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The Role of Oxidative Stress and Antioxidant Supplementation in Pregnancy Disorders

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Running Head: Oxidative Stress and Pregnancy Disorders

Abstract 250 words max

Oxidative stress is widely implicated in reproductive performance including infertility, miscarriage, diabetes-related congenital malformations and pre-eclampsia. Maternal obesity is a strong risk factor for pre-eclampsia, and recently, in an animal model of maternal obesity we have reported evidence of oxidative stress in the oocytes of obese animals prior to pregnancy as well as in early stage embryos. This adds to the growing evidence for a greater focus on the pre-conceptual period in prevention of pregnancy disorders including those related to oxidative stress. Our research has also focussed on the role of free radicals and antioxidant capacity in pre-eclampsia. Assessment by measurement of markers of lipid peroxidation or of antioxidant capacity has provided unequivocal evidence for oxidative stress in this disorder. Partial failure of the process of placentation has been implicated, with recent evidence proposing that ischaemia reperfusion in the placenta may contribute to oxidative stress in trophoblast. Endoplasmic reticulum stress in the placenta may also play a role. We and others have performed randomised controlled trials to determine whether early supplementation with vitamins C and E in women at risk of pre-eclampsia may be beneficial but these studies have shown no evidence for prevention of pre-eclampsia. Whether this represents an inappropriate antioxidant strategy or whether supplementation has been too late in gestation to be beneficial is not known. Other potential approaches to prevention of pre-eclampsia through amelioration of oxidative stress include provision of supplements in the pre-conceptual period, selenium supplements, anti-peroxynitrite strategies and statins.

Oxidative stress, defined as an imbalance between pro-oxidants and antioxidant capacity, has been implicated in sub-optimal reproductive performance from the earliest stages of development through to labour and delivery. Reactive oxygen species (ROS) are substances with one or more unpaired electrons; because of this ROS are highly reactive, interacting with lipids, proteins or DNA leading to oxidation and cellular malfunction which may initiate pathological processes. The most commonly produced of the ROS in mammals is superoxide. Depletion of antioxidant capacity, whether through low abundance of non-enzymatic (e.g. vitamins C, E, glutathione) or enzymatic (e.g. superoxide dismutase, glutathione peroxidases, catalase) antioxidants renders the cell vulnerable to oxidative attack, even under physiological situations where redox status is maintained through careful balance of a low level of synthesis of reactive oxygen species and the pathways of cellular defence (Raijmakers MTM et al, Current Pharm Design 2004; Forman HJ et al Biochemistry 2010).

Oxidative Stress and Fertility.

Gametes are vulnerable to oxidative attack (Ruder EH et al, Human Reproduction Update 2009; Aitkin RJ and Iuliis Mol Human Reproduction 2010). Reduced fertility in men has been associated with oxidative damage to sperm. Human sperm is vulnerable to oxidant attack as it contains a very high content of polyunsaturated fatty acids, which are necessary to facilitate fusion with the oocyte, but are rich in double bonds which are prone to oxidation. The spermatozoa of subfertile patients contain high levels of 8-hydroxy-20-deoxyguanosine (8OHdG), the oxidation product formed when DNA is subjected to attack by ROS (Kodama H et al., Fertil and

