Male Infertility and antioxidants

Martins da Silva, Sarah J.

Published in:
Reproductive BioMedicine Online

DOI:
10.1016/j.rbmo.2019.08.008

Publication date:
2019

RBMO

COMMENTARY

Male Infertility and antioxidants: one small step for man, no giant leap for andrology?

Sarah J. Martins da Silva*

ABSTRACT

Oxidative stress is detrimental to spermatozoa and is acknowledged to be a common pathology in infertile men. Antioxidant supplements, therefore, represent a logical therapeutic approach, although the recent Cochrane review recommends cautious interpretation of publications and findings to date. This commentary considers whether male fertility supplements have a place in current reproductive medicine practice. Importantly, although sperm selection for intracytoplasmic sperm injection is a common research theme, survey data show that men would prefer medication to achieve natural conception, rather than treatment to improve assisted reproductive technology (ART) success. A total of 27.1% (n = 112), 26.6% (n = 110) and 24.5% (n = 101) respondents indicated they (or their male partner) would undertake medical treatment to attempt natural conception for up to 6 months, 12 months and 2 years, respectively. A total of 63% indicated that they would be prepared to participate in a clinical trial and 57% would defer ART by 6 months to do so. This information represents the beginnings of a dialogue with patients and stakeholders and should be used to shape research efforts.

Assisted Reproductive Technology (ART) registries consistently document male factor as the most frequent underlying or contributory cause for infertility worldwide. Despite its prevalence, most male factor infertility is idiopathic, and generally no cure is available. Specifically, no drug that can be prescribed to reliably improve male fertility, nor added *in vitro* to improve sperm function, is available. Therapeutic interventions, therefore, rely on ART, which is invasive and expensive. 'Take home baby' rates after intracytoplasmic sperm injection (ICSI) remain frustratingly static, with 25–30% live birth rate per (fresh) treatment cycle (*McLernon et al., 2016*); despite these realities, however, most will seek medical intervention and many are prepared to face significant financial hardship to pay for it. The need for effective treatment for male infertility is clearly unmet.

Diagnostic semen analysis is the cornerstone of investigation of male fertility. It does not, however, evaluate sperm function to inform patient treatment pathway or prognosis. Sperm function tests aim to examine attributes required for conception but, although they are useful as research tools, they are of little clinical benefit, particularly given the widespread use of ICSI.

Reactive oxygen species (ROS) are by-products of cellular metabolism and are essential for normal sperm function, including capacitation, hyperactivation, acrosome reaction and fertilization. Oxidative stress occurs when production of ROS exceeds natural antioxidant defences, leading to cellular injury. Excessive ROS damages the sperm cell membrane, which affects sperm motility and the ability to fertilize an oocyte, and also damages sperm DNA (*Aitken and Curry, 2011*). Key contributors to ROS include smoking, obesity and poor diet as well as environmental factors, including pesticides and pollution.

Spermatozoa are particularly vulnerable to oxidative stress because they have little cytoplasm and, therefore, limited endogenous antioxidant protection. Sperm DNA damage caused by ROS impairs natural conception, affects success of ART and may contribute to a higher risk of miscarriage (*Simon et al., 2017*). A variety of approaches can be used to test sperm DNA damage (*Cissen et al., 2016*), which are available commercially. Analysis is conducted on unprepared semen after cryopreservation

Reproductive and Developmental Biology, School of Medicine, Ninewells Hospital and Medical School, University of Dundee, Dundee DD1 9SY, UK

*Corresponding author. E-mail address: s.martinsdasilva@dundee.ac.uk (S. J. M. da Silva). https://doi.org/10.1016/j.rbmo.2019.08.008 1472-6483/
Declaration: The author reports no financial or commercial conflicts of interest.

and shipping. As such, results may be arguably different to a fresh semen sample, or one prepared for ART. The different tests also measure subtly different aspects of sperm DNA damage, with no defined clinical thresholds, although consensus opinion suggests that DNA fragmentation index (DFI) over 30% is significant. Nonetheless, evidence is mounting that DNA repair can follow fertilization, and that elevated DFI does not necessarily negatively affect the outcome of ART (*Antonouli et al., 2019*). More frustratingly, rather than identifying spermatozoa with low levels of DNA damage to select for ICSI, sperm DNA damage tests render them clinically unusable.

