants to counteract intrinsic ROS activity (Garrido *et al* 2004), trials with oral antioxidants have yielded varied, and sometimes contradictory, results. These discrepancies may be due to the large variation in study designs and patient selection (Tremellen 2008, Agarwal *et al* 2004), and, although conventional sperm parameters do not change, there might still be longer-term positive influences on DNA integrity. Men with evidence of DNA damage that underwent antioxidant therapy showed a reduced incidence of early pregnancy loss in a small study (Gil-Villa *et al* 2009), indicating that there may be other benefits of antioxidants and trace elements than what is visible through the microscopic lens.

4.1.1 Folate

Folate has an important role in female fertility and healthy offspring, but its role in male fertility is less understood. As folate is an important substance for DNA synthesis and repair, and a simultaneously water-and lipid-soluble antioxidant alike (Joshi *et al* 2001, Ebisch *et al* 2006), it is thought to influence germ cell development and to protect the sperm membrane from ROS. Investigating the relationship between antioxidants (vitamin C, E and beta-carotene), zinc and folate intake on sperm aneuploidy, an inverse relationship between folate and sperm aneuploidy has been found (Young *et al* 2008). The study, however, used a self-reported questionnaire, which may be open to bias, and it is also possible that people with higher intake of folate-rich foods lead a generally healthier lifestyle and diet. Another study did not discover any significant difference in seminal plasma folate levels among 44 infertile and 176 fertile men (Chen *et al* 2001a). A larger, observational study, came to the same results, but saw an inverse correlation between DNA fragmentation and seminal plasma folate levels in fertile men (Boxmeer *et al* 2009). The lack of correlation in the infertile group may be due to that other, more severe pathologies caused the infertility rather than folate deficiency, or that seminal plasma folate levels do not correlate with semen quality– which does not neccessarily mean that supplementation is of no use.

Supplementation with folate (folic acid) has yielded various results. Treatment with 10 mg for 30 days increased plasma and seminal fluid folate levels but had no effect on sperm concentration, motility or DNA content (Landau *et al* 1978), although the treatment period may have been too short to notice any changes in sperm quality. Infertile men with round-cell idiopathic syndrome had increases in sperm number and motility after 3 months supplementation with 15 mg folinic acid/day (Bentivoglio *et al* 1993), possibly via increasing the seminiferous tube cellular cohesion and thus prevent a premature release of immature germ cells (Forges *et al* 2007). Due to the poor study design without blinding or placebo, hovewer, these results are not of major scientific value. Two double-blind, randomised placebo-controlled trials (RCTs) with infertile men noticed significant increases in normal sperm concentration after supplementation with zinc and folic acid (Ebisch *et al* 2006, Wong *et al* 2002),

given as a combination because zinc deficiency impairs folate intestinal absorption (Tamura and Kaiser 1991, Favier *et al* 1993, Wong *et al* 2002). Folic acid alone, however, yielded less impressive results compared to the combination treatment (Ebisch *et al* 2006).

None of these studies looked at the pre-treatment levels of homocysteine[1], which could have been interesting, and all the studies referred to applied dosages higher than the Recommended Daily Allowance (RDA) standards (400 micrograms/day for folic acid), even exceeding the upper limit of safety set to 1 mg/day (McNulty 1995). Although mainly water soluble, excess folate and other antioxidants alike may have counterproductive effects, which will be seen below. To sum up, there is not enough good evidence in favour of folate treatment to improve male fertility.

4.1.2 Selenium

Selenium is a mineral and essential component of many metabolic pathways fundamental to human health. These include thyroid activity, antioxidant defences, detoxification of heavy metals, immune function, synthesis of DNA and RNA, and male reproduction (Brown and Arthur 2001, Oguntibeju *et al* 2009). Body levels depend on dietary selenium that varies with the soil concentration (Dodig and Cepelak 2004). Selenium makes up several so-called selenoproteins, including the glutathione peroxidase enzymes (GSHPXs) that are vital to antioxidant processes, spermatogenesis and sperm function (Shalini and Bansal 2005, Brown and Arthur 2001, Pfeifer *et al* 2001). GSHPXs are actively expressed in spermatids, the mid-piece mitochondrial capsule and in mature sperm (Oguntibejo *et al* 2009). In animal models, absence of selenium impairs sperm motility and eventually leads to testicular degeneration (Oguntibeju *et al* 2009, Roveri *et al* 2001, Foresta *et al* 2002). Selenium also acts anti-inflammatory via affecting the genetic machinery (Ginn-pease and Whisler 1998, Handel *et al* 1995, Rahman and MacNee 2000), which makes it an interesting substance to research in regards to inflammation-related testicular pathologies.

[1] Homocysteine is an amino acid, biosynthesised from methionine, which is related to cardiovascular health impairment. Folic acid, vitamins B6 and B12 may reduce its levels (Lonn *et al* 2006)

Selenium levels and their relationship with sperm quality is a matter of debate. In patients with varicocele, a correlation between seminal plasma selenium and sperm count, motility and normal morphology has been noted (Camejo *et al* 2011). The levels of rescued GHSPX[2] activity has been reported to be significantly lower in infertile men, and particularly low in oligoasthenoozospermic ejaculates (Foresta *et al* 2002). On the contrary, Roy *et al* (1990) found no relationship between seminal plasma selenium and sperm count or motility.

Another pattern has been observed in Nigerian men: the azoospermic cases had significantly higher levels of seminal plasma selenium than the controls and oligospermic group, but had lower serum selenium levels. The ratio of serum selenium to seminal plasma selenium was 4:1 in oligospermic and 1: 2 in azoospermic subjects, whereas the fertile controls had an approximated 1:1 ratio. A significant inverse correlation was observed between serum selenium level and sperm count, and seminal plasma selenium correlated with motility, morphology and viability of the sperm (Akinloye *et al* 2005). This points towards that a possible derangement in the uptake, storage or metabolism of selenium is associated with fertility problems, rather than a deficiency or excess as such.

In an interesting cohort study by Bleau *et al* (1984), semen selenium was positively correlated to sperm count. Regarding motility there was an optimal window: between 50-69 ng/ml. Lower selenium levels were associated with lower pregnancy rates, whereas high-end levels were linked to higher miscarriage rates, pointing out the risks with over-supplementation. Similarly, in a murine experiment, both the selenium excess- and deficient group exhibited reduced fertility, germ cell number and differentiation and increased oxidative stress markers (Shalini and Bansal 2005).

Selenium-supplementation has yielded various results. In a non-controlled trial, selenium and vitamin E given to nine oligoasthenoteratozoospermic men lead to marked improvements in all sperm parameters (Vezina *et al* 1996), but the study size and lack of placebo are major drawbacks. A double-blind RCT administering selenium, or selenium together with vitamin A, C and E, resulted in significantly improved motility in men with selenium deficient diets. Only 56% of the patients “responded” to treatment by showing changes in serum selenium (Scott *et al* 1998).

[2] An selenium-containing antioxidant that protects membrane lipids against peroxidation (Brigelius-Flohé 1999)

Seminal plasma levels were not assessed, leaving it open if selective selenium accumulation in the reproductive glands could have occurred. It is also possible that this mineral acts intracellularly – with the seminal or serum levels not reflecting its actual biological activity.

In a randomised, open study, selenium and vitamin E decreased sperm malondialdehyde (MDA)[3] concentration and improved motility (Keskes-Ammar *et al* 2003). This was, however, based only on the 20 patients that returned for control, and the study used B-vitamins as "placebo" – possibly altering sperm characteristics as well.

Iwanier and Zachara (1995) conducted an open study on selenite and selenium-rich yeast given to subfertile men for 12 weeks. The yeast group saw significantly improved GSHPX activity whereas no effect was seen in the selenite group, and in both groups, no effect on any sperm parameter was seen. The same lack of sperm improvement was repeated in an RCT on healthy men with high-dose supplementation for 48 weeks (Hawkes *et al* 2009). This may, however, have been due to patient selection, or inappropriately high dosage (200-300 micrograms/day, RDA is 40-55 micrograms/day). Too long a treatment may also have lead to pro-oxidative effects, especially since these men had no evidence of selenium deficiency in the first place.

It seems that both supraphysical and deficient states of selenium are equally detrimental to fertility and supplementation must therefore be handled with caution - avoiding excessive doses and considering the initial selenium status. More research is also needed to investigate the fate of different types of oral selenium and its biodynamics in the testis.

4.1.3 Zinc

Zinc is a trace element found in seminal plasma where it is important for scavenging ROS (Colagar *et al* 2009), repairing damaged DNA, and regulating transcription and translation (Ebisch *et al* 2007, Ho and Ames 2002). The prostate gland and sperm themselves contain large amounts of zinc (Zaichick *et al* 1997), and zinc deficiency in animal models is linked to oxidative damage of DNA, lipids

[3] An important, highly toxic, product of polyunsaturated fatty acid peroxidation used as a marker for lipid peroxidation (Del Rio *et al* 2005).

and proteins (Oteiza *et al* 1995), suggesting that zinc has a positive effect on male fertility.