Sterility 1997). De Iuliis and colleagues have recently reported the presence of 8OHdG adducts in human spermatozoa to be highly correlated with DNA strand breaks, determined using the TUNEL assay (De Iuliis et al., 2009). In a recent review Aitken and De Iuliis list the possible mechanisms which may result in DNA strand breaks in human sperm (Aitken and De Iuliis 2010). These include reduced antioxidant capacity in epididymal plasma or seminal fluid (as may occur in smokers), infection and iatrogenic ROS synthesis. These authors propose that the latter is predominantly derived from mitochondria and may be exacerbated in damaged sperm. It is suggested that damaged sperm, characterised by retention of residual cytoplasm, abnormal chromatin remodelling or abnormally high content of polyunsaturated fatty acids, will default to preferential activation of pathways of apoptosis. This in turn will lead to excess mitochondrial ROS synthesis. Mitochondrial oxidative phosphorylation necessarily leads to synthesis of free radicals through electron 'leakage' from the electron transfer pathway leading to generation of superoxide ($O_2^{\bullet}$) and hydroxyl radicals ($OH^{\bullet}$) and mitochondrial activation in association with apoptosis can lead to excessive ROS synthesis and 'auto' attack of the already vulnerable sperm, DNA oxidation and subsequent malfunction. Improved sperm function *in vitro* by addition of antioxidants has been repeatedly shown (Baker HW et al Fetility and Sterility 1996; Donnelly ET et al, Mutagenesis 2000) and it might be anticipated that antioxidant supplements would improve fertility in sub-fertile men. Several studies suggest that antioxidant supplementation may be of benefit in those subfertile men with proven oxidative damage in the sperm but there is no consensus of opinion in regard to the appropriate supplement or dose, and infertile men are not routinely tested for DNA fragmentation (Deepinder F et al, Endocr Pract 2008). One study has reported the

potential benefit of antioxidant supplementation (1 g vitamin C and 1 g vitamin E daily for 2 months) in men with already proven high levels of DNA oxidative damage and one failed ICSI attempt. The majority of the men demonstrated a reduction in DNA fragmented spermatozoa and improved ICSI success (Greco E, Human Reproduction 2005). An improvement in fertility was also found in men with a high DNA fragmentation index instructed to consume a diet rich in antioxidants or commercial multivitamins containing beta-carotene, vitamin C, vitamin E, and zinc for at least 3 months (Gill Villa AM Fertility and Sterility 2009). Our group have recently systematically reviewed the effect of oral antioxidants on male subfertility and concluded that supplementation could improve sperm quality and/or pregnancy rates; however large adequately powered trials using individual antioxidants are required (Ross et al., 2010).

Oxidative stress also influences fertility in species other than man. An interesting corollary from the animal kingdom, recently published, is the recognition that amongst males of several avian species, those which are more brightly coloured are the more fertile. In the Great Tit (Parus major), the intensity of the yellow colour of the male breast has been related to the degree of carotenoid sufficiency, which in turn has been found to protect the sperm from lipid peroxidation, and thereby improve chances of reproductive success (Helfenstein F et al Eco Letters 2010).

Oocyte quality is also affected by oxidative stress, and lower fertility rates in cigarette smokers, or in association with high levels of alcohol consumption, have been have been linked to increased ROS synthesis (Paszkowski T et al, Clin Chim Acta 236; 1995; Jensen TK et al, BMJ 1998;317; Eggert J et al, Fertil Steril 2004; 81; Ruder

EH et al 2008). As in many cellular processes, a low level of ROS synthesis fulfils an important role in cell signal transduction pathways, and in the oocyte, is a prerequisite for the first meiotic phase (MI) and also a requirement for folliculogenesis. However excessive ROS synthesis will impair oocyte maturation (MII) and inadequate intracellular antioxidant capacity, particularly low concentrations of reduced glutathione (GSH), can limit successful ovulation and fertilisation (Ruder et al, 2008). Whilst there is good evidence that dietary antioxidant supplements can modulate fertility in rodents, there is as yet very limited evidence to suggest that periconceptional antioxidant supplementation should be recommended to improve fertility in women (Ruder et al, 2008; Cetin I et al, Human Reprod Update 16; 2010).

In vitro fertilization is also affected by excessive ROS in the embryo culture media, and the routine practice of incubation in a low oxygen tension prevents embryo arrest and enhances the chance of successful fertilization. (Ruder et al;, 2008)

