Don't burn your sperm – Antioxidants and IUI outcome [not all IUI]

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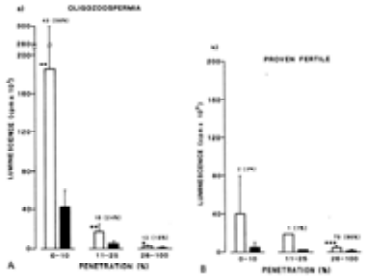
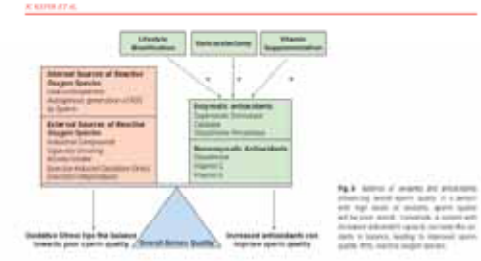
Background
Generation of ROS by spermatozoa and biological function

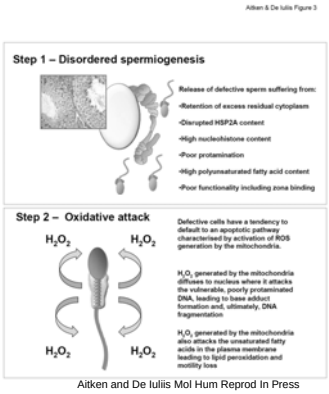


Aitken et al., 1989 J Androl 10, 214-220.

Oxidative stress



Kefer JC, et al., 2009 Int J Urol. 16:449-57.



Aitken and De Iuliis Mol Hum Reprod In Press

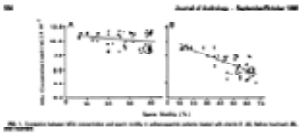
Oxidative damage –
the whole circle
RJA developed hypothesis

How do we approach treatment? Identification of the pathology

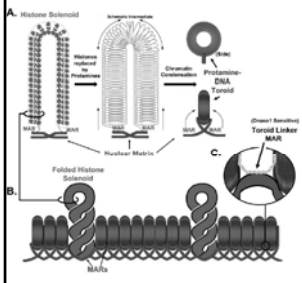
- Non specific administration of AO to 'sub fertile men' proven to be of no value e.g. Rolf *et al.,* (1999) performed randomised control trial with AO and showed no significant differences with placebo [Hum Reprod 14,1028-
- If identified problem then *may be* appropriate.
- Problem could be oxidative stress e.g. excessive ROS production or …….
- more appropriately manifested as increased damage to the sperm e.g. MDA production, DNA damage.
- If these are identified - is there any known benefit?

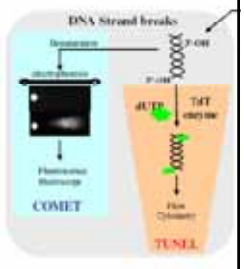
Potential targeted treatment – promising.

- Kessopoulou *et al* 1995 performed a double blind randomised placebo cross over trial with Vit E [600mg/day] to treat men with high levels of ROS. [Kessopoulou *et al* Fertil Steril 64, 825-831].
- Significant improvement in zona binding (p=0.004) consistent with improvement in plasma membrane of the spermatozoon.
- Sulieman et al. 1996 showed significant improvement in motility and pregnancy rates in men with high MDA levels treated with Vit E. [Sulieman et al., 1996 J Androl 17, 530-537.



Damage to the paternal genome manifested as DNA damage



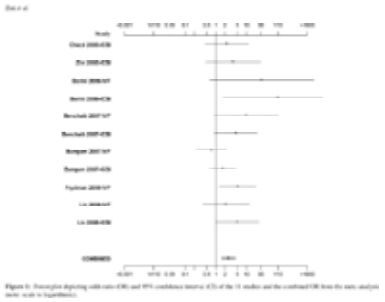


Ward In press Mol Hum Reprod

Delbes et al., In press Mol Hum Reprod

Increased pregnancy loss associated with sperm DNA damage meta analysis

Zini *et al.*, 2008 Hum Reprod. 23, 2663-2668..



One step further to identifying pathology? DNA damage of the cells – treatment works?

- 64 men with high levels fo DNA damage randomised with AO vs. placebo treatment. Greco *et al.*, 1996 J Androl 17, 530-537.

Table 2. *Comparison of basic sperm parameters and the incidence of DNA fragmentation in the antioxidant treatment group before and after the treatment period**

Time of Analysis	Sperm Concentration (× 10⁶/mL)	Percent		
		Sperm Motility	Normal Sperm Forms	TUNEL-Positive Spermatozoa
Before treatment	18.6 ± 17.3†	40.3 ± 35.1‡	10.5 ± 8.1‡	33.1 ± 7.7‡
After treatment	27.5 ± 24.6†	41.6 ± 22.0†	8.0 ± 7.1†	9.1 ± 7.2‡

* Data are mean ± SD. TUNEL indicates terminal deoxyribonucleotidyl transferase–mediated dUTP nick-end labeling.
† $P > .05$.
‡ $P < .001$.

Interestingly some responders and some non responders.