An analysis of 72 fertile and infertile men saw a significant positive correlation between seminal fluid zinc levels and sperm count and morphology. Smokers tended to have lower levels of zinc than non-smokers, and fertile men had significantly higher levels ($P< 0.001$) (Colagar *et al* 2009). Several studies have confirmed a positive correlation between serum or seminal zinc levels and various sperm parameters or fertility (Caldamone *et al* 1979, Marmar *et al* 1975, Mankad *et al* 2006, Chia *et al* 2000, Zhao and Xiong 2005, Camejo *et al* 2011, Ebisch *et al* 2006), whereas others found no significant differences in zinc levels between fertile and infertile men, or with various aspects of sperm quality (Fuse *et al* 1999, Carpino *et al* 1998, Wong *et al* 2001, Bakalczuk *et al* 1994, Lin *et al* 2000). Carpino *et al* (1998) presented an interesting thesis in their study, suggesting that increased amounts of unbound zinc decreases fertility due to excessive accumulation of zinc ions in spermatozoa, likely affected by sperm membrane dysfunction. The proportions of bound and unbound zinc change at ejaculation, and the authors claim that the only valid measurement is the amount of bound zinc in the ejaculate (Carpino *et al* 1998). Indeed, in this study there was no difference in seminal plasma total zinc content in the fertile and infertile groups, but subfertile men had significantly higher levels of unbound zinc in their ejaculate compared to healthy controls. This is in line with *in vitro* inhibition of motility with zinc added to the medium (Lindholmer 1974, Riffo *et al* 1992), and with one study in which the zinc content of seminal plasma was negatively correlated with motility (Danscher *et al* 1978). It therefore, again, seems as though the actual levels of a substance may not be as important as its bioavailability and metabolism. Also, if different measurements are used, the results are likely to be anharmonius.

Supplementation with zinc in a small, double-blind RCT yielded significant increases in sperm concentration in subfertile men, with no effect in fertile (Ebisch *et al* 2006). Interestingly, the blood levels of zinc did not change with the intervention, possibly due to the same mechanisms as for selenium, or due to a lack of deficiency.

Folate and zinc combination treatment lead to significant increases in sperm count in subfertile men (Wong *et al* 2002), and smaller increases were observed with zinc alone in both subfertile and fertile men.

Worth noticing is that zinc alone lead to slight reductions in motility, and that there were, again, no changes in serum zinc levels after therapy.

Zinc alone, and with other vitamins, lead to significant improvements in motility, fertilising capacity, and reductions in DNA fragmentation and oxidative stress markers in men with asthenozoospermia (Omu *et al* 2008). Neither healthy controls nor a placebo-group were included, and the study was very small, making it difficult to draw any conclusions.

On the whole, there are vague suggestions that point towards benefits of zinc therapy, but this must be backed up by both biodynamics- and clinical studies. The repeated detrimental effect on motility may be linked to changes in zinc form after ejaculation, and warrants further research. It may also be that the effect of zinc depends on the integrity of the spem membrane, so combination treatments are possibly more efficient.

4.1.4 Vitamin C

Vitamin C is not only a common antioxidant found in fruits and vegetables, but is also found in seminal plasma at very high concentrations (Jacob *et al* 1992). A positive correlation has been found between seminal plasma ascorbate and normal sperm morphology (Thiele *et al* 1995) and other sperm parameters (Ebenesunun *et al* 2004). In addition, azoospermic semen samples with evidence of ROS have been linked to lower ascorbic acid levels (Lewis *et al* 1997).

A small, placebo controlled trial demonstrated a positive effect on sperm quality supplementing with 1000 mg/day for one month (Dawson *et al* 1992), and in 10 patients maintained on an ascorbic acid controlled diet, the low-vitamin C-group had significant reductions in DNA integrity (Fraga *et al* 1991). A high-dose (2g/day), open trial with 14 oligospermic men resulted in significant improvements in motility and morphology and higher sperm concentration after two months treatment (Akmal *et al* 2006). This study lacked both control and placebo groups, and all the above studies were low-powered, indicating the need for further, larger studies.

High-dose supplementation with vitamins C and E or placebo showed no changes in sperm quality, neither in the fertile, nor in the asthenozoospermic group (Rolf *et al* 1999). The lack of results may have been due to a short treatment period (56 days), that it was conducted on a population without evidence of oxidative stress, or using a too high dose.

Another, placebo-controlled trial of vitamin C and E supplementation for two months, although producing no changes to standard semen parameters, lead to marked reductions in DNA fragmentation (Greco *et al* 2005a). The same regime, albeit without a placebo-group, produced improvements in implantation and clinical pregnancy rates with ICSI after treatment (Greco *et al* 2001b). These results suggest that although semen parameters may not change, there might still be a clinical benefit of supplementation with vitamin C, but if these three studies would have yielded different results using vitamin C alone is an open question.

4.1.5 Vitamin E

Vitamin E consists of a group of tocopherols, lipophilic antioxidants that protect PUFAs against oxidative stress (Buettner 1993) with their main action in the cell membrane (Ford and Whittington 1998). Alpha-tocopherol is also an important local antioxidant in seminal plasma (Bolle *et al* 2002) and the concentration of this antioxidant in spermatozoa has been related to the percentage of motile sperm (Therond *et al* 1996).

In a placebo-controlled study by Suleiman *et al* (1996) oral vitamin E improved spontaneous pregnancy rates and sperm motility, and reduced MDA levels. A reduction in MDA levels and DNA fragmentation was confirmed by Greco and his co-workers (2005) after supplementing with vitamins C and E with a similar study design. Fertilising capacity measured by the zona binding test was significantly improved by oral vitamin E in a cross-over RCT on men with evidence of excess seminal plasma ROS (Kessopoulou *et al* 1995), and an open study on normospermic men with previous IVF failure supplementing with 200 mg vitamin E/day lead to significant reductions in MDA levels and improvements in fertilisation rates per cycle after 1 month treatment (Geva *et al* 1996). On the contrary, a high-dose vitamin C and E treatment presented no significant changes to any semen parameter (Rolf *et al* 1999), neither did 600, 800 and 1200 mg tocopherol/day for three weeks (Moilanen and Hovatta 1995). As vitamin E excess may also have pro-oxidative effects (Bolle *et al* 2002), the dosage and initial antioxidant status may also have influenced the results.

If oral supplementation with vitamin E increases its levels in seminal plasma has both been confirmed (Moilanen and Hovatta 1993) and disputed (Whittington 1997, Kessopoulou *et al* 1995).

The seminal plasma levels, however, do not seem to be related to the sperm membrane concentration (Therond *et al* 1996), so it is possible that the positive effects of vitamin E supplementation are independent from its seminal plasma concentrations. Although it is questionable if conventional sperm parameters improve by oral vitamin E treatment, it seems to improve the chances of conception via other mechanisms.

4.1.6 Mixed compounds

Mixed compounds of trace elements are likely to affect fertility in a broader way than single-agent supplements. A multi-formula antioxidant treatment (Menevit) given during three months, showed no improvement in IVF/ICSI fertilisation rates, but produced increased pregnancy rates compared to placebo (Tremellen *et al* 2007). Menevit contains vitamins C, E, selenium, garlic and lycopene, all shown *in vitro* to neutralise free radicals (Agarwal 2004, Opara and Rockway 2006, Heber and Lu 2002, Prasad *et al* 1995, Chung 2006). There was also selenium, folate and zinc in the formula, protecting DNA from oxidative damage (Pfeifer *et al* 2001, Kvist *et al* 1987, Huang *et al* 1999). Vitamins C and E have also been shown to reduce sperm DNA fragmentation (Greco *et al* 2005), and may have accounted for the improvement in pregnancy rates. Sperm quality was not assessed and the trial was small for having pregnancy rates as an end-point.

In a trial with vitamins C, E and glutathione for 2 months, there were significant improvements in sperm count and a reduction in 8-hydroxy-2`-deoxyguanosine, a biomarker of oxidative stress (Kodama *et al* 1997), but the trial only included 14 cases and was without placebo controls. The effect of glutathione on the results cannot be substantial, as this agent has negligible oral bioavailability (Witschi *et al* 1992). Multiagent formulas that mimic the dietary composition of micronutrients offer a sounder option than the skewed overload of one substance, but do present us with difficulties regarding their scientific evaluation – which substance did what?