Obese women have a high rate of infertility, and assisted conception is often associated with a low success of oocyte fertilization, or of failure of embryo development (Pandey S and Battacharya S; Womens Health 2010). Several biomarkers of oxidative stress are increased in the blood of obese non-pregnant and pregnant individuals (Iyer A et al Nature Rev Endocrinol 2010; Jarvie E et al, Clin Science 2010). In a recent study we addressed the hypothesis that oxidative stress may be a contributory factor to reduced fertility in obese pregnancies (Igosheva N et al, PlosOne 2010). We explored the hypothesis that increased substrate availability

for mitochondrial respiration may lead to oxidative stress in the oocyte and developing embryo. It is proposed that a high plane of nutrition may lead to excessive enrichment of the reproductive milieu (Robker RL et al, J Clin Endocrinol Metab 2009) and high rates of metabolism may compromise oocyte and embryo development, potentially through excessive mitochondrial ROS synthesis. We determined whether obesity in the mouse is associated with increased mitochondrial activity in oocytes and early stage embryos. C67BL/6J mice were fed a highly palatable diet or normal laboratory chow. After six weeks of the diet, females were induced to superovulate by hCG and oocytes were collected by puncture of pre-ovulatory follicles. Zygotes and blastocysts were collected after successful mating with lean males after 24 and 84hr post hCG. Mitochondrial membrane potential, an indirect measure of mitochondrial activity, was determined with a mitochondrial specific membrane fluoroprobe (TMRM). Hyperpolarization of the membrane was observed in oocytes and zygotes retrieved from the obese females when compared to those from the lean animals. Redox status was assessed by measurement of the oxidative status of the pyridine nucleotide (NAD(P)H) and the flavine nucleotide FAD^{++} , by measurement of autofluorescence. Both showed evidence of increased oxidation in oocytes and zygotes from the obese females. Direct measurement of free radical generation in *in vitro* 'real time' using the fluorescent dye dihydroethidium (HEt) also showed clearly that obesity was associated with increased ROS synthesis. Measurement of reduced glutathione using the fluorescent dye monochlorabimane (MCB) provided evidence of reduced cellular antioxidant capacity. ROS can also affect mitochondrial abundance and copy number and we found in the oocytes that mitochondrial DNA copy number was increased together with expression of nuclear genes encoding mitochondrial DNA transcription factors

(mtTFAM and NRF1). The ability of zygotes to develop to the blastocyst stage was also reduced in the obese mice. To our knowledge this is the only study to have directly addressed redox status in oocytes and early embryos in obese animals and supports the hypothesis that oxidative stress may play a role in suboptimal fertility in obesity.

Using the same model of murine obesity our laboratory also reported previously that the offspring later become hypertensive, demonstrate glucose intolerance and are fatter than controls (Samuelsson et al, Hypertension 2008). We also reported that the adult (3 month old) male offspring of the obese dams display a decrease in the mitochondrial electron transport chain as shown by reduced mitochondrial-linked complex II-III (Shelley, McConnett et al, Am J Physiol 2009). Thus abnormal mitochondrial function is an accompaniment of obesity from the earliest stages of life. Whether this played a role in development of the phenotype described in the adult offspring of the obese dams, or whether it was a consequence remains to be determined but there is growing evidence that that the mitochondrion, which itself is particularly susceptible to DNA damage and has a modest capacity for repair, may be involved in the epigenetic processes associated with the developmental of adulthood disorders arising from nutritional imbalance *in utero* and in early post natal life (Simmons RA Rev End Met Dis 2007). Figure?

Oxidative Stress and Early Pregnancy Loss.

Oxidative stress has also been implicated in early miscarriage. Jauniaux and Burton have suggested that miscarriage may arise from premature oxygenation of the early embryonic environment. Using an O_2 probe in women prior to first trimester

termination they showed a steep rise in placental pO_2 between 8 and 12 weeks of gestation, coincident with the establishment of maternal perfusion. Oxygenation was accompanied by upregulation of a battery of antioxidant defences including increased expression of catalase, glutathione peroxidise and Cu/Zn and Mn superoxide dismutase (Jauniaux E et al, Am J Pathol 2000). The same group showed an increase in markers of oxidative stress in placental tissue from early pregnancy losses compared with controls and suggested that increased ROS generation may arise from a consequence of the premature establishment of maternal placental perfusion (Burton G, Jauniaux E, J Soc Gynecol Invest 2004). Whilst it might be anticipated that antioxidant supplements may provide some protection against miscarriage, metanalysis of all relevant studies suggests no substantive evidence for antioxidant supplements providing any benefit (Rumbold A, Middelton, Crowther, Cochrane Review 2005).

