Chapter 11
Effect of Antioxidants on Sperm Genetic Damage

Yves Menezo, Don Evenson, Marc Cohen, and Brian Dale

Abstract According to worldwide statistics, between one in four and one in five couples have fertility problems. These problems are equally distributed between males and females. Modern lifestyle has obviously increased these problems: endocrine-disrupting chemicals, such as plastic polymer catalysts, alkylphenols, phthalates and so on, and cosmetic additives seem to be strongly involved in this fertility problem. Many of these compounds increase oxidative stress (OS) and thus impair spermatogenesis. The oocyte has only a finite capacity, decreasing with maternal age, to repair sperm-borne decays. To decrease this DNA repair burden, reducing the sperm DNA damages linked to OS is tempting. Antioxidant vitamins are often given haphazardly; they are not very efficient and potentially detrimental. A detailed analysis of the sperm nucleus is mandatory (DNA fragmentation or lack of nuclear condensation) prior to any treatment. Here we discuss new concepts in OS and the corresponding therapeutic approaches.

Keywords Oxidative stress • Gametes DNA damages • Fertility • Antioxidants • Homocysteine

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Introduction

According to the 2002 National Survey of Family Growth by the Centers for Disease Control and Prevention (CDC), infertility affects approximately 12 % of the reproductive-age population. In the USA, this includes 7.3 million women and their partners. But by the World Health Organizations (WHO) (2004) estimation, infertility could now affect one couple in five in Western countries to one in four in the world-wide population [infecundity, infertility, and childlessness in developing countries (Demographic and Health Surveys, DHS, Comparative reports No. 9 ORC Macro and WHO 2004)]. Male reproductive failure is thought to be the cause of 50–70 % of infertility cases in Western countries: isolated males account for one-half of these and are a contributor in the other half (Krausz 2011). This, together with lifestyle variations, has led to a delay in first delivery in couples from around 25 years of age in the previous generation to over 30 years of age. Over the past half century, routine classic semen analysis has focused only on the morphology, number, and motility of sperm cells. The advent and large-scale development of assisted reproductive technology (ART) and especially intracytoplasmic sperm injection (ICSI), which bypass a natural "selection," have caused an upheaval. A better appreciation of sperm DNA integrity, especially due to the pioneering work of Evenson et al. (1980), has changed the scientific and therapeutic approach to male infertility. Importantly, this has led to the discovery that infertile men with both normal and abnormal sperm parameters may have significantly higher sperm DNA damage. The major factors affecting sperm DNA integrity include DNA fragmentation and formation of DNA adducts (primary and secondary structure) and chromatin decondensation (tertiary structure). Oxidative stress (OS) is one of the major causes of DNA and chromatin damage (at least for primary and secondary structure) and sperm quality (Kao et al. 2008). OS causes DNA fragmentation, formation of a-basic sites, and formation of DNA adducts, which can be partially the result of chemical covalent interactions between by-products of lipid (polyunsaturated fatty acids, PUFAs) peroxidation, i.e. malonedialdehyde and 4 hydroxynonenal, with nuclear bases (Badouard et al. 2008). In theory, these chemical insults can be repaired by the zygote and the very early embryo using reserves accumulated in the growing oocyte (Menezo et al. 2007a; Jaroudi et al. 2009). The oocyte has an important and redundant, yet limited, DNA repair capacity that decreases with age. However, the oocyte must also repair female genome DNA damage (Lopes et al. 1998; Zenzes et al. 1998), thereby contributing to an overall increase in the total amount of DNA needing repair. Approximately two million DNA repair operations are needed during the first 24 h following fertilization (Menezo et al. 2010). If the DNA repair capacity is overwhelmed, the embryo will initiate apoptosis and developmental arrest. However, a point of greater concern is that some sperm DNA damage, if not repaired, may lead to mutations. Therefore, paternal transmission of damaged DNA may compromise embryonic development and subsequently alter post-natal development (Ji et al. 1997; Zenzes 2000; Zini and Sigman 2009; Robinson et al. 2012).

In animal models, ICSI using sperm with fragmented DNA leads to a high risk of genetic disease transmission and severe pathologies (Fernandez-Gonzalez et al. 2008).