4.2 Proteins and Enzymes

4.2.1 Carnitine

Carnitine is a protein important for metabolism, intracellular fatty-acid transportation and mitochondrial oxidation, and is found at high concentrations in the epididymis (Stanley 2004, Agarwal and Said 2004). Both free and acetyl-L-carnitine also affect sperm maturation and motility (Jeulin and Lewin 1996), and act antioxidant (Agarwal and Said 2004).

The level of both free and total carnitine is undisputedly lower in infertile than fertile men (Li *et al* 2006, Gürbüz *et al* 2003, Zöpfgen *et al* 2000, Soufir *et al* 1984) and carnitine in seminal plasma has been correlated to both sperm concentration, morphology and motility (Menchini-Fabris *et al* 1984, Gürbüz *et al* 2003). Despite this, oral intake of carnitines only slightly increases the seminal plasma levels, so it is thought to act intracellularly or at the sperm membrane (Lenzi *et al* 2003).

A cross-over RCT on infertile men produced significant increases in motility and sperm concentration (Lenzi *et al* 2003), although the results became significant only after the exclusion of several outliers. The difference in motility was 11% in the treatment group and 8.8 % in the placebo group, which, due to sample size, reached the significance treshhold. Another RCT confirmed some positive effects, albeit not significant, on motility, particularly in the group with poorest initial values (Lenzi *et al* 2004). Based on the void of changes in morphology or concentration, this group concluded that the effect of carnitine is post-testicular. A similar trial confirmed increases in motility, especially in the patients with lowest baseline values, reductions in atypical forms, and found correlations between the kinetic improvements and increases in the radical scavenging capacity of the semen (Balercia *et al* 2005). All these studies, although being small, also showed positive impacts of carnitine on pregnancy rates.

One low-powered RCT showed no significant difference between the treatment and placebo- groups after 24 weeks carnitine treatment (Sigman *et al* 2006), but included only 21 patients. Interesting to note, however, was the linear improvements in motility with time in both groups, pointing to the efficacy of the placebo-effect - even on sperm quality.

Another theory was presented by Garolla *et al* (2005). They suggest that a certain level of GSHPx, related to the function of sperm mitochondria, is needed to achieve a beneficial effect of carnitine – if a mitochondrial dysfunction is present, carnitines will not improve motility. Although their results were based on only 30 patients, they do present an interesting theory of how carnitine supplementation may, or may not, work.

A meta-analysis including nine studies found significant improvements in pregnancy rate, motility and progressive motility without any changes in sperm concentration and semen volume with carnitine treatment (Zhou *et al* 2007).

A more recent, open study on men with various sperm defects supplementing with 1g of L-carnitine for 3 months revealed highly significant improvements in morphology and significant reductions in abnormal forms (Abd El-Baset *et al* 2010). In the fertile control group no such changes occurred, indicating that carnitine treatment may selectively “repair” sperm quality in cases requiring this. There was, however, no placebo group in this study.

These results are in favour of carnitine treatment, particularly to men suffering form poor sperm motility, but further placebo-controlled studies, including those with DNA integrity as an end-point, are required. Carnitine, however, seems to do no harm and can be considered a safe, possibly effective treatment to men in which motility is the major obstacle to natural conception.

4.2.2 Coenzyme Q10

Coenzyme Q10 (CQ-10) is an antioxidant highly concentrated in seminal fluid and, in its reduced form ubiquinol, in sperm mitochondria (Mancini *et al* 1994, Alleva *et al* 1995, Mancini *et al* 1998). This points towards a role in sperm motility and energy metabolism, as well as in protecting the sperm membrane from ROS damage (Lewin and Lavon 1997). CQ-10 may be reduced in both sperm cells and seminal fluid of men with idiopathic or varicocele-associated asthenozoospermia (Balercia *et al* 2002). The idiopathic cases had significantly lower levels of CQ-10, proposing that abnormalities with antioxidant pathways may be an important cause of idiopathic infertility.

Several studies have shown positive effects on sperm motility in infertile subjects after CQ-10 supplementation (Mancini *et al* 2005, Lewin and Lavon 1997, Safarinejad 2009, Balercia *et al* 2008). One open, small trial lead to increases in

seminal fluid and sperm CQ-10 levels, and noted a correlation with the latter and significant increases in motility (Mancini *et al* 2005). Despite the poor design of this trial, the direct relationship between kinetic features and CQ-10 concentration is interesting. Another study of similar design, CQ-10 supplementation increased fertilisation rates after ICSI, but produced no changes in sperm parameters (Lewin and Lavon 1997). The 17 men included had a history of ICSI failure, which may implicate other problems rather than those directly related to semen quality, and the observed increase in fertilisation rates can thus be seen as a promising observation – or as the magic of placebo.

A placebo-controlled RCT of 212 men with idiopathic oligoasthenoteratospermia presented significant increases in sperm density and motility, and positive correlation with the percentage normal sperm, and improvements in acrosome reaction after 300 mg CQ-10 daily for 26 weeks. In addition, serum FSH and LH levels decreased significantly (Safarinejad 2009). This group, however, only took the most significant improvement measurements and used that to compare with placebo - not pooling or using the end-stage values. As there is a large natural variation in individual motility scores, this may have influenced the results. The change in motility from baseline was, however, impressive - 30.7% versus 2% in the placebo group.

Another double-blind RCT for 6 months duration with 200 mg CQ-10 /day in men with idiopathic infertility produced marked increases in motility, especially in patients with lower baseline values (Balercia *et al* 2008), which is in line with several other studies, and indicates a good therapeutic potential of CQ-10 among this group of patients.

For a tabular overview, all studies above are summarised in appendix IV and ***Figure 2***.

4.3 Natural Antioxidants from Food

The discussion on antioxidants and trace elements is not complete without considering their natural source – our daily food. The modern, western diet with its emphasis on refined ingredients and ready-made meals may be a clue to the increasing fertility problems. Although preparation, storage, cultivation and ripening status affect the amounts of bioavailable antioxidants in food (Lester *et al* 2004), plant-based antioxidants have an undisputed positive effect on general health. Some well-researched sources include fruits and vegetables, seeds, wine, tea, coffee and common spices (Dimitrios 2006) (see Appendix V for details). Apart from the widely known vitamins, many plants also contain phenolic compounds, shown to possess higher antioxidant potential than Butylated hydroxyanisole (BHA)[4] and other synthetic antioxidants. Phenolic antioxidants do not only scavenge free radicals, but also act on cell signalling pathways and modulate numerous pathways counteracting inflammation (Williams *et al* 2004, Soobrattee *et al* 2005). Another interesting aspect of phenolics is their potentiating interaction with other antioxidants, for example augmenting the effect of vitamin C (Rice-Evans *et al* 1995). A combination of phenols and vitamins, as found naturally, may thus provide us with more antioxidants than expected. Phenolics contribute to the colour and aroma of food (Hagen *et al* 2007), so the recommendation to eat as many different colours and varieties of food as possible may be scientifically justified. However, the antioxidant potential of phenols are usually tested *in vitro*, and *in vivo* studies are required to assess their actual bioavailable potency after passage through the digestive tract, as is their effect on male fertility.

The sulphuric glucosinolates, found in cruciferous vegetables such as cabbage, broccoli and cauliflower represent other interesting fertility-modulating agents from the vegetable kingdom. The glucosinolates are metabolised to yield indole-3-arbinol and sulforaphane, antioxidants that stimulate endogenous radical scavenging enzymes, including glutathione S-transferases (Kelloff *et al* 1996). Glucosinolates have also been shown to protect against prostate cancer (reviewed in Kristal and Lampe 2002). Indole-3-carbinol also affects oestrogen metabolism, reducing the level of the estrogen metabolite C16α-hydroxyoesterone in animal and experimental models (Osborne 1999), and if the same occurs in men is worth

[4] BHA: a synthetic phenolic antioxidant (Hocman 1988)

investigating. The effect of natural plant constituents on human male fertility is very poorly researched -and may be fruitful as future research targets.

4.4 Herbal supplements

Most medicinal herbs not only contain antioxidants, but also other therapeutic constituents that may have an impact on reproduction. Several herbs have been used traditionally to improve male fertility and some of these are briefly discussed below. None of the following herbs have enough scientific evidence to back up their traditional use, but have been included due to relevance and scientific interest.

Ginseng is a common supplement to increase vigour and potency and to improve fertility. An open study on subfertile men and healthy controls showed that 4 g ginseng extract per day for 3 months increased sperm count, motility, total testosterone, free testosterone and dihydrotestosterone in all groups. Prolactin levels fell and gonadotrophin levels were either increased or decreased, depending on the initial status (Salvati *et al* 1996). In a phase I trial on healthy men with Kan Jang[5], valerian and ginseng, ginseng reduced the amount of spermatozoids with dyskinetic forms (Mkrtchyan *et al* 2005). Unfortunately, there were no placebo groups in any of the trials, so a strong placebo-effect cannot be ruled out.