A multitude of alternative approaches can be taken to assess oxidative stress. Of the four DNA bases, guanine is the most susceptible to oxidative damage. Levels of 8-hydrodeoxyguanosine (8OHdG) can be measured and are generally correlated with estimates of DNA fragmentation. In human semen, ROS can usually be measured by fluorescence or chemiluminescence. Antioxidant enzymes (superoxide dismutase, glutathione, catalase, or all three) can be quantified, or a combination of ROS and antioxidants evaluated, termed oxidation redox potential (ORP). Alternatively, oxidative balance in semen can be evaluated indirectly, e.g. oxygen radical absorbance capacity assay. Metabolites of lipid oxidative damage can also be quantified, including malondialdehyde (MDA) and 4-hydroxynonenal. Although levels of DFI, 8OHdG, ROS, ORP and MDA have a degree of agreement, however, this is imprecise.

Antioxidants are protective against ROS, and those naturally found in semen include vitamins C, E, B9 (folic acid), trace elements zinc and selenium, and micronutrients such as carnitines and carotenoids. Perhaps not surprisingly, antioxidant supplements have been investigated as potential treatments for male infertility. The Cochrane meta-analysis 'Antioxidants for male subfertility' was recently updated and includes 61 randomized controlled trials and 6264 infertile men (*Smits et al., 2019*). It concluded that antioxidant therapy was associated with an increase in clinical pregnancy rate (CPR) and live birth rate (LBR). Although this conclusion should represent an epic and defining moment in clinical andrology, it is not time to celebrate just yet, because this review has huge and significant limitations. Only seven studies (a total of 750 men and 124 births) evaluated LBR, and only 11 trials (786 men, 105 pregnancies) evaluated CPR. Evidence demonstrating any difference or reduction in miscarriage is also insufficient.

Perhaps then, it is somewhat surprising that a large variety of commercially available vitamin and dietary supplements (VDS) are available for male fertility. Convenient, prescription-free and non-invasive, they are increasingly popular for men (and women) attempting to conceive. Prenatal and pregnancy VDS retail value sales grew by 34% between 2008 and 2013, and currently represent a market estimated to be worth US$4.7 billion worldwide. Ingredients and doses for a representative sample of male fertility VDS are shown in the TABLE. All contain folic acid (vitamin B9), selenium and zinc. Most contain vitamin C, vitamin E, or both, and coenzyme Q10 and L-carnitine with a variety of other trace elements and micronutrients. The component amounts, however, are substantially lower than those reported in scientific publications (*Showell et al., 2014*). For example, 15 mg folic acid daily for 3 months increased sperm count and motility, yet male fertility supplements contain 800 µg or less. A total of 200 µg selenium daily resulted in improved sperm motility, yet 26–150 µg is present in VDS. A total of 250 mg zinc twice daily had beneficial effects on sperm motility and fertilizing capacity, yet supplements contain up to 40 mg. Intriguingly, magnesium supplementation for 3 months showed no association with improvement in sperm motility, yet several supplements contain it. Conversely, 600 mg N-acetyl cysteine significantly increased sperm motility yet few VDS contain this micronutrient. Two fertility supplements in this sample contain pine bark extract, also known as h. Inclusion of this super-antioxidant seems to be based on a study of 19 male patients in which improvement in morphology and mannose binding scores is reported in capacitated spermatozoa after 90 days' supplementation (*Roseff, 2002*).