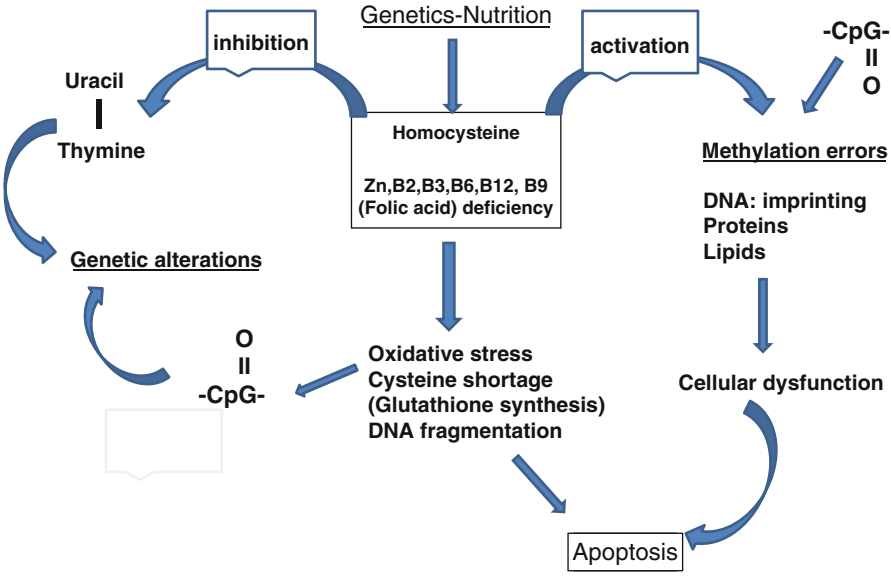

Fig. 11.1 Interrelations between Methylation, homocysteine and oxidative stress. CpG=O, CpG islands with oxidized guanine. CpG: phosphodiester bond between Cytosine (C) and Guanine (G)

There are at least 12 base oxidation products (Menezo et al. 2012); the most important is 8 oxo guanine (G). 8 oxo G causes G->T transversions, a source of mutations. If left unrepaired, 8 oxo G affects methylation of the adjacent cytosine (CpG, phosphodiester bond between the cytosine and guanine sites): these CpG sites in DNA represent mutational hotspots (Wachsman 1997; Franco et al. 2008). This observation strongly suggests a link between reactive oxygen species (ROS) decays, genetic alterations and carcinogenesis (Fig. 11.1) induced by aberrant DNA methylation (Cerda and Weitzman 1997; Franco et al. 2008). G is also an important component of telomeres (TTAGGG repeats). Critically short telomeres are associated with sperm DNA fragmentation (Rodriguez et al. 2005). Thus, OS, in a kind of vicious circle, also directly damages telomeres and accelerates their shortening. Telomere shortening induces defects in meiosis, fertilization and embryo development and thus leads to infertility (Keefe and Liu 2009). Telomere dysfunction is a masterpiece in reproductive aging. In addition, 8 oxo G may affect codons of the sulfur-containing amino acid cysteine (UGU, UGC), involved in the synthesis of glutathione, the universal "physiological" free radical scavenger. 8 oxo G may also affect the methionine (AUG) codon. Methionine is the major effector in methylation processes (through S adenosyl methionine: SAM): it yields the methyl groups, and the by-product formed is homocysteine. This aspect can be a supplementary link between defective methylation and OS. ROSs are at the crossroads between genetic and epigenetic changes, potentially affecting cell quality. Seminal plasma has a finite potential for scavenging ROSs either by enzymatic action [superoxide dismutase (SOD), catalase and glutathione peroxidase (GPX)] or by small molecules:

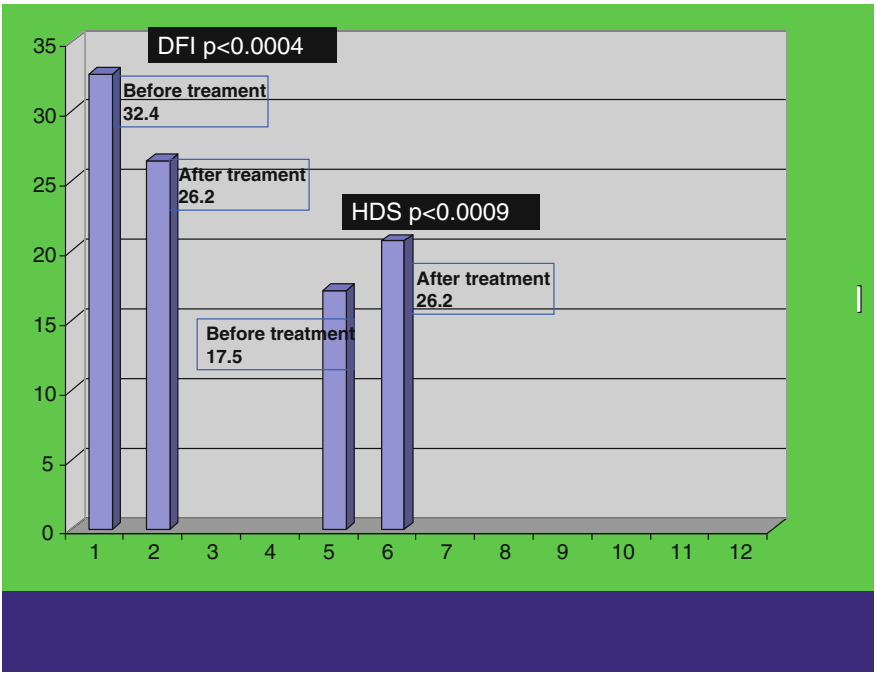

Fig. 11.2 variations in DFI and HDS, post 3 months treatment with vitamins C, E and A and Selenium (From Menezo et al. 2007b). The treatment decreases fragmentation (DFI) but increases the nucleus decondensation (HDS) inthe same order of magnitude