The Peruvian food tuber *Leypidium meyenii*, also known as Maca, is a dietary supplement rich in proteins, unsaturated fats and minerals, apart from nine kinds of glucosinolates (Wang *et al* 2007). Its tradition as a fertility enhancer to both man and livestock is long, and modern research has shown positive effects on both sexual behaviour and spermatogenesis in men and rodents alike (Gonzales *et al* 2006, Gonzales *et al* 2002, Gonzales *et al* 2004, Gonzales *et al* 2003, Piacente *et al* 2002, Cicero *et al* 2001, Cicero *et al* 2002). In double-blind RCTs, maca has shown aphrodisiac effects (Gonzales *et al* 2002) and to improve sexual performance in men with mild erectile dysfunction (Zenico *et al* 2009). In an open study on 9 healthy men it increased sperm count and motility without affecting serum gonadotrophin or testosterone levels (Gonzales *et al* 2001). The lack of placebo group and blinding paired with a very low power makes this study more of

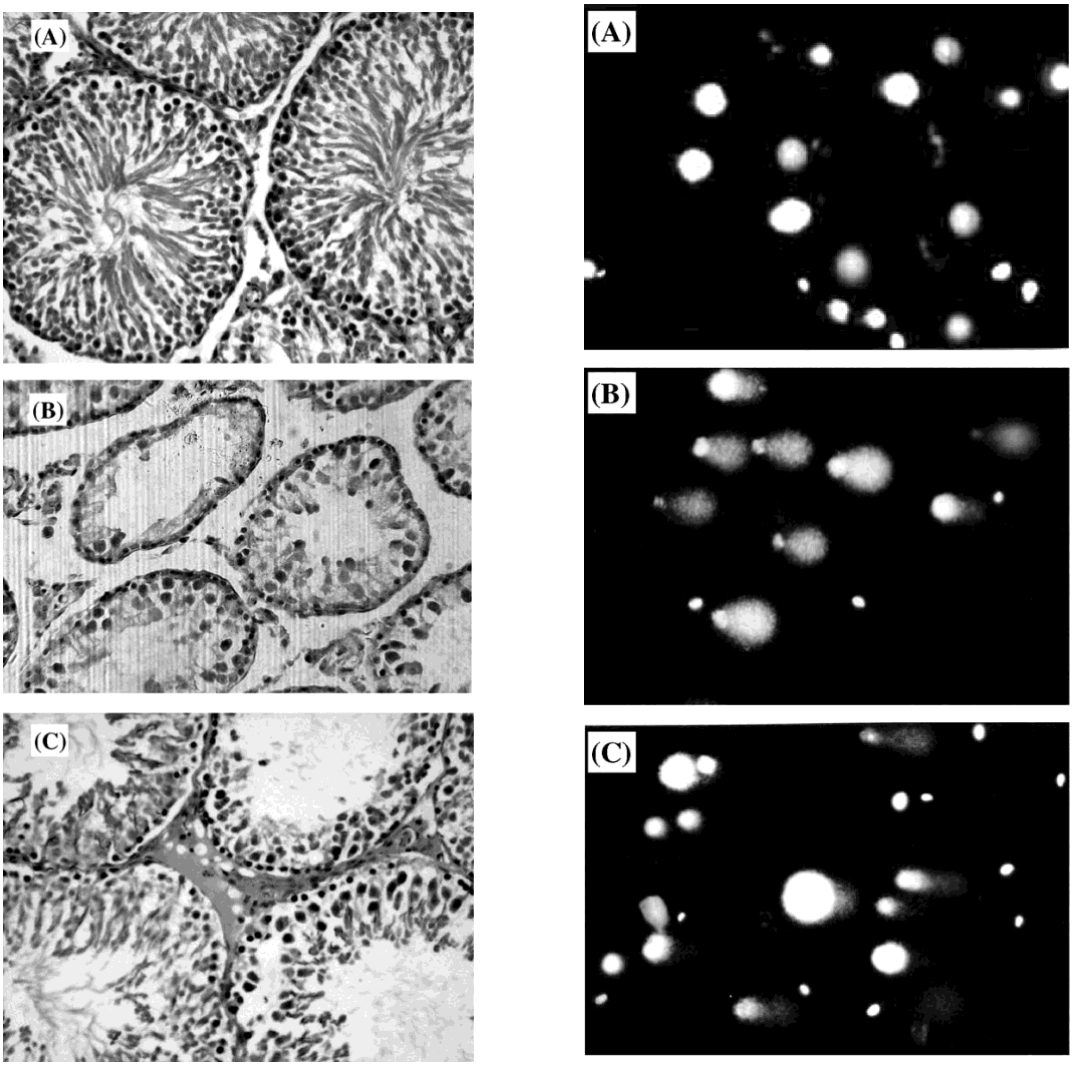
[5] Kan Jang is a standardised combination of *Andrographis paniculata* and *Eleuthrococcus senticosus*, two herbs with immunomodulatory properties (Panossian *et al* 2002).

a curiosity rather than scientific evidence. Maca is also an antioxidant, which may have contributed to its positive effect on spermatogenesis (Yucra *et al* 2007, Wang *et al* 2007). However, its historical use as a fertility-enhancer must be backed up with well-designed human research before any recommendations can be done.

Goji berries (*Lycium barbarum*) have an important position in the Chinese herbal pharmacopeia for various ailments including male infertility (Gao *et al* 2000). It is considered one of the strongest natural antioxidants available, and also contains vitamins, trace elements, carotenoids, flavonoids and immunomodulating polysaccharides (Wang *et al* 2010). Its antioxidant activity *in vivo* in humans has been confirmed in a double-blind RCT (Amagase *et al* 2009), as well as in numerous animal and *in vitro* studies (Ren *et al* 1995, Huang *et al* 2001, Kim *et al* 1999, Wu *et al* 2004, Wu *et al* 2006). In a murine model, the polysaccharides protected testicular tissue against hyperthermic and H_2O_2 damage (see ***Figure 1.*** below), increased superoxide dismutase activity[6], restored testosterone levels in damaged rat testis, as well as improving copulative function and sperm quantity and quality (Luo *et al* 2006). Interesting, the optimal dose seemed to be 10-50 mg/kg per day - a higher dose of polysaccharides did not improve the results.

[6] A marker of antioxidant activity (Amagase *et al* 2009)

Figure 1. *Effect of Gojiberry Polysaccharides on Murine Fertility*



Left side. Typical photomicrographs (× 200) of the seminiferous tubules of rat testis (H&E staining). (A) Normal control showing normal morphological features, with all the successive stages of spermatogenesis, and lumen filled with spermatozoa. (B) Negative control (43 °C heat-exposed) showing serious destruction and deterioration of the tubules in the testis, lumen is filled with cellular debris and is devoid of spermatids and sperms. (C) The administration of LBP (10 mg/kg·per day) after 43 °C heat-exposure showing partial recovery effect of LBP on the seminiferous tubules of the damaged testis.
Right side. Examples of comet photomicrographs (× 200) of mouse testicular cells. (A) Normal control (H_2O). (B) Negative control (H_2O_2). (C) LBP (50 μg/mL)+ H_2O_2.

(taken from Luo *et al* 2006)

With its plethora of highly concentrated trace elements and phytonutrients, goji berries seem like a promising candidate to conduct human fertility studies on.

Astaxanthin is a carotenoid substance produced by the algae *Haematococcus pluvialis* with a very strong antioxidant potential (Iwamoto *et al* 2000, Goto *et al* 2001). A double-blind RCT tested the effect of astaxanthin on subfertile men during 3 months, resulting in significant reductions in seminal ROS and serum inhibin B levels, improvements in straight line velocity, a slight positive effect on sperm morphology and motility, and highly significant difference in monthly pregnancy rates (10.5 % in the placebo- versus 54.5% in the treatment group) (Comhaire *et al* 2005). This group hypothesised that testicular ROS may stimulate excessive secretion of Inhibin B, and that astaxanthin may counteract this. The higher pregnancy rates in the treatment arm was thought to result from functional improvement of sperm, but, considering the low power, the pregnancies might have occurred due to chance alone.

