The Role of Oxidative Stress and Antioxidant Supplementation in Pregnancy Disorders

Lucilla Poston
The brightest males have better chance of reproductive success!

Sperm of colourful males are better protected against oxidative stress


Carotenoid-based colourful trait in the Great Tit (_Parus Major_) associated with resistance to lipid peroxidation in sperm
Release of defective sperm prone to oxidative damage

- Retention of excess residual cytoplasm
- Disrupted chromatin remodelling
- High polyunsaturated fatty acid content

Oxidative Attack

- Apoptosis activates ROS generation
- Mitochondrial $\text{H}_2\text{O}_2$ attacks poorly protaminated DNA and PUFAs

Antioxidant Supplements Beneficial?

- In vitro > in vivo

Adapted from; Aitken and Iuliis: On the Possible Origins of DNA Damage in Human Spermatazoa. Molecular Human Reproduction 2010; 16: 3-13
Oxidative stress influences human oocyte development

• Low level ROS generation implicated in normal meiosis of primary oocyte (MI) and folliculogenesis

• MII division inhibited by excessive ROS

• Adequate antioxidant defences (e.g., GSH) required for successful ovulation and fertilisation

Lifestyle and supplements

Oocyte oxidative stress associated with cigarette smoking and alcohol intake (Ruder Eh et al, Human Repro Update 2008)

Pre-conceptional multivitamin supplements may enhance fertility (Chavarro JE et al, Obstet Gynecol 2007)

IVF

• ROS implicated in embryo arrest
  Reduced oxygen tension improves IVF embryo viability (Betts DH et al, Mol Human Reproduction 2008)
Maternal Obesity affects Fertility and Reproductive Success

- Reduced conception/increased miscarriage rate
- Impaired immediate outcomes of assisted reproductive technologies
Mitochondria in oocyte and embryo

- Maternally inherited.
- Involved in oocyte maturation, fertilization, embryo development.
- Excessive energy substrates perturb mitochondrial metabolism in oocytes and embryos, compromising development.
Maternal Diet Induced Obesity in Mice
Increased mitochondrial activity (membrane potential↑) and altered distribution in oocytes and zygotes

Igosheva N et al, PLoSOne 2010
Maternal Diet-Induced Obesity in Mice
Intracellular redox potential shifts towards oxidation in oocytes and zygotes

(NAD(P)H & FAD$^{++}$ autofluorescence)

Igosheva N et al, PLoSOne 2010
Maternal diet-induced obesity increased oxidative stress in oocytes and zygotes

(Het fluorescence)

\[\text{HEt fluorescence, \%}\]

\[\text{Rate of ROS production,\%}\]

control

obese

zygotes oocytes

zygotes

Igosheva N et al, PLoSOne 2010
Maternal Diet-Induced Obesity

Attenuated antioxidant defence in oocytes and zygotes

(Glutathione by MCB fluorescence)

Igosheva N et al, PLoSOne 2010
Pre-eclampsia

The Maternal Syndrome

- Hypertension
- Glomerular endotheliosis
- Proteinuria, ATN
- Microangiopathic haemolysis/thrombocytopenia DIC
- Hepatic necrosis, rupture
- ARDS
- Oedema
- Cardiomyopathy
- Eclampsia, stroke
- Hepatic necrosis, rupture
- Rupture
- ARDS
- Oedema
- Cardiomyopathy
- Eclampsia, stroke
From Cindrova-Davies T. Gabor Than Award Lecture: Pre-eclampsia- From placental oxidative stress to maternal endothelial dysfunction. Placenta 2009.
VIP trial
Vitamins in Pre-eclampsia
5597 women assessed for eligibility

- 3187 excluded
  - 832 not meeting inclusion criteria
  - 1564 refused to participate
  - 643 no response
  - 148 other

- 3 randomised in error
- 3 withdrew consent

2404 validly randomised

- 1199 allocated Vitamin C & E
  - 3 lost to follow up
  - 1196 women analysed (1393 fetuses/neonates)

- 1205 allocated placebo
  - 6 lost to follow up
  - 1199 women analysed (1391 fetuses/neonates)
Synergy between ascorbic acid and α tocopherol

Ascorbate regenerates α-tocopherol

[Diagram showing the interaction between ascorbate and α-tocopherol, indicating the regeneration process.]
Vitamin C and E Supplements Do Not Prevent Pre-eclampsia in High Risk Women

<table>
<thead>
<tr>
<th>Condition</th>
<th>Treatment</th>
<th>Placebo</th>
<th>Risk Ratio (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-eclampsia</td>
<td>181 (15%)</td>
<td>187 (16%)</td>
<td>0.97 (0.80 to 1.17)</td>
<td>0.754</td>
</tr>
<tr>
<td>Low birthweight (&lt;2.5Kg)</td>
<td>387 (28%)</td>
<td>335 (24%)</td>
<td>1.15 (1.02 to 1.30)</td>
<td>0.023</td>
</tr>
<tr>
<td>SGA (&lt;5&lt;sup&gt;th&lt;/sup&gt; centile)</td>
<td>294 (21%)</td>
<td>259 (19%)</td>
<td>1.12 (0.96 to 1.31)</td>
<td>0.161</td>
</tr>
</tbody>
</table>