glutathione, hypotaurine, ascorbic acid and gamma tocopherol (and not beta tocopherol, the commercial form). For infertile men with high levels of sperm DNA fragmentation, it is tempting to increase the antioxidant capacity by providing nutraceutical antioxidants. The positive effect of such complementation is far from being demonstrated (Agarwal et al. 2004). Moreover, we recently demonstrated that complements may have a deleterious effect on sperm nucleus condensation (Menezo et al. 2007b) (Fig. 11.2). In this chapter, we will point out two types of complement treatment: (a) the modern theories on OS and (b) the effective action of these reducing substances on cell physiology, with particular emphasis on the overexposure that is often observed.

Approaches to Determining Oxidative Stress in Sperm

First of all, two important and unavoidable prerequisites must be mentioned: high-quality reproducible evaluations of the main DNA decays must be made. Determination needs to be made as to whether the decays observed are stable in time without any treatments or if there are fluctuations of known/unknown origin. DNA fragmentation, tertiary structure (compaction/decondensation), presence of

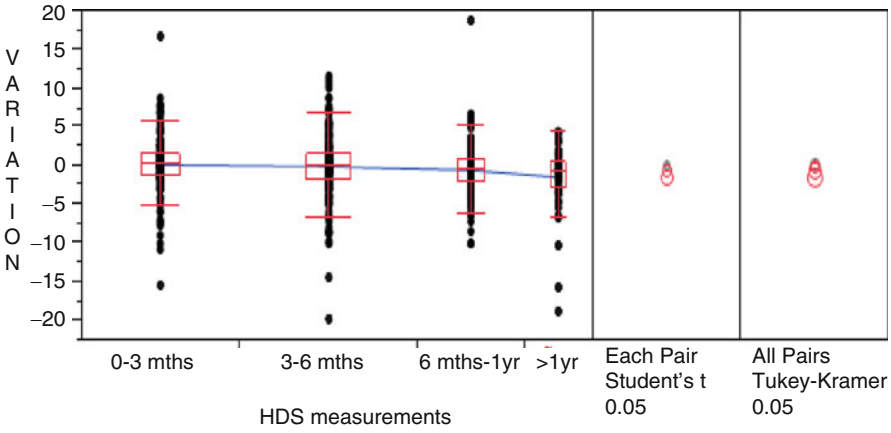

Fig. 11.3 Variations in HDS measurements (SCSA) over time. Each patient is its own control ($N=560$ patients). The curve shows that under normal conditions, without treatment, sperm nucleus condensation does not show any modification during 6 months and then it slightly decreases

adducts and oxidized bases are the key parameters linked to successful embryogenesis. Fragmentation can be estimated by two robust techniques: the SCSA test (Evenson et al. 1980, 2002), which measures by flow cytometry the percentage and extent of sperm DNA fragmentation, and the TUNEL (d-UTP nick-end labelling), which may have some artefacts (Muratori et al. 2010). Decondensation can be quantified using the SCSA test (HDS: high DNA stainability) and staining with toluidine/aniline blue (Hammadeh et al. 1996). Chromomycin A3 (CMA3), a glycosidic antibiotic from *Streptomyces griseus* which is used as a fluorescent stain of DNA, has been used to quantify sperm maturity, i.e. defect in protamination. But in reality CMA3 has no specificity towards histones or protamines: it is a DNA dye. Indeed, CMA3 seems to have the same feature as TUNEL in determining rather DNA decays (Belloc et al. 2009). Sperm DNA fragmentation and nuclear lack of condensation in the nucleus are largely, but not totally, independent parameters (Wyrobek et al. 2006). Failed condensation does not increase the risk of DNA fragmentation. In fact, the balance between the two parameters is clearly demonstrated by two observations: (1) age increases DNA fragmentation and decreases decondensation (Wyrobek et al. 2006); (2) strong antioxidants decrease fragmentation to a certain extent but increase decondensation (Menezo et al. 2007b). This likely means that an increase in ROS production and vulnerability (with age) allows for a better padlocking of the protamine-cysteine bridges. A balance must be respected between pro- and antioxidants, and the glutathione/glutathione peroxidase (GPX) system is the epicenter of the regulations involving padlocking of sperm nuclei and protection against ROS insults (Menezo et al. 2012).