The majority of herbs in Traditional Chinese Medicine (TCM) used to treat infertile men have shown anti-oestrogenic and antioxidant properties (Tempest *et al* 2008). Although low levels of oestrogens are required for gonadal function, at levels similar to women, this steroid may have negative effects on male fertility (Séralini and Moslemi 2001). Synthetic antioestrogens, such as tamoxifen or clomiphene citrate, are a treatment option for idiopathic subfertile men (Ghanem *et al* 2010). The glucosinolates, discussed above in the sections on cruciferous vegetables and maca, have shown anti-oestrogenic activity, as have *Peonia rubra* (radix), *Phellodendron amurense* (cortex), *Moutan radicis* (cortex) and *Varricariae pyaramidata* (semen) from the Chinese pharmacopoeia (Tempest *et al* 2008), none if which have been investigated in regards to male fertility.
Oestrogen levels may also be reduced by modulating aromatase, the enzyme that controls the androgen to estrogen ratio (Séralini and Moslemi 2001, Karaer *et al* 2004). In patients with subnormal levels of testosterone and excess of oestrogens, synthetic aromatase inhibitors may be a treatment option, albeit not much researched (Raman and Schlegel 2002). Natural aromatase inhibitors include flaxseeds (Adlerkreuz 2007, Adlerkreutz *et al* 1993, Wang *et al* 1994) and chrysin, a flavone in *Passiflora incarnata* (Pelissero *et al* 1996). Chrysin is also an antioxidant and anti-inflammatory agent (Cho *et al* 2004, Pushpavalli *et al* 2010), adding to its potential as a future research target.

Apigenin, a flavone found in Chamomile *spp.* (Jeong *et al* 1999), and the flavones myricetin, gossypetin and liquiritigenin from *Impatiens balsamina*, *Pyrrosa petiolosa* and *Dalbergia odorifera*, respectively (Paoletta *et al* 2008), have also shown significant aromatase inhibitory activity *in vitro*. Natural aromatase inhibitors against idiopathic male infertility are interesting but require more studies, both clinical and *in vivo*, as well as studies investigating their long-term effects.

Inflammation is another cause of male subfertility, and the pro-inflammatory prostaglandin E2 (PGE2), normally absent from semen, has been found in men with oligozoospermia (Mayerhofer *et al* 2002). Cyclooxygenase isoenzyme 2[7] (COX-2) drives pro-inflammatory pathways and can be inhibited by several herbs. Pycnogenol, for example, a product from the bark of *Pinus maritime*, inhibits this enzyme (Baumann *et al* 1980, Rohdewald 2002), and improved sperm morphology significantly in a small, open trial by Roseff and Gulati (1999), opening up for larger studies. For more anti-inflammatory herbs, see Appendix VI.
Although it is too early to recommend any of these herbs as a treatment against idiopathic male infertility, they may well be worth investigating further.

[7] An enzyme that converts arachidonic acid to proinflammatory prostaglandins (Morita 2002)

Figure 2. *Summary of Therapeutic Agents and their Effects*

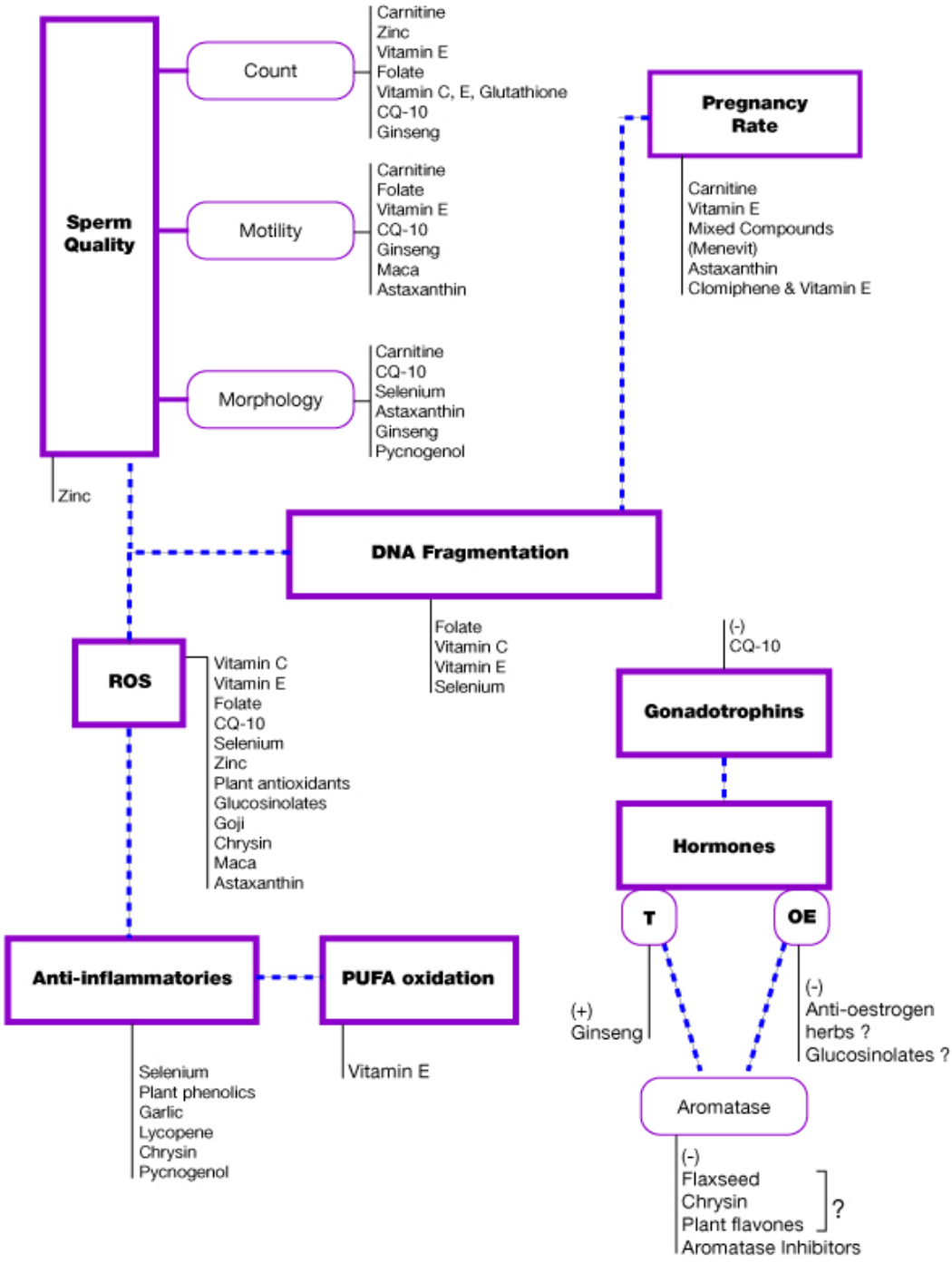


Figure 2. This picture summarises the studies above in a graphic way. It serves the purpose of giving an overview among all substances affecting semen quality from all types of human studies, but does not indicate actual treatment options, which will require more research to be fully evaluated.

------ :a relationship between the two factors.

(+): the agent increases the factor, (-): the agent decreases the factor.

? : there is uncertainty regarding the agent(s) in question, if they affect the factor *in vivo* or not.

PUFA: Polyunsaturated fatty acids. T: Testosterone OE: Oestrogen

(For detailed explanation: see Appendix III)

5. Detrimental Agents

Having discussed the therapeutics, let us briefly consider what dietary factors may be detrimental to semen quality. It is reasonable to believe that if large quantities of food that increase ROS generation or disrupt the fine-tuned endocrine balance are consumed, no fertility-boosting supplement in the world can live up to their promises. Due to space restrictions, this section is a very rough overview of the most investigated agents to reduce or avoid, and other authors have provided more extensive reviews.