Follow up for ICSI in treated men – encouraging but no placebo/control

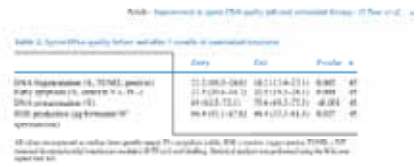
Greco *et al.*, 2005, Hum Reprod 20,2590-2594

Table V. Implantation and pregnancy in two sequential ICSI attempts performed in the antioxidant-responsive group before and after antioxidant treatment

Time of attempt	Attempts	Embryos transferred	Clinical pregnancies[a]	Pregnancy rate (%)[b]	Gestational sacs[c]	Implantation rate (%)[d]
Before treatment	29	89	2	6.9[e]	2	2.2[e]
After treatment	29	92	14	48.3[e]	18	19.6[e]

[a]With at least one gestational sac with cardiac activity.
[b]Percentage of attempts resulting in a clinical pregnancy.
[c]With cardiac activity.
[d]Percentage of embryos transferred that gave rise to a gestational sac with cardiac activity.
[e]$P < 0.05$.
[f]$P < 0.01$.

Unfortunately field is littered with uncontrolled trials



Tunc et al., 2009 RBM Online 18, 761-768

Review by Armand Zini [Zini *et al.*, 2009 J Assist Reprod Genet 26,427-432] concluded that:

'the data suggest that dietary antioxidants maybe beneficial in reducing DNA damage, particularly in men with high levels of DNA fragmentation. …Most of the clinical studies are small'.

In vitro AO in protecting sperm DNA from exogenous ROS

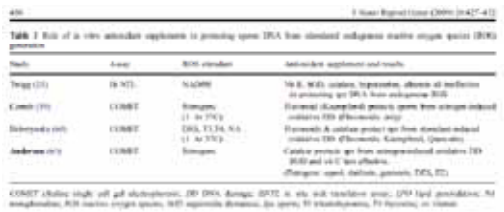
Table 2 Role of in vitro antioxidant supplements in protecting sperm DNA from exogenous ROS

Study	Assay	Exogenous ROS	Antioxidant supplement and results
Lopes (55)	TUNEL	X+XO	GSH+hypotaurine protect spz from X+XO-induced DD
			catalase and n-acetylcysteine individually protect spz from X+XO induced DD
Potts (56)	TUNEL	H_2O_2+Fe+ADP	S. plasma (>60%v/v) lowers oxidative spz damage (↓DD, LPO)
Sierens (57)	COMET	H_2O_2	Isoflavones, vit C & E protect spz from H_2O_2-induced DD
			(Isoflavones: genistein, equol). Dose effect noted.
Russo (58)	COMET	(1) H_2O_2,	Propolis lowers oxidative spz damage (↓LPO, DD, LDH)
		(2) Benzopyrene,	(Propolis – a natural resinous hive product)
		(3) H_2O_2+Fe+ADP	

ADP adenosine diphosphate; *COMET* single cell gel electrophoresis; *DD* DNA damage; *DFI* DNA fragmentation index; Fe iron; *GSH* glutathione; *LDH* lactate dehydrogenase; *LPO* lipid peroxidation; *S. plasma* seminal plasma; *Spz* sperm; *TUNEL* terminal nucleotidyl transferase dUTP nick end labeling; *X* xanthine; *XO* xanthine oxidase

Zini *et al.*, 2009 J Assist Reprod Genet 26,427-432

In vitro AO in protecting sperm DNA from stimulated endogenous ROS



Zini *et al.*, 2009 J Assist Reprod Genet 26,427-432

In vitro AO in protecting sperm DNA from semen processing

Table 4 Role of in vitro antioxidant supplements in protecting sperm DNA from semen processing

Study	Assay	Semen processing	Antioxidant supplement and results
Chi (62)	COMET	Centrifugation	EDTA or catalase lower centrifugation-induced sperm ROS
		(1000 rpm x2)	EDTA or catalase lower centrifugation-induced sperm DD
		+ 1 hr incubation	EDTA or catalase have no protective effect on LPO
Donnelly (63)	COMET	Percoll DGC	Vit C or E do not lower baseline sperm ROS & DD
Hughes (64)	COMET	Percoll DGC	Vitamins C_1 E or urate lower sperm DD after DGC
			Vitamins C+E or AC increase sperm DD after DGC
Donnelly (65)	COMET	Percoll DGC	GSH, hypotaurine or both do not alter baseline sperm DD
		+ H_2O_2	

AC Acetyl cysteine; *COMET* alkaline single cell gel electrophoresis; *DD* DNA damage; *DGC* density gradient centrifugation; *GSH* glutathione; *LPO* lipid peroxidation; *ROS* reactive oxygen species; *vit* vitamin

Zini *et al.*, 2009 J Assist Reprod Genet 26,427-432

How do we approach treatment? Identification of the pathology

- Reasonable data to support *in vivo* administration.
- For *in vitro* administration data less robust
- Great care in preparing cells as those that are susceptible to damage are more ‘delicate’.
- NOT a substitute to having high quality systems in place in the beginning – remember many centres have very low IUI success.
- Likely to have a weight of data in next 2-3 years on treatment of ‘oxidative tress and DNA damage’