Concerning the stability of the chromatin structure over time, our study in a group of 503 patients, which controlled for HDS after various time periods (0–3, 3–6, 6–12 months, more than 1 year), demonstrated that HDS values did not vary between 0 and 6 months. After 6 months, it slightly but significantly decreased, confirming the general feature observed for age ($r=-0.22$, $p<0.001$) (Fig. 11.3). The DFI is also

stable over time without treatment; the DFI measured in 45 patients (1 sample every month for 8 months) showed no significant variation. DFI is a much more stable parameter than the ones given by conventionnal semen analysis; moreover it is not subject to seasonal variatons contrariliy to sperm concentration and quantity.

8 OHdG is an important marker for OS. Quantification can be done using several techniques including both liquid chromatography and fluorescence, flow cytometry/immunofluorescence or mass spectometrty after DNA hydrolysis. These tests are not easy to carry out (Badouard et al. 2008). A strong correlation exists between DNA fragmentation and oxidative damage as determined by SCSA fragmentation and 8 OHdG (Oger et al. 2003) and TUNEL fragmentation and 8 OHdG (De Iuliis et al. 2009; Aitken et al. 2010). Malonedialdehyde (MDA) formation results from a peroxidation of lipids; it most likely induces the formation of DNA adducts (Badouard et al. 2008): MDA is a marker of sperm immaturity (Montjean et al. 2010) and the related excessive presence of polyunsaturated fatty acids (PUFAs). No correlation has been found between TUNEL and MDA sperm content. DNA adduct determination is complex and requires both liquid chromatography and mass spectrometry measurements.

To demonstrate the effect of antioxidant treatments, there are only two possibilities: (1) double blind versus placebo and (2) control before and after treatments (paired samples) once the stability of the parameter without treatment has been demonstrated. The patient is his own control.

Current Treatments and Their Pitfalls

Current treatments are generally based on bits of information from the literature. Therefore, supplements may vary widely in terms of composition and concentration. Oral intake of some molecules may increase their concentration in serum but not necessarily in the testes. It must be clear that the active compounds must pass into the blood and then various organs. Pregnancy should be the ultimate parameter in judging treatment efficacy.

Selenium

Selenium (Se) is a very popular compound in supplements, especially for anti-ageing. It could be a logical choice since it is a cofactor of phospholipid hydroxyperoxide glutathione peroxidase GPX4 or PhGPX (Ursini et al. 1997), involved in the compaction of the sperm nucleus through a padlocking of the protamine-cysteine bonds. However, there are negative effects that counteract this positive observation. First, there is no Se deficiency described in the scientific literature for the Caucasian population. Second, According to Bleau et al. (1984), seminal plasma Se must be in a strict range of values, between 50 and 70 nG/mL. Increased values are associated with decreased motility and a higher incidence of asthenospermia, followed by higher abortion rates.

This observation was confirmed by Li et al. (2012) in 100 patients: the concentration found for Cu, Mn and Se in the seminal plasma of pathological sperm was higher than in the normal sperm group (Cu, $p=0.024$; Se, $p=0.002$; Mn, $p=0.002$). Third, animal studies have demonstrated that if supplementation increases serum values, then it does not influence testicular values. Fourth, increasing Se serum values is not totally harmless. According to Hawkes et al. (2009), Se at high doses induces thyroid pathologies which reduce sperm motility: we have observed two cases in our hypofertile population. Finally, according to WHO regulations (Guidelines for drinking-water quality, fourth edition, World Health Organization 2011), a high selenium concentration in human drinking water, together with aluminium, bacterial contamination, radioactivity, pesticides and nitrates, is one of the six parameters used to regulate safety. According to Brack et al. (2013), serum Se value is never a systemic biomarker of oxidative stress in chronic human pathologies. From a basic point of view, selenites increase apoptosis through a generation of free radicals (superoxides) by mitochondria (Zhao et al. 2009). Mitochondria are already the primary source of DNA damage in sperm cells (through oxidative phosphorylation). Finally, Se at high concentrations could displace Zn and alter DNA methylation and, thus, impair genetic stability. Iron and copper are to be avoided, unless a strong deficiency is established; their role in increasing free radical formation is widely known (Haber-Weiss and Fenton reactions).