Figure 3. *Summary of Detrimental Dietary Factors and their Mechanisms.*

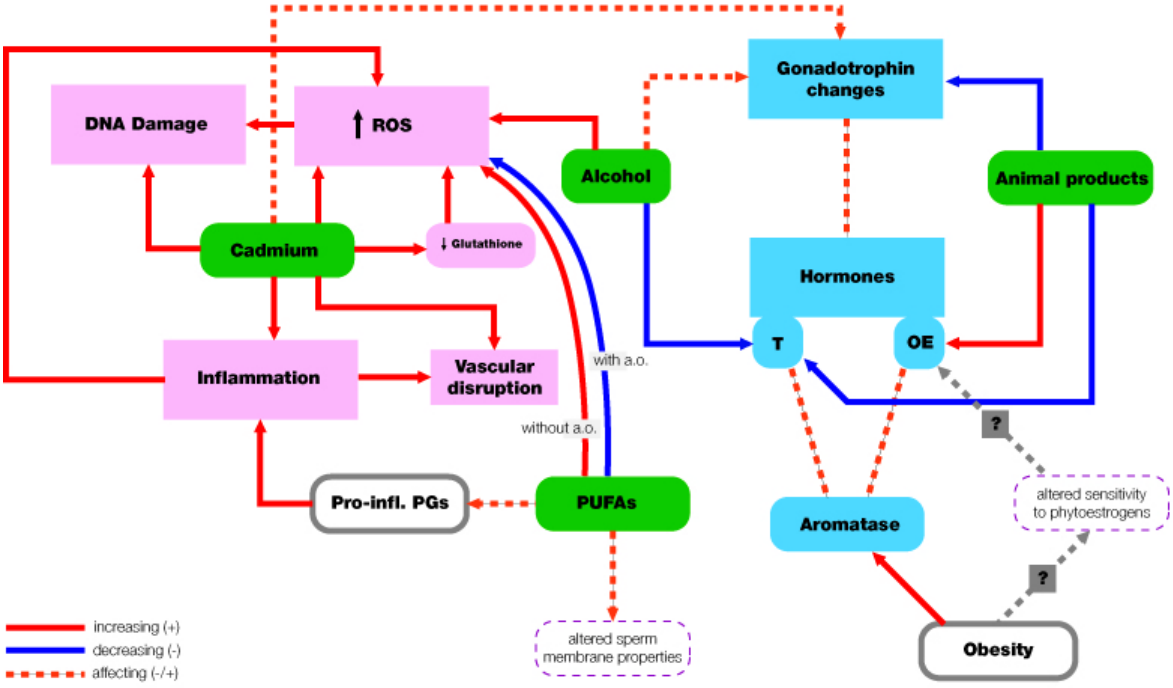


Figure 3. Cadmium, found mainly in offal, seafood and sunflower seeds (Franz *et al* 2008, Satarug *et al* 2009) is a heavy metal particularly detrimental to male fertility (Siu *et al* 2009, Benoff *et al* 2000). Cadmium increases vascular disruption via negative effects on E-cadherin (Prozialek *et al* 2008, Prozialek and Lamar 1999). It is also linked to hormonal disturbance and modification of gonadotrophins (Lafuente *et al* 2004, Gunnarson *et al* 2007, Laskey and Phelps 1991), as well as increasing testicular ROS (Hsu and Guo 02, Acharya *et al* 03) via depletion of glutathione (Valko *et al* 2005) and other antioxidant agents (Sen Gupta *et al* 2004). Increased DNA damage after exposure has also been observed (Xu *et al* 03, Naha and Chowdhury 06).

Alcohol increases the production of ROS and, in excess, disrupts the endocrine balance (Maneesh *et al* 2006), and has consistently been proven detrimental to male fertility (Gaur *et al* 2010, Pajarinen *et al* 1996, Gavaler *et al* 1983, Van Thiel and Gavaler 1982, Amory 2007, Mathusami and Shinnaswamy 2005, Donnelly *et al* 1999, Olsen *et al* 1997, Anderson *et al* 2010), thus reducing alcohol intake is a plausible recommendation to subfertile men.

Excess oestrogens have also been proposed to disturb male fertility (Malekinejad *et al* 2006, Andersson and Skakkebaek 1999). The effect of phytoestrogens[8] on male fertility is controversial - some studies have shown neutral or beneficial effects (Mitchell *et al* 2001, Song *et al* 2006), and others detrimental (Atanassova *et al* 2000, Fraser *et al* 2006, Gray *et al* 2006), especially in obese men (Chavarro *et al* 2008). Since adipose tissue has a high aromatase activity (Czajka-oraniec and Simpson 2010, Mahmoud et al 1998), this may also be a clue to the link between oestrogen excess and obesity, and there is no scientific consensus on whether to recommend or avoid phytoestrogen consumtion to subfertile men.

Animal products, especially diary products, have been linked to endocrine disruption, lowered testosterone and an excess of oestrogens (Heap and Hamon 1979, Pepe-Zambito *et al* 2008, Malekinejad *et al* 2006,Daxenberger *et al* 2001, Aksglaede *et al* 2006, Ganmaa and Sato 2005, Maruyama *et al* 2010, Andersson and Skakkebaek 1999). Men with higher intakes of fat and protein (generally from animal products) have also been shown to have poorer semen quality than those with a higher intake of vegetables, fruit and carbohydrate (Mendiola *et al* 2010).

The composition of polyunsaturated fats (PUFA) affects male fertility, especially regarding membrane fluidity and fertilising capacity (Wathes *et al* 2007, Christophe *et al* 1998, Lands 1992). The high content of PUFAs in sperm plasma membrane (Hurtado de Catalfo and de Gomez Dumm 2002, Cinti *et al* 1992, Saether *et al* 2003, Tran *et al* 2003) renders sperm particularly susceptible to ROS damage (Jones *et al* 1979, Alvarez and Storey 1995, Aitken *et al* 1989, Aitken *et al* 1993a, Aitken *et al* 1993b), and oxidative stress also seems to negatively affect the phospholipid content of the sperm membranes with a possible link to pro-inflammatory prostaglandins (Zalata *et al* 1998, Comhaire and Mahmoud 2006).

[8] Plant-derived compounds that bind oestrogen receptors (Andersson and Skakkebaek 1999)

Due to their sensitivity to ROS, an increased intake of PUFAs without adequate antioxidant (a.o in the picture) defences may therefore be detrimental to male fertility. To summarise, subfertile men can be recommended to avoid excessive amounts of cadmium-rich foods, alcohol and animal products, and protect oneself against the effects of these with an adequate antioxidant defence.

6. Summary and Conclusions

There is a lack of well-designed studies on dietary agents and semen quality, and this is reflected in the discrepant and sometimes contradictory results achieved from the available material. The variations in patient selection, treatment protocols and dosages, types of supplements used, presence or lack of placebo group, healthy controls and blinding, paired with limited power makes it difficult to draw any conclusions about many substances. Despite this, there are some recommendations that can be given to affected men.

Due to high levels of cadmium, offal and shellfish consumption should reduced and the same applies to alcohol, animal and dairy products, that may increase oxidative stress and disrupt the endocrine balance if taken in excess. There is reasonably sound evidence to suggest that carnitine- and CQ-10 supplementation may be beneficial, especially to patients with poor sperm motility and evidence of increased oxidative stress. Antioxidants in general are likely to decrease both ROS generation and DNA fragmentation, thus including these as a therapeutic target both for natural conception and IVF is feasible. However, antioxidants seem to follow the rule “too much of the good is less good” - if the initial levels of oxidative stress in the testis is low, or if the dosages are overestimated or self-medicated without medical supervision, the effect may be, at worst, counterproductive. For example, vitamins C, E, folate and selenium can act both pro-and antioxidative, indicating the importance to adhere to standard RDA doses unless otherwise instructed. It seems that the classic thought of homeostasis can be applied in this respect too – eating moderate amounts of many different things, or, in the case of supplements, taking a low-dose multivitamin instead of a single-agent mega dose seems to be a healthier option than using high doses of a single agent.

Subfertile men and medical personnel must be aware of the more or less appropriate treatments available, and point out the risks with mega-dose supplementation.

The antioxidant defence can also be improved by dietary changes and increasing the intake of herbs, fruits, vegetables and spices. To eat a colourful, varied diet may be the easiest way to obtain a broad variety of plant antioxidants and other phytonutrients with positive effects on both general health and spermatogenesis alike, albeit not well investigated in relationship to male fertility.

Summa summarum – go for a broad, RDA-dose approach to supplements with initial vitamin- and mineral status in mind, consider adding carnitine and CQ-10, and, vary, colour and spice up your everyday diet.

7. Discussion

Despite concerns about human fertility, little work has been done to evaluate the impact of food on it. Dietary manipulations are “prescribed” to counteract several other afflictions, but in regards to male fertility, there are still no recommendations widely available. Many studies on dietary supplements have solely used standard semen analysis as their method of assessment. Additional tests such as DNA fragmentation index and semen ROS levels may give a more complete picture, as is the use of several, pre-trial assessments of sperm quality to reduce the impact of natural variation. How the biodynamics of an element affects the results of supplementation and if and how that can be manipulated is another question. Taking selenium as an example, it seems that the actual seminal plasma levels are less important, but that intracellular uptake and metabolism may be altered in infertile men. If excessively high doses are administered, this may put an extra eliminatory stress on the organism, thus having a counterproductive effect. We also know too little about the blood-testis barrier, and if a dysfunction there stands in relationship to the effect of supplementation. Good fertility studies also require a large number of patients, time and funding - means that the greengrocers, dietary associations or supplement-producers are unlikely to have, and which may be one of the causes of the meagre research material.

In the quest for an agent against male infertility, it is easy to forget how each being is different, having a unique genetic makeup, lifestyle, reproductive pathologies, diet and reaction to supplements, making both the evaluation and treatment choice more complex and difficult to perform. Along the same lines, generalised treatments springing from a “one fits all”-mentality can be criticised.

The vast differences in results from the studies above cannot be interpreted without acknowledging that people may react differently to any treatment– an agent tested on the “wrong” population is unlikely to yield any significant results. Perhaps it is better to regard each patient as unique, evaluating his individual situation and history, and apply supplementation accordingly. Correcting a deficiency may be beneficial, but uniform supplementation less so. One must also not forget the natural availability of vitamins and trace elements. Vitamin C, for example, is found in most fresh foods, whereas selenium is almost absent from certain soils and may therefore be more important to supplement with. Or maybe it is safer to rely on the trace elements in our diet? Our daily food and how it is metabolised and utilised is a largely unknown territory. There is an enormous amount of different phytonutrients to investigate - lycopene, anthocyanidins, and green tea-catechins in addition to the herbs mentioned above are just some substances potentially affecting male fertility. The question is only; who is interested the funding?