Results from published studies of combined agents, e.g. zinc and folic acid, are disappointingly inconsistent (*Showell et al., 2014*). This presumably reflects the heterogeneous population spanned by male infertility. It is also possible that a threshold of severity exists beyond which antioxidant supplementation is unable to rescue and reverse. Nonetheless, most commercial fertility VDS formulations have not been tested in randomized controlled trials, and data are limited for those that have. For example, the effect of FertilAid (Fairhaven Health, Bellingham, WA, USA) was studied over a 90-day period, but only 14 men were included. Total normal motile sperm count increased after antioxidant supplementation, but this did not reach statistical significance ($P = 0.05$), nor were any significant differences found in individual semen characteristics (*Clifton and Ellington, 2009*). Profertil (LenusPharma, Vienna, Austria) was reported to improve fertility in men with a 2-year history of unexplained subfertility and subclinical varicocele. No control group was used, baseline semen parameters were all within the fertile range according to World Health Organization (WHO) diagnostic criteria, and no statistical analysis was included in the published report (*Schauer et al., 2009*). Another study reported improved hyaluronan binding after 3 months of treatment with Profertil (*Lipovac et al., 2014*). Significant pre-existing differences were found in hyaluronan binding between treatment and control groups at baseline ($P = 0.02$).

Research studies are also needed to address relevant study outcomes. Fertilovit M Plus supplementation (Gonadosan Distribution GmbH, Bregenz, Austria) in 160 infertile men resulted in a significant improvement in all sperm characteristics, but not to a point where ICSI could be avoided (*Ajayi et al., 2013*). Men treated for 4 months with a single sachet of Proxeed (Sigma-tau HealthScience, Indianapolis, IN, USA) daily showed significant improvement in sperm motility, although effect on fertility was not assessed (*Busetto et al., 2012*). Alternatively, treatment with Profertil for 3 months was found to significantly increase sperm motility and morphology in 132 subfertile males, compared with 73 subfertile controls (*Imhof et al., 2012*). A total of 34 pregnancies (25.8%) were reported in the treatment group, compared with 11 (15.1%) in the control group, which is notable, albeit not statistically significant ($P = 0.07$). Furthermore, *Tremellen et al. (2007)* examined the effect of

TABLE INGREDIENTS AND DOSES IN 12 REPRESENTATIVE BRANDS OF COMMERCIALLY AVAILABLE VITAMIN AND DIETARY SUPPLEMENTS FOR MALE FERTILITY

	FertilAid	*Fertility Support for men*	*Fertilman*	*Fertilovit M Plus*	*Fertilsan M*	*Fertimax*	*Menevit*	*Orthomol Fertil Plus*	*Profertil*	*Proxeed Plus*	*Vitamen*	*Wellman Conception*
Acetyl L-carnitine										1.0g		
Calcium		20 mg										
Chromium	120 µg	20µg										50 µg
Coenzyme Q10	PB		5 mg	15 mg	15 mg	40 mg		15 mg	15 mg	40 mg	30 mg	2 mg
Copper	2 mg							1 mg				1 mg
DHA								65 mg				
EPA								90 mg				
Garlic oil							333 µg					
Ginseng extract	PB											30 mg
Glutathione			5 mg	50 mg					80 mg			2.5 mg
Iodine	150 µg											
Inositol												40 mg
Iron		5 mg	15 mg									6 mg
L-arginine		300 mg	250 mg		500 mg				250 mg		200 mg	10 mg
L-carnitine	PB	100 mg	100 mg	300 mg	500 mg	400 mg		440 mg	440 mg	2.0 g	100 mg	50 mg
L-citrulline				300 mg								
L-taurine		100 mg	50 mg								100 mg	
Lycopene				4 mg			6 mg	[a]				1.5 mg
Maca extract	PB											250 mg
Magnesium	120 mg	20 mg	51 mg									60 mg
Manganese	2 mg	5 mg										0.5 mg
N-acetyl cysteine				50 mg	80 mg			80 mg				
Omega 3 FA								170 mg				
Pine bark extract					100 mg							30 mg
Selenium	100 µg	100 µg	50 µg	100 µg	60 µg	50 µg	26 µg	80 µg	60 µg	100 µg	100 µg	150 µg
Vitamin A (beta carotene)	5000 iU	2320 iU 5 mg			3 mg			3 mg[a]				2500 iU
Vitamin B1	1.5 mg	20 mg										12 mg
Vitamin B2	1.7 mg	20 mg	2 mg									5 mg
Vitamin B3	20 mg	20 mg	15 mg									18 mg
Vitamin B5	10 mg	20 mg										10 mg
Vitamin B6	2 mg	20 mg	10 mg					3.5 mg			10 mg	10 mg
Vitamin B7												150 µg
Vitamin B9	500 µg	400 µg	400 µg	500 µg	800 µg	200 µg	500 µg	800 µg	800 µg	400 µg	400 µg	400 µg
Vitamin B12	25 µg	20 µg						9 µg		3 µg	20 µg	75 µg
Vitamin C	250 mg	200 mg	80 mg	100 mg	200 mg	180 mg	100 mg	250 mg		180 mg	100 mg	90 mg
Vitamin D	400 iU	2.5 µg	10 µg		10 µg						1000 iU	600 iU
Vitamin E	150 iU	200 mg	20 mg	100 mg	120 mg	30 mg	400 iU	120 mg	120 mg		150 iU	30 mg
Vitamin K	80 µg											
Zinc	30 mg	30 mg	20 mg	25 mg	40 mg	15 mg	25 mg	40 mg	40 mg	20 mg	25 mg	15 mg