Zinc (Zn) Is an Interesting Divalent Cation

First of all, according to the CDC, the National Center for Health Statistics (NCHS), 15 % of the Caucasian population suffers from Zn deficiency. Zn is a cofactor in around 200 enzymes, and especially Zn superoxide dismutase and metallothionein (MT) capture of superoxide and hydroxyl radicals. The sperm of fertile men have mRNA coding for most of the MTs (Garcia Herrero et al. 2011). Zn counteracts the negative impact of cadmium. Zn restriction and repletion affect DNA integrity in healthy men (Song et al. 2009; Ho 2004), decreasing the DNA repair capacity. There is no reason that spermatogenesis should not be regulated by this important process. Zn is an important cofactor in homocysteine recycling, at two levels, dihydrofolate reductase and methionine synthase (Fig. 11.4). Homocysteine is a molecule considered to strongly disturb the reproductive system, in both males and females, causing as a consequence OS and inhibition of DNA methylation (Fig. 11.1). Thus, Zn supplementation seems important, if not mandatory. Omu et al. (2008), working on Zinc supplementation associated or not with vitamins C and E, observed no difference in the outcome measures between zinc only and zinc with vitamin E and a combination of vitamins E and C. However, it has been shown that most of the compounds added to supplements, i.e. Zn pipecolate, Zn oxide, Zn gluconate, Zn sulfate and others, are only marginally efficient due to their poor bioavailability. To be active, Zn must be added in a chelated form www.efsa.europa.eu/ (*European Food Safety authority: EFSA-Q-2005-035, EFSA-Q-2005-133, EFSA-Q-2005-034, EFSA-Q-2005- 038, EFSA-Q-2005-166, EFSA-Q-2005-033, EFSA-Q-2005-132, EFSA-Q-2005-036, EFSAQ-2005-130).*

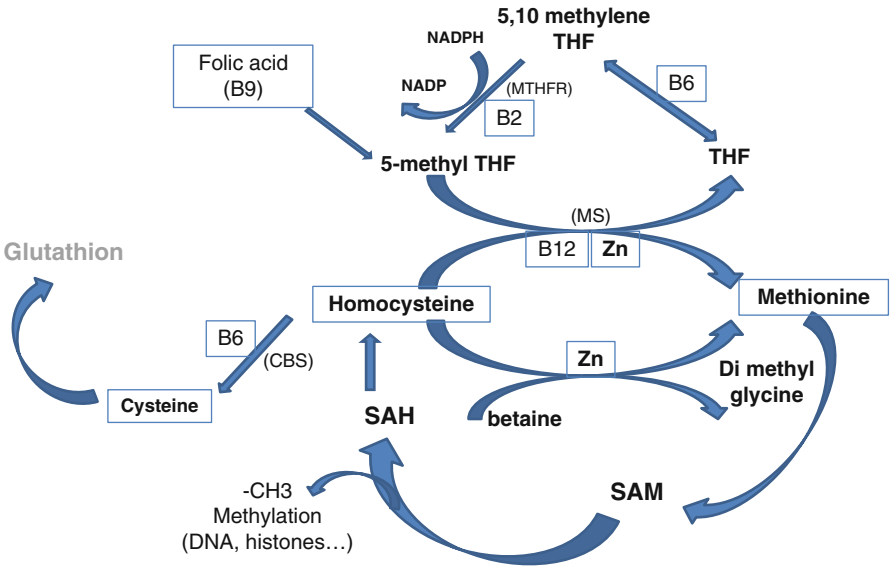

Fig. 11.4 The one carbon cycle. Importance of group B vitamins and Zinc in Homocysteine recycling SAH: S-Adenosyl Homocysteine; SAM: S-Adenosyl Methionine; THF tetrahydrofolate CBS: cystathionine beta synthase; MS Methionine synthase; MTHFR: Methyltetrahydrofolate reductase Zn: Zinc

Vitamin C (Ascorbic Acid)

Vitamin C is one of the natural antioxidants present in the testes, and its concentration in seminal plasma is ten times the concentration observed in serum (Lewis et al. 1997). It was the first supplement used by Fraga et al. (1991) to prevent smoking-related damage. But according to Kandar et al. (2011), vitamin C and uric acid concentrations are similar in the seminal plasma of smokers and non-smokers. It is probable that vitamin C intake can help to reduce DNA fragmentation to a certain extent. However, it is a handicap for sperm nucleus condensation (Menezo et al. 2007b). First, it prevents the primary oxidation of the sperm membrane, and second, it has the capacity to open the disulfide bonds padlocking the DNA. This capacity to open disulfide bonds is of general concern (Giustarini et al. 2008; Donnelly et al. 1999).

Vitamins A and E

These to vitamins may improve the quality of spermatogenesis (Almbro et al. 2011). Vitamin E is given in the form of alpha tocopherol. It may protect membrane lipids from peroxidation and thus increase sperm motility. It probably has no effect on DNA protection. However, the concentrations of the various forms of tocopherol in seminal plasma are very low when compared to serum (Benedetti et al. 2012),

so it is not obvious that an increase in the blood has any impact on testicular concentration. After its neutralization with a free radical, vitamin E may be regenerated by vitamin C and is therefore a better complement (Greco et al. 2005). A decrease in carotene is very rarely a marker of pathologies. This is not the case for vitamin E: its concentration is significantly lower in several pathologies (Brack et al. 2013).