The effect of broader dietary interventions also needs evaluation – the Mediterranean diet being one example that improves pregnancy rates in ART (Vujkovic *et al* 2010). Both epidemiological and intervention studies are needed for a more complete picture. Nevertheless, providing patients with some general knowledge that ignites self-evaluation of their diet and lifestyle could be a helpful aid towards better fertility.

Science is always looking for a magic bullet, the one and only factor that can alleviate the problem of investigation. The magic bullet sought to combat poor sperm quality is yet to be discovered. Scientific research aside, with the fascinating complexity of human reproduction in mind, perhaps we are more likely to be successful if a magic, individualised mixture paired with dietary changes is applied, rather than a bullet?

Word count: 6958

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Appendix I

Databases

AMED (Allied and Complementary Medicine), BIOSIS, Cochrane Collaboration, Medline, OVID, Pubmed, PubmedCentral, Science Direct, SpringerLink, Wiley Interscience,

Webpages

Mediherb.com, Mediwire.com, Medscape.com, Phytotherapies.org, Repro-med.net

Journals

Fertility and Sterility (1995-2010)

Human Reproduction (1990-2010)

Journal of Ethnopharmacology (1990-2008)

Journal of Nutrition (1998-2010)

Phytomedicine (1994-2008)

Proceedings of the New York Academy of Sciences (1995-2010)

Appendix II

Key Words Used for Database Searches

Animal products, anthocyanidins, aromastat, aromatase, assisted reproduction, astaxanthin, ayurvedic, brassicaceae, calcium, carnitine, catechins, chrysin, coenzyme Q10, constituents, crocus sativa, dairy, diet, dietary, enzymes, fats, fatty acids, fertility, FSH, food, foodstuff, fruits, ginseng, glucosinolates, glutathione, goji, goji berries, gonadotropins, green tea, herb, herbal, herbal medicine, hormonal, hormones, human, human spermatogenesis, human reproduction, ICSI, idiopathic male infertility, infertility, lepidium meyenii, LH, lycium barbarum, lycopene, maca, male, male infertility, male reproduction, meat, men, minerals, phenol, phenolic, phyto, phytoestrogen, phytotherapy, polyunsaturated fatty acids, selenium, semen, semen parameters, soil, sperm, spermatogenesis, sperm quality, subfertility, TCM, tomato, trace elements, transfatty acids, turnera diffusa, vegetables, vitamins, vitamin C, vitamin E, vitamin D, vitamin K, xenoestrogen, zinc.

Appendix III

Figure 2. This picture shows the relationship between various dietary substances and which parameter related to fertility that they improve. Broken lines again show that there is a relationship between the two factors. Next to sperm quality are the individual substances that have shown a positive effect on sperm count, motility and morphology. Under pregnancy rate are the substances that have shown positive effects on pregnancy rates listed. Under DNA fragmentation and to the right of ROS are the agents listed that have reduced ROS generation. Below anti-inflammatories are substances that have shown anti-inflammatory activity. Under PUFA oxidation are some agents that have been proven to reduce the oxidation of PUFAs. CQ-10 has been found to decrease the levels of gonadotrophins, ginseng to increase testosterone, and the anti-oestrogen herbs and glucosinolates to reduce oestrogen. The question mark next to glucosinolates here is due to that the studies have only involved animals and women, so the effect in men is not yet elucidated. Under aromatase are several agents that have shown aromatase inhibitory qualities in various *in vitro* tests, but if these act so *in vivo*, on men, is not yet known.

Figure 3. This picture tries to illustrate some negative dietary associations with poor semen quality. Red arrows indicate that the factors increases the other factor, the blue arrows show the opposite. Broken lines indicate a relationship between the two factors. For example, inflammation has been shown to increase the amount of ROS in the testis, pro-inflammatory prostaglandins (PGs) increase inflammation, and there is a relationship between PUFAs and pro-inflammatory PGs. PUFAs are also related to altered sperm membrane properties, but in a more uncertain way (therefore the broken circle around the latter). PUFAs without adequate antioxidant (a.o) defence increase ROS, whereas PUFAs with enough a.o decrease it. Cadmium increases both inflammation, vascular disruption, reduces glutathione and increases ROS generation and DNA damage. There is also a relationship between cadmium ingestion and changes in gonadotrophins, which then affects hormonal levels. The hormonal levels are also influenced by aromatase activity that is increased in obesity. Obesity has been suggested to increase the sensitivity to phytoestrogens and potentially to increase oestrogen levels (therefore the question mark), but this is still uncertain. Animal products have been shown to increase systemic oestrogen and decrease testosterone levels. Alcohol raises the amount of ROS and decreases testosterone, as well as changing the balance of gonadotrophins.

This picture is not aiming at providing a complete picture, but is a rough outline of different relationships that influence sperm quality.

Appendix IV.

Summary of research on different parameters of human male fertility after treatment with antioxidants, vitamins and minerals.

Agent	Effect	Treatment Protocol	Reference
Pregnancy rate			
Vitamins C & E	No change	56 days 1g vitamin C, 800mg vitamin E	Rolf *et al* 1999
Menevit	Significant improvement of pregnancy rates	3 months, multiformula	Tremellen *et al* 2007
Vitamin E	Increase in spontaneous pregnancy rate	6 months, 300 mg/day	Suleiman *et al* 1996
Carnitine	Improvement of pregnancy rate	Meta-analysis	Zhou *et al* 2007
DNA fragmentation			
Vitamin C & E	Reduction	2 months 1 g/day of vitamins C and E	Greco *et al* 2005
Vitamin C	Reduction	Dietary manipulation/various amounts of supplementation with vitamin C, 28-32 days.	Fraga *et al* 1991
Zinc, zinc & vitamins C/E	Reduction	3 months supplementation with zinc and/or vitamins C+/-E (400 mg Zn, 20 mg Vit E, 1 g Vit C/day)	Omu *et al* 2008
Sperm concentration			
Vitamin C & E	No significant change	2 months 1 g/day of vitamins C and E	Greco *et al* 2005
Vitamin C	Improvement	2 months, 2 g vitamin C/day	Akmal *et al* 2006
Vitamin C, E & glutathione	Increased sperm count slightly	2 months, 200 mg vitamins C & E, 400 mg glutathione	Kodama *et al* 1997
Vitamin E & selenium	Increased	6 months	Vezina *et al* 1996
Folic acid	Increased	10 mg daily for 30 days	Landau *et al* 1978
Folic acid	Increased	15 mg during 3 months	Bentivoglio *et al* 1993
Folic acid & zinc	Increased normal sperm count	26 weeks, 5 mg folic acid, 66 mg zinc sulphate	Ebisch *et al* 2006
Folic acid & zinc	Increased normal sperm count	26 weeks, 5 mg folic acid, 66 mg zinc sulphate	Wong *et al* 2002
Carnitine	No change	Meta-analysis	Zhou *et al* 2007
Carnitine	Inreased in oligozoospermic men	1 g/day during 3 months	Abd El-Baset *et al* 2010.
CQ-10	Increased	300 mg during 26 weeks	Safarinejad 2009
Selenium	No significant difference	3 months selenomethionine 100 µg/day	Scott *et al* 1998