[a] Mixed carotenoids containing beta carotene, lutein and lycopene.

PB, proprietary blend containing L-carnitine, Maca (root), grape seed extract, Asian ginseng (root) and Coenzyme Q10.

Menevit (Bayer, Pymble NSW) on ART outcomes and showed no significant differences in oocyte fertilization rate or embryo quality between intervention and placebo groups. A statistically significant improvement in CPR, however, was found compared with control.

In summary, routine semen analysis can identify those at risk of male factor infertility but has limitations. Sperm function tests currently have little clinical value and treatment options for male infertility are almost exclusively limited to ART. Despite a wealth of studies examining antioxidants, evidence that they improve the chance of live birth is still insufficient, despite a multitude of VDS being commercially marketed for this very purpose. A concerted research effort is needed to move forward from this position.

Involvement of patients and stakeholders in research is changing and evolving. Public involvement and an acknowledgement of the value of insight from those who live with a health condition are increasingly being recognized. Patient contributions will be different to expert knowledge but help to ensure research quality and relevance. To this end, we commissioned a SurveyMonkey questionnaire, to gather opinion from patients affected by male infertility (n = 470). Over 90% said that male infertility was equal to or more important than female infertility. Research into male infertility was rated as highly important (average response 8/10, total score 3726/4700), as was the need for development of new treatment options. Overwhelmingly, the preferred treatment for men (or their partners) would be medication (tablets 58.3% or injections 24.6%) to achieve natural conception rather than medication to improve IVF/ICSI success. Similar numbers of respondents indicated they (or their male partner) would be prepared to take treatment for male infertility for up to 6 months (27.1%; n = 112), up to 12 months (26.6%; n = 110) and up to 2 years (24.5%; n = 101) to attempt natural conception. 62.6% (286/457) indicated that they would be prepared to participate in a clinical trial and 57% (253/444) defer IVF/ICSI by 6 months to do so. Not surprisingly, live birth was rated as the most important outcome after a research study (259/467 respondents; 55.5%), although pregnancy rate and time to achieve pregnancy are also important to patients. Miscarriage rate was important to 31 out of 467 (6.6%) patients. A minority indicated the importance of research in generating knowledge to help others or to enhance scientific understanding.

In conclusion, there is a demand and an unmet need for new treatment options for male factor infertility, and a desire of patients to avoid ART if possible. We need better diagnostics to identify patients who might benefit from intervention and randomized controlled trials with outcomes that are relevant to patients live birth, time to pregnancy, miscarriage and side-effects. Results are awaited from NIH funded MOXI (males, antioxidants and infertility; NCT02421887) and FAZST (folic acid and zinc supplementation; NCT01857310) trials but current evidence that male fertility and live birth really improve after antioxidant intake is insufficient, and the stark reality remains that no definitive treatment to prescribe for idiopathic male factor infertility is available.