CoQ10 (Ubiquinone)

CoQ10 deficiency is very rare; it occurs in cases of recessive autosomal mutations, some cancers, diabetes, and muscular and cardiovascular pathologies (Villalba et al. 2010), and not in the relatively young population consulting for infertility. Commercially available CoQ10 (ubiquinone) is poorly absorbed by the intestine (Liu and Hartmann Liu and Artmann 2009; Villalba et al. 2010). Marginal increases in CoQ10 can be observed in seminal plasma after long-term treatments but with no improvement in fertility (Mancini and Balercia 2011). In fact, the reduced form of ubiquinone, ubiquinol, which has only recently become available, affects DNA quality with a simultaneous increase in decondensation and, for some patients, a severe decrease in spermatogenesis (Amar et al., personnal communication).

Superoxide Dismutase

Our experience using superoxide dismutase (SOD) of vegetal origin, 'orally effective', (as modified to resist in the stomach environment), was that it yielded the same results as the mixture Se plus vitamins A, C, E: a decrease in DFI but a symmetric increase in nucleus decondensation (Menezo et al. 2007b) (Fig. 11.2).

Reduced Glutathione

Is the universal 'physiological' free radical scavenger. However, its 'effective' capacity after oral absorption is highly questionable for two reasons: (a) it is destroyed in the stomach and (b) it is not transported to the cells.

Carnitine

Carnitine is not, strictly speaking, an antioxidant molecule: it is a regulator of lipid metabolism and limits peroxidation of membrane phospholipids. A marginal positive effect has been described.

New Approaches to Treatment

Information given on the structure of the nucleus by spermiogrammes is scant: in extreme cases, abnormal forms can give an idea on fragmentation, but not on decondensation. In our experience, decondensation and fragmentation affect only marginally the fertilization process. There is no universal treatment and the type of sperm problem must be clearly established. There are so far four main DNA detrimental figures: (1) single defective condensation HDS > 25 %, (2) single moderate fragmentation 25 % < DFI < 35 %, (3) very high single fragmentation with DFI > 35 %, (4)simultaneously elevated fragmentation and decondensation. There exists a common consensus that DFI > 30 % is an upper tolerable level; beyond this limit fertility problems appear, especially for natural conception or even IUI, but less for IVF and ICSI (Bungun et al. 2007, 2011). Spermatozoa may be subject to a burst of ROS during their transit in the female genital tract and at the time of capacitation (De Lamirande and Gagnon 1995). It must be clear that the critical level depends upon the quality (i.e. DNA repair capacity) of the fertilized oocyte. Beyond a 50 % DFI, the chance of conceiving is virtually non-existent (we had one pregnancy with ICSI in a young woman 27 years of age). Concerning the lack of condensation, the problem is rather more complicated as the oocyte is not able to repair anomalies in the tertiary structure of the sperm nucleus. Our upper limit is 25 % for condensation (HDS) defects; this may vary with the technique: aniline/toluidine blue is more variable as it is operator dependent. Beyond these limits, embryo developmental arrests are observed as early as the one-cell stage.

One of the critical point in the understanding of ROS related DNA damages is the correlation between ROS insults and methylation (Tunc and Tremellen 2009). Homocysteine is the key parameter link between these two aspects (Fig. 11.1): "Homocysteine: cause and consequence of oxidative stress" in various pathologies (Tunc and Tremellen 2009; Hoffman 2011; Menezo et al. 2011). DNA damage and low folic acid are correlated (Boxmeer et al. 2009), but in general, folic acid and vitamin B12 are potent modulators of fertility (Ebisch et al. 2007; Boxmeer et al. 2009). As mentioned earlier, Zn (involved in homocysteine recycling) deficiency affects 15 % of the Caucasian population, and its importance has been underlined by Omu et al. (2008). Thus, it seems that all the group B vitamins must be present and not only folic acid (B9), associated with chelated Zinc, as blocks may occur at various steps of the recycling process (Fig. 11.4). Glutathione has a dual role in spermiogenesis – as a free radical scavenger but also an unavoidable partner in nucleus condensation. Quercetine, or quercetol, is a flavonoid (Fig. 11.5). It passes the blood barrier (Moskaug et al. 2005) and several organs such as muscle, and its metabolites formed in the blood, glucuronide, sulfate and methyl quercetin, have an extended life span. The protecting and supporting effect of quercetin has been widely known since the late 1980s. It has been demonstrated that it prevents sperm lipid peroxidation (Moretti et al. 2012) and protects sperm against various environmental toxicants (hydrocarbons), including estrogens or tar and ammonia originating from cigarette smoking (Bohn et al. 2010). Foods rich in quercetin increase the synthesis of 15 mRNAs involved in DNA repair and 11 mRNAs linked to apoptosis.