Sperm motility			
Vitamin C & E	No difference	2 months 1 g/day of vitamins C and E	Greco *et al* 2005
Vitamin C	Improvement	2 months, 2 g vitamin C/day	Akmal *et al* 2006
Zinc, zinc & vitamins C/E	Improvement	3 months supplementation with zinc and/or vitamins C+/-E (400 mg Zn, 20 mg Vit E, 1 g Vit C/day)	Omu *et al* 2008
Carnitine	Increase	6 months, l-carnitine 2 g/day and l-acetyl-carnitine 1 g/day	Lenzi *et al* 2003,
Carnitine	Increase	6 months, l-carnitine 3g/day and l-acetyl-carnitine 3 g/day	Balercia *et al* 2005
Carnitine	Increase, but not significant	6 months, l-carnitine 2 g/day and l-acetyl-carnitine 1 g/day	Lenzi *et al* 2004
Carnitine	No sign. change	24 weeks, 2 g L-carnitine, 1 g L-acetyl-carnitine/day.	Sigman *et al* 2006
Carnitine	Increase	3 months, 2 g L-carnitine/day	Garolla *et al* 2005
Carnitine	Increase	2 months after 2 months intermittent NSAID, Carnitines: Carnitene 1 g/12 hours and Nicetile 500 mg/2 hours	Vicari *et al* 2002
Carnitine	Increase	1g/day during 3 months	Abd El-Baset *et al* 2010
Carnitine	Increase	Meta-analysis	Zhou *et al* 2007
Selenium	Increase	3 months oral supplementation selenomethionine 100 µg/day	Scott *et al* 1998
Vitamin E	Increase	300 mg/day during 6 months	Suleiman *et al* 1996
Vitamin E & Selenium	Increase	6 months, vitamin E 400 mg, Selenium 100-200 µg/day	Vezina *et al* 1996
Vitamin E & Selenium	Increase	3 months 400 mg vitamin E, selenium 225 µg/day	Keskes-Ammar *et al* 2003
Folic acid	Increase	15 mg during 3 months	Bentivoglio *et al* 1993
CQ-10	Increase, but not significant	60 mg/day, for a mean of 103 days	Lewin and Lavon 1997
CQ-10	Increase	300 mg during 26 weeks	Safarinejad 2009
CQ-10	Increase	200 mg during 6 months	Balercia *et al* 2008
Sperm morphology			
Vitamin C and E	No sign.change	2 months 1 g/day of vitamins C and E	Greco *et al* 2005
Vitamin C	Improvement	2 months, 2 g vitamin C/day	Akmal *et al* 2006
CQ-10	Improvement	300 mg during 26 weeks	Safarinejad 2009
L-carnitine	Significant improvement	1g/day during 3 months	Abd El-Baset *et al* 2010
Carnitine	Improvements	Meta-analysis	Zhou *et al* 2007
Change in seminal ROS			
Carnitine	Reduction	3 months, l-carnitine 2 g/day and acetyl-carnitine 1 g/day	Vicari and Calogero 2001
L-carnitine	Reduction	1d/day during 3 months	Abd El-Baset *et al* 2010
Antioxidant mix	Reduction	6 months, 600 mg acetylcysteine or 30 mg beta-carotene and 180 mg vitamin E daily plus EFAs	Comhaire *et al* 2000
Vitamin E	Reduction	3 months, 600 mg/day	Kessopoulou *et al* 1995
MDA levels			
Vitamin E & Selenium	Reduction	3 months 400 mg vitamin E, selenium 225 µg/day	Keskes-Ammar *et al* 2003
Vitamin C & E	Reduction	2 months 1 g/day of vitamins C and E	Greco *et al* 2005
Vitamin E	Reduction	300 mg/day during 6 months	Suleiman *et al* 1996

Fertilisation capacity			
Vitamin E	Increase	3 months, 600 mg/day	Kessopolou *et al* 1995
Vitamin E	Increase	3 months, 200 mg/day	Geva *et al* 1996
Zinc, zinc & vitamins C/E	Improvement	3 months supplementation with zinc and/or vitamins C+/-E (400 mg Zn, 20 mg Vit E, 1 g Vit C/day)	Omu *et al* 2008
CQ-10	Improvement	60 mg/day for a mean of 103 days	Lewin and Lavon 1997
Acrosome reaction			
CQ-10	Improvement	300 mg during 26 weeks	Safarinejad 2009
Various sperm parameters			
Vitamin E	No significant change	600, 800, 1200 mg during 3 weeks	Moilanen and Hovatta 1995
Vitamin C	Improvements	200 mg/1 g vitamin C during 1 month	Dawson *et al* 1992
Selenium	No sign. change	12 weeks, 200 micrograms/day	Iwanier and Zachara 1995
Selenium	No sign. change	48 weeks, 300 micrograms/day	Hawkes *et al* 2009
Folic acid	No sign. change	1 month, 10 mg/day	Landau *et al* 1978
CQ-10	No sign. change	60 mg/day for a mean of 103 days	Lewin and Lavon 1997

Appendix V

Table of Antioxidant Herbs and Foods

Common name	Latin name	Reference
Herbs		
Rosemary	*Rosmarinus officinalis*	
Sage	*Salvia officinalis*	
Oregano	*Oreganum onites*	
Cinnamon	*Cinnamomum zeylanicum*	
St Johns Wort	*Hypericum perforatum*	
Cumin	*Syzygium aromaticum*	Wojdylo *et al* 2007
Meadowsweet	*Filipendula ulmaria*	Shilova *et al* 2006
Amaranth	*Amaranthus paniculatus*	
Coriander	*Coriandrum sativum*	Ali *et al* 2008
Marigold	*Calendula officinalis*	
Citrus	*Citrus aurantius*	
Grape	*Vinis vitifera*	
Rosemary	*Rosmarinus officinalis*	Gladine *et al* 2007.
Rosemary	*Rosmarinus officinalis*	
Sage	*Salvia officinalis*	
Oregano	*Oreganum onites*	
Thyme	*Thymus officinalis*	
Ginger	*Zingiber officinalis*	
Mint	*Mentha piperita*	
Chamomille	*Chamomilla recutita*	Yanishlieva-Maslarova *et al* 2001,
Linden	*Tillia cordata*	Atoui *et al* 2005.
Fruits		
Pomegranate	*Punica granatum*	
Guava	*Pisidium guava*	
Mango	*Mangifera indica*	Stangeland *et al* 2009
Berries	*Ribes nigrum, Rubus spp, Fragarica spp.*	Hakkinen *et al* 1998, Belitz and Grosch 1999, Wang and Lin 2000, Yanishlieva-Maslarova *et al* 2001, Manach *et al* 2004
Citrus fruits	*Citrus spp.*	Yanishlieva-Maslarova *et al* 2001, Beecher 2003, Manach *et al* 2004.
Plums, prunes, apples, pears, kiwi		Belitz and Grosch 1999, Yanishlieva-Maslarova *et al* 2001,Manach *et al* 2004.
Rhubarb		Manach *et al* 2004.
Vegetables		
Aubergine		
Chicory		
Artichoke		
Parsley		
Beans		
Kale		
Leek		Manach *et al* 2004.
Spinach		Bergman *et al* 2001.
Drinks		
Black, green tea		Manach *et al* 2004, Beecher 2003, Triantaphyllou *et al* 2001, Koleva *et al* 2003.
Rooibos		
Maté		Kroyer 2005

Red wine	
Cider	
Orange juice	
Coffee	
Chocolate	Manach *et al* 2004.
Chocolate	Sanchez-Gonzales *et al* 2005, Beecher 2003.

Note 1. This is not aiming at providing an exhaustive list of antioxidant foodstuff. Other authors have produced more extensive lists.

Appendix VI

Table of Anti-inflammatory Herbs

Herb	Latin name	Method	Reference
Chili pepper	*Capsicum annuum*		
Allspice	*Pimenta officinalis*		
Basil	*Ocimum basilicum*		
Bay leaves	*Laurus nobilis*		
Black pepper	*Piper nigrum*		
Licorice	*Glycyrrhiza glabra*		
Nutmeg	*Myristica fragrans*		
Oregano	*Origanum onites*		
Sage	*Salvia officinalis*		
Thyme	*Thymis vulgaris*	LPS-stimulated macrophage model	Mueller *et al* 2010
Echinacea	*Echinacea angustifolia*	LPS-stimulated macrophage model	Stevenson *et al* 2005
Echinacea	*Echinacea spp.*	*In vitro* bioactivity assay on peripheral blood mononuclear cells.	Senchina *et al* 2006
Green tea	*Camelilla sinensis*	Arthritic mice, *in vivo* assay of inflammatory markers	Haqqi *et al* 1999
Green tea	*Camelilla sinensis*	*In vitro* cancer cells	Fujik *et al* 2000
Cat`s claw	*Uncaria tomentosa*	Murine macrophage cell line assay for inflammatory markers	Sandoval *et al* 2000
Curcuma	*Curcuma longa*	Carrageenin-induced paw edema, murine	Joe *et al* 1997 Mukhopadhay *et al* 1982
Curcuma	*Curcuma longa*	Human double-blind crossover trial against rheumatoid arthritis	Deodhar *et al* 1980
Curcuma	*Curcuma longa*	LPS-stimulated macrophage model	Aggarwal and Shishodia 2004
Ginger	*Zingiber officinalis*	Human, *in vitro*, on synoviocyte cells	Srivastava and Mustafa 1992
Royal jelly	-	*In vitro*, activated macrophages	Kohno 2004
Boswellic acid	*Boswellia serrata*	LPS-stimulated monocyte model	Syrovets *et al* 2005

Note 1. The anti-inflammatory activity is based on a significant reduction of inflammatory markers such as IL-6 and TNF-alpha, enhancement of IL-10, and reduction of COX-2 or iNOS.

Note 2. The herbs/food were selected for their presence and recognition in European cooking/herbal medicine. A wide spectra of more exotic varieties have been excluded, as these are not widely available.

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