REFERENCES

Aitken, R.J., Curry, B.J. **Redox regulation of human sperm function: from the physiological control of sperm capacitation to the etiology of infertility and DNA damage in the germ line.** Antioxid. Redox. Signal 2011; 14: 367–381

Ajayi, R., Okhowat, J., Spitzer, D., Schechinger, B., Zech, N.H. **Impact of antioxidative supplementation on semen quality according to MSOME criteria.** Journal of the Brazilian Society of Assisted Reproduction 2013; 17: 27–31

Antonouli, S., Papatheodorou, A., Panagiotidis, Y., Petousis, S., Prapas, N., Nottola, S.A., Palmerini, M.G., Macchiarelli, G., Prapas, Y. **The impact of sperm DNA fragmentation on ICSI outcome in cases of donated oocytes.** Arch. Gynecol. Obstet. 2019

Busetto, G.M., Koverech, A., Messano, M., Antonini, G., De Berardinis, E., Gentile, V. **Prospective open-label study on the efficacy and tolerability of a combination of nutritional supplements in primary infertile patients with idiopathic astenoteratozoospermia.** Archivio italiano di urologia, andrologia: organo ufficiale [di] Societa italiana di ecografia urologica e nefrologica / Associazione ricerche in urologia 2012; 84: 137–140

Cissen, M., Wely, M.V., Scholten, I., Mansell, S., Bruin, J.P., Mol, B.W., Braat, D., Repping, S., Hamer, G. **Measuring Sperm DNA Fragmentation and Clinical Outcomes of Medically Assisted Reproduction: A Systematic Review and Meta-Analysis.** PloS one 2016; 11e0165125

Clifton, G.D., Ellington, J.E. **Prospective study of FertilAid vitamin in men with low sperm quality.** Journal of Andrology 2009

Imhof, M., Lackner, J., Lipovac, M., Chedraui, P., Riedl, C. **Improvement of sperm quality after micronutrient supplementation.** European e-Journal of Clinical Nutrition and Metabolism 2012; 7: 50–53

Lipovac, M., Bodner, F., Schütz, A., Kurz, H., Riedl, C., Mair, J., Imhof, M. **Increased hyaluronan acid binding ability of spermatozoa indicating better maturity, morphology, and higher DNA integrity after micronutrient supplementation.** European Medical Journal 2014; 1: 60–65

McLernon, D.J., Steyerberg, E.W., Te Velde, E.R., Lee, A.J., Bhattacharya, S. **Predicting the chances of a live birth after one or more complete cycles of in vitro fertilisation: population based study of linked cycle data from 113 873 women.** BMJ 2016; 355: i5735

Roseff, S.J. **Improvement in sperm quality and function with French maritime pine tree bark extract.** The Journal of reproductive medicine 2002; 47: 821–824

Schauer I., Jost R., Imhof M. Micronutrients as an alternative to fertility treatment in men with subclinical varicocele 2009.

Showell, M.G., Mackenzie-Proctor, R., Brown, J., Yazdani, A., Stankiewicz, M.T., Hart, R.J. **Antioxidants for male subfertility.** The Cochrane database of systematic reviews 2014; 12

Simon, L., Zini, A., Dyachenko, A., Ciampi, A., Carrell, D.T. **A systematic review and meta-analysis to determine the effect of sperm DNA damage on in vitro fertilization and intracytoplasmic sperm injection outcome.** Asian journal of andrology 2017; 19: 80–90

Smits, R.M., Mackenzie-Proctor, R., Yazdani, A., Stankiewicz, M.T., Jordan, V., Showell, M.G. **Antioxidants for male subfertility.** The Cochrane database of systematic reviews 2019; 3CD007411

Tremellen, K., Miari, G., Froiland, D., Thompson, J. **A randomised control trial examining the effect of an antioxidant (Menevit) on pregnancy outcome during IVF-ICSI treatment.** The Australian & New Zealand journal of obstetrics & gynaecology 2007; 47: 216–221

Received 14 June 2019; received in revised form 22 August 2019; accepted 23 August 2019.