Poston et al, Lancet 2006
WHO multicentre randomized trial of Vitamin C and E supplementation among pregnant women at high risk for preeclampsia and low nutritional status

Villar J, Purwar M, Merialdi M, Zavaleta N, Ngoc NTN, Anthony J, Poston L, Shennan A
on behalf of the WHO Vitamin C and Vitamin E trial group

Villar et al BJOG 2009
NO evidence for protection against pre-eclampsia by Vitamins C and E

• Poston et al, Lancet 2006 (high risk women)
• Rumbold et al, NEJM 2006 (nulliparous women)
• Villar et al, BJOG 2009 (high risk, developing countries)
• Roberts et al, NEJM 2010 (low risk women).
No association with vitamin C intake and pre-eclampsia.

Trend (p<0.01) towards protection against severe pre-eclampsia, eclampsia and HELLP

BJOG 2009; 116: 964-974
Antioxidant Status is Improved by Vits C and E

Poston et al, Lancet 2006
Effect of antioxidants on plasma isoprostane concentrations: 8-epi PROSTAGLANDIN $F_{2\alpha}$

Chappell et al, AMJOG 2002
Vitamins C and E reduced \( \gamma \) Tocopherol in Women at Risk of PE

![Graph showing the effect of vitamins on gamma tocopherol levels across different gestation periods.](image-url)
In vitro hypoxia-reperfusion in Trophoblast increases HIF-1α and sFlt-1; prevented by vitamin C and E
Are we giving antioxidants too late?
After adjustment in multiple regression model, regular use of multivitamins associated with 45% reduction in pre-eclampsia risk compared with non-use (odds ratio 0.32-0.95)
A Dietary Pattern Characterized by High Intake of Vegetables, Fruits, and Vegetable Oils Associated with Reduced Risk of Preeclampsia in Nulliparous Pregnant Norwegian Women

23,423 nulliparous pregnant women in Norwegian Mother and Baby Cohort Study.

<table>
<thead>
<tr>
<th></th>
<th>Preeclampsia</th>
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<tbody>
<tr>
<td></td>
<td>Yes, n = 1267</td>
</tr>
<tr>
<td>Vegetable</td>
<td>-0.122 ± 0.027</td>
</tr>
<tr>
<td>Processed food</td>
<td>0.160 ± 0.027</td>
</tr>
<tr>
<td>Potato and fish</td>
<td>0.049 ± 0.029</td>
</tr>
<tr>
<td>Cakes and sweets</td>
<td>-0.013 ± 0.028</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM. The scores are not adjusted for confounders (Mann-Whitney U-test).

Brantsaeter Al et al, Journal of Nutrition 2009
Are we giving wrong antioxidants?
TOENAILS and Oxidative Stress

Median *toenail* selenium concentrations in preeclamptic subjects significantly lower than in matched controls (P=.001). Being in the *bottom tertile of toenail selenium* was associated with a 4.4-fold (95% CI 1.6-14.9) greater incidence of the condition.

*Rayman, Bode and Redman, AmJOG 2003.*
Selenium Deficiency in Pre-eclampsia

Mistry et al, Hypertension 2008
LOX-1 expression in small omental arteries

Inhibition of LOX-1 expression by scavenging peroxynitrite-a novel approach?

Inhibitor of peroxynitrite prevents PE serum induced increase in LOX-1

Sankaralingam S et al, Hypertension 2009
Peroxynitrite (ONOO-) as a target in pre-eclampsia?

Antioxidant vitamins do not scavenge ONOO-.

Melatonin scavenges ONOO-, reduces NFkB and AP-1 translocation to nucleus, and inhibits iNOS and COX-2.
Novel antioxidant strategies for prevention of pre-eclampsia?

- Start earlier in pregnancy?
- Melatonin
- Selenium
- NADPH oxidase inhibitors
Summary

• Oxidative stress strongly implicated in fertility and pregnancy outcome
• Antioxidant supplementation requires more vigorous testing in sub-optimal fertility
• Vitamin C and E supplementation in pregnancy does not prevent pre-eclampsia
• Other strategies should be explored
• *or* is oxidative stress and ‘innocent bystander’?
Thanks to

Researchers
• Natalia Igosheva
• Josie McConnell
• Tom Fleming
• Michael Duchen
• Andrey Abramov
• Annette Briley
• Andrew Shennan
• Lucy Chappell
• The VIP team
• Jose Villar
• Mario Merialdi and the WHO team

Funders
• The Wellcome Trust
• BBSRC
• Tommy’s Baby Charity
• World Health Organisation