

New concepts for antioxidants treatments of sperm DNA structure alteration

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Introduction:

The major factor affecting ICSI success and source of concern is sperm DNA quality i.e. essentially fragmentation and decondensation. Although this technique has shown a high efficiency, its full safety is far from demonstrated: the injection of spermatozoa with damaged DNA, in a system where natural selection mechanisms are bypassed, may lead to birth defect and transmission of genetic diseases or cancer (Aitken et al. 2003, Fernandez-Gonzales et al. 2008). ROS affect sperm DNA quality not only by inducing fragmentation but also through formation of DNA adducts. We recently found even in our best samples, beside 8oxo deoxyguanosine, 1, N6-ethenoadenosine and 1, N2-ethenoguanosine, arising from a reaction with 4-hydroxy-2-nonenal, the main aldehyde compound released during lipid peroxidation (Badouard et al. 2008).

It is therefore tempting to try to reduce or avoid the generation of DNA alterations related to free radical exposure using intake of anti-oxidants. However if oral intake of high levels of vitamins (especially A and C) does reduce DNA fragmentation, it also increases sperm decondensation thus negatively affecting initiation and regulation of paternal gene activity during preimplantation development (Ménézo et al. 2007a)... This is why we have tried to find new nutritional complements in order to be more specific according to the DNA structure defects and to avoid the negative effects on sperm tertiary structure.

Material and Methods:

The patients involved in this study were waiting for oocytes donation or have faced at least two failures of IVF/ICSI, with no evidence of maternal age or ovarian poor response. They were tested for sperm DNA fragmentation (using SCSA in a first series of attempts and then TUNEL assay, Roche) and decondensation (SDI), using SCSA first and then Aniline blue considered as more reliable than Chromomycin A3 in our hands).

For treatments, according to our previous results (Ménézo et al. 2007a), 3 groups were considered:

Treatment A (Fertibiol): Carmine, Coenzyme Q, Asthaxanthine + Zeaxanthine (10 times stronger than vitamin C), Pycnogenol and Tyrosine for patients with isolated very high fragmentation >40%. For this group of patients, the oxidative status was controlled in serum (Zn, tocopherol alpha and gamma, GPX, oxidized LDL, vitamin B6, 9.12 and homocysteine)

Treatment B (Procrelia): Zinc, polyphenols, carotenoids, flavonoids and Lycopene and betalaines, from prickly pear (*opuntia*) that protect the poly-unsaturated fatty acids of sperm

International Congress of Andrology ICA 2009 Barcelona. Spain

membranes, for patients with high fragmentation , between 20 and 40% and moderate decondensation below 20% . It contains low doses of Vitamin C for recycling of folic acid, but neither SOD nor selenium for their potential risk in increasing fragmentation (mitochondria mediated apoptosis)

Treatment C: Zinc and prickly pear extracts (soft antioxidants) and mixture of group B vitamins, no selenium and no SOD for patients with High decondensation >20% associated with moderate fragmentation (below 20%).

Results:

In a first experiment against placebo (maltodextrin), involving 48 patients, treatment B (procrelia: Treatment B) gave a significant improvement for motility: 49.3 +/-1 vs. 28 +/-6.2 associated with a significant decrease in DFI: 9.6 +/- 4.1 vs. 28.4 +/-5.6.

Then starting the treatment (Treatment A) for patients having a DFI from 40 to 78%, 11/34 had a strong decrease in DFI, below 20%. 16 had a slight decrease or no change and 7 (including 5 smokers) had a slight increase

In a first small cohort of patients (21), we can observe that folic acid and zinc associated with “soft” antioxidants (Treatment C, 3-6months): 10/21 has a significant decrease (10 to 60%) in decondensation. For 5 of these couples the treatment resulted in a clinical pregnancy, 4 of them are now ongoing (>3mths). 10/21 did not get any change in decondensation (SDI) and 1/21 had an increase in SDI (25 to 28%).

The overall decrease is significant (p<0.03).

Discussion:

The time of “simple and strong” antioxidants treatments: large scale use of vitamins A, E, C associated with selenium has to be carefully revisited. A Clear determination of the Decays i.e. DNA fragmentation or decondensation, has to be established before any type of treatment. Selenium is now known as a mitochondria pro-apoptotic factor even increasing DNA fragmentation. Vitamin C associated or not with SOD strongly decreases fragmentation, however it may alter the correct condensation of sperm DNA blocking the formation of disulfide bridges; at very low doses, it helps in the folic acid pathway (Methyl THF formation) It has also to be considered that the product of oxidation of an antioxidant is not toxic.

The best example is given by hypotaurine: its oxidation product Taurine is an osmolyte protecting sperm. This is also the reason of our choice for polyphenols, pycnogenol and betalaines (leading to quinine like compounds), Terpene (activating Heat shock proteins), Asta and ZeaXanthine. These low antioxidants have to be associated with Folic acid and Zn, introduced for their ability to maintain chromatin stability. One must consider that DNA fragmentation or oxidation has to be reduced in order to avoid the overwhelming of DNA repair capacity of the oocyte (Menezo et al. 2007b), in order to allow an harmonious embryo development and thus avoid time bombing effect to the offspring (Fernandez-Gonzales et al. 2008); Yet abnormal tertiary structure of the nucleus i.e. presentation of the DNA in the oocyte precludes early embryo development (Rousseaux et al. 2008) via chromosomal anomalies.

Oocyte capacity to restore a correct tertiary structure is very limited (Menezo et al. 2007b). Soft anti-oxidants seems to have, at least an interesting efficiency towards sperm motility; decrease in motility is usually linked to sperm membrane lipid peroxidation. In the same approach, DFI is decreased.

Zinc and folic acid have been shown to improve some semen parameters including count (Ebisch et al; 2006, Wong et al. 2002). More interesting is the association of these 2 compounds with polyphenols, betalaines, flavonoids and carotenoids: it improves the secondary and the tertiary structure of the nucleus (condensation) contrarily to what is observed with vitamin A and C (Menezo et al. 2007a). This is due to the multiple roles of PHGPx (Ursini et al. 1997) also involved in prevention of ROS linked decays in fine, large scale blind treatments with strong anti-oxidants must be now avoided. Selective treatments have to be prescribed according to the specific decays of sperm nucleus: fragmentation and /or decondensation to avoid a “worse than bad” story.

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