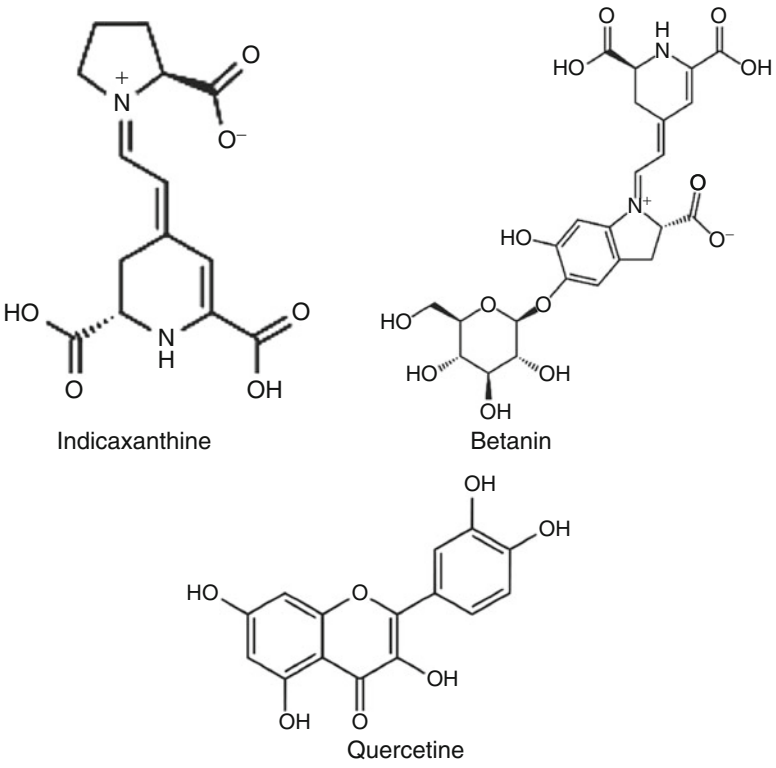

Fig. 11.5 Structure of Indicaxanthine, Betanin and quercetin

It up-regulates gamma-glutamylcysteine synthetase (CS) and glutathione synthetase expression and acts directly on promoters (Moskaug et al. 2005; Bohn et al. 2010). In vitro it may have a marginal protecting effect during sperm processing. Betalains are aromatic indole derivatives (yellow or red); they are antioxidant, lipoperoxyl radical scavengers (Tesoriere et al. 2004). They might have positive health effects in humans, slowing anarchistic cell division, and promote apoptosis of certain cancerous cells in vitro (Sreekanth et al. 2007). Betanine and indicaxanthine (Fig. 11.5) are the best known betalains. The most interesting fruit containing quercetine and betalains is *Opuntia* (prickly pear). We have used these observations in treating our patients.

Defective condensation (HDS > 25 %)

This study was performed in two private IVF units: 39 male patients with a severe history of infertility and numerous failed ART attempts were involved in our program. All of them had a sperm decondensation index (SDI), measured by aniline blue

Table 11.1 Effect of treatments with vitamins of group B, quercetine and betalains (Condensyl) on HDS treatment of 4 months (Wilcoxon test for paired samples)

	Decondensation (m, SD)		
	Before		After
All over patients (34)	36.4 (10.4)	$p<0.002$	23.6 (14.6)
Patients reacting positively (29)	34.4 (10.3)	$p<0.002$	23 (10.5)

staining, over the critical threshold of 25 %. The male patients took, orally, one pill containing all the B group vitamins (B2:1.4mG, B3: 16mG, B6: 2mG, B9: 200μG, B12:1 μG) and Zn: 15 mG (*under a chelated form of Zn bisglycinate*), which are involved in the recycling of homocysteine (Condensyl, Nurilia, France), for at least 4 months. Then a control of SDI was performed. Four patients did not make the post-treatment control. Two of them started a spontaneous pregnancy after the end of treatment, before the control. Of the 34 remaining patients, 5 showed no improvement. Overall (including the five stable patients), a highly significant effect of the treatment was observed (Table 11.1). To be precise, for three patients (not included in the study), a period of 6 months or more was necessary for a complete recovery of normal condensation under the critical threshold of 20 %.

Isolated Suboptimal DNA Fragmentation (25 %<DFI<35 %)

Our program has enrolled more than 100 patients in 4 different IVF units, patients with an infertility of 3 years or more with at least two failed ART procedures. The complement (Procrelia) contains all the ingredients of Condensyl plus citrus flavonids, green tea polyphenols and dunalliela carotenoids (Procrelia). The results, in terms of pregnancy, are not yet available (Menezo et al. in press); however, we have found a 27 % decrease in DNA fragmentation, but with no effect on decondensation. Tunc and Tremellen (2009), using a rather similar treatment (folic acid+lycopene+zinc+selenium+garlic oil, Menevit) observed a 50 % decrease in DNA fragmentation, but with no improvement in sperm numeration or motility. At this level of fragmentation the question could be whether we should treat these patients. The corresponding female age, and the oocyte capacity for DNA repair, is at this point one of the keys to answering that question: the answer is yes if the 'maternal' age is over 35.

Very High isolated DNA fragmentation (DFI>35 %)

Eighty patients are enrolled in this study. All of them had an infertility lasting at least 5 years, with 2 or more IVF/ICSI/IMSI failures. In this case, Fertibiol (Nurilia France) is used; it is a strong antioxidant cocktail containing all the group B vitamins,

chelated Zn, ubiquinol (the soluble form of CoQ10), astaxanthin, and green tea extract (containing more than 30 % polyphenols) and Pycnogenol. In a first approach with ten voluntary patients, we have observed that this treatment cannot be pursued over 5 weeks. Otherwise a strong decrease in spermatogenesis is observed in 20 % of the patients (Amar et al., personnal communication). After a 5-week treatment, a decrease in fragmentation is observed (up to 200 %), but a classic decondensation of the sperm nucleus is observed (Menezo et al. 2007b). Thus, the treatment for defective condensation is mandatory, and for these patients the 5-week Fertibiol treatment was followed by 4 months of Condensyl in order to fix nucleus compaction. Seventeen patients have completed the entire treatment regime. The DFI changed from 27.4 % (SD: 13.6) to 10.8 % (SD: 4.1) and the HDS from 22.4 % (SD: 10.8) to 11.9 % (SD: 6.1). Both variations before and after treatment are highly significant: $p < 0.001$, Wilcoxon test. Four ongoing spontaneous pregnancies obtained, three other have been obtained post IVF attempts (total 7/17: 41%). One pregnancy ended in a miscarriage.

High DFI and High Sperm Nucleus Decondensation (DFI and HDS > 25 %)

The same protocol as for isolated high DFI must be followed. DNA fragmentation must be corrected first (5 weeks of strong antioxidants, Fertibiol) followed by a correction of decondensation for 4 months. Sixty patients are enrolled in this protocol; the results on pregnancy rates are not yet available.

Conclusions

The negative impact of sperm DNA damage on human fertility is no longer a matter for debate. It is obvious that ROSs are strong effectors in compromising DNA quality. Aerobic conditions are normal in life, i.e. the generation of ROSs. ROSs are formed during the intermediate steps of oxygen reduction. However, modern life has obviously increased fertility problems through pesticide-induced ROS generation: xenoestrogens, endocrine-disrupting chemicals involved in plastic technology such as polychlorinated biphenyls, bisphenol A, phthalates and alkyl phenols, di-(2-ethylhexyl) phthalate (DEHP) (Ambruosi et al. 2011). There are little data as to whether it is possible to increase DNA repair capacity in the oocyte; that is why in ART, the yield in terms of take-home baby rate per oocyte retrieved is no higher than 5 % (Patrizio and Sakkas 2009). In addition, in vitro techniques to select the best spermatozoa are still weakly controlled. High-magnification sperm selection (IMSI) has not demonstrated any advantage in terms of selecting sperm cells with the best DNA secondary and tertiary structure (Montjean et al. 2012; Tanaka et al. 2012). Selection with hyaluronic acid seems more relevant and consistent

(Parmegiani et al. 2010; Wilding et al. 2012). A combined technique of IMSI and PICSI, termed PIMSI, has also been proposed (Wilding et al. 2012).

In any case, in vivo improvement of spermatozoa before starting a pregnancy or before ART is of paramount importance. Ingestion of vitamin and oligo-element cocktails is no longer reasonable. If we consider the Hippocratic "primum non nocere" precept, they are highly questionable. The real decays in sperm nuclei must be correctly defined, i.e. either DNA fragmentation (oxidation) or tertiary structure anomalies (nuclear condensation). It is obvious, however, that this type of treatment may be marginally useful for a part of the infertile population. Analyses of serum and, to a lesser extent, of seminal plasma are usually poor and give little indication of the real testicular situation. In infertility, the relationship between homocysteine recycling and OS seems particularly relevant: homocysteine seems to be the epicenter of both male and female problems, even if they are not completely understood. This is the case for the positive effect of homocysteine recycling, *via* methyl donor supply, on sperm nuclear condensation/maturity, which is probably more multifactorial than currently defined (Junca et al. 2012); methylation and condensation seem to be very tightly linked and interacting. Considering the B group vitamins, it is surprising that folic acid alone is sometimes given as nutraceutical complement. Looking at the methionine cycle (Fig. 11.4) it is obvious that at least B2, B6 and B12 are also strong partners in this process. A strict equilibrium must be maintained when reducing OS to avoid nuclear decondensation. In conclusion, a complete evaluation of sperm nucleus quality should be mandatory following IVF/ICSI failure(s).

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