Oxidative Stress and Pre-eclampsia.

Pre-eclampsia, which affects approximately 2-7% of all pregnancies, is a syndrome associated with multi-organ dysfunction, characterised by new onset hypertension (blood pressure $\geq$140/90 mmHg) and proteinuria ($\geq$300 mg/L) after 20 weeks' gestation (Brown et al., 2001). Other complications including stroke, convulsions, pulmonary edema, liver failure and thrombus formation make this a potentially life threatening condition for the mother and child (Villar J, Say L, Gulmezoglu AM et al. Eclampsia and pre-eclampsia: a worldwide health problem for 2000 years. In Critchley H, MacLean A, Poston L, Walker J *'Pre-eclampsia'.* London (UK) RCOG Press 2003; 189-207). Infant mortality and morbidity may also be may compromised

by placental insufficiency leading to poor fetal growth. The precise mechanisms that lead to pre-eclampsia, which often occurs without warning and may follow a precipitous course are not known, but failure of the normal processes of placentation, followed by inadequate placental perfusion would seem to be a necessary prelude to the cascade of molecular events which culminate in the maternal syndrome (Redman CWG and Sargent IL, Placental Stress and Pre-eclampsia, Placenta 2009). This is characterised by a marked exaggeration of the normal, mild inflammatory response which occurs in the pregnant woman, and is accompanied by vascular endothelial cell and platelet activation, and impairment of vascular endothelial dilator function (Redman CW, Sargent IL. Preeclampsia and the systemic inflammatory response. Semin Nephrol. 2004 Nov;24(6):565-70).

The normal process of placentation involves remodelling of the maternal spiral arteries, the small resistance arteries of the uterine circulation responsible for blood supply to the placental intervillous space. The normally thick muscular wall of the spiral artery is rendered flaccid and non-contractile as the cytotrophoblast (placental epithelial cells) invade the decidua and myometrium of the uterine wall (Pijnenborg et al., 1980; Jauniaux et al., 2006). In pre-eclampsia this process is often incomplete and some vessels retain the smooth muscle layer (Whitley GS, Cartwright JE. Cellular and Molecular Regulation of Spiral Artery Remodelling: Lessons From the Cardiovascular Field. Placenta. 2010 Mar 30; doi:10.1016/j.placenta.2010.03.002). This in turn leads to reduced placental perfusion. The resultant hypoxia, together with intermittent reperfusion, is hypothesised to provoke ROS synthesis in the placenta **(**Burton, Hwang, Cindrova Davies, Placenta 2009; 23; S43**).** Numerous

studies describe elevation of markers of oxidative stress in placental tissue from women with pre-eclampsia (Raijmakers, 2004 Current Pharmaceutical Desgin; Burton, Hwang etc 2009). Periods of ischaemia followed by reperfusion are associated with conversion of xanthine dehydrogenase to xanthine oxidase which is a potent source of superoxide ($O_2^{\bullet}$) and xanthine oxidase activity is increased in the placentae of affected women (Many A, Hubel CA, Fisher SJ, Roberts JM, Zhou Y. Invasive cytotrophoblasts manifest evidence of oxidative stress in preeclampsia. Am J Pathol. 2000 Jan;156(1):321-31). Recently, Burton *et al* have shown that hypoxia in placental tissue leads to endoplasmic reticulum (ER) stress, and activation of the unfolded protein response (UPR) (Burton GJ, H-W Yung Placenta 2009; 23; S43). ER stress leads to accumulation of misfolded proteins, which is the trigger for the unfolded protein response (UPR) that aims to restore ER homeostatic balance. Failure of this mechanism, which attempts to reconfigure the folding of proteins can lead to activation of apoptotic pathways and protein synthesis inhibition, which have been implicated by Burton and colleagues in the pathway to fetal growth restriction. Moreover ER stress is coupled to ROS synthesis, and ROS synthesis will increase with the degree of maternal spiral artery malfunction (Burton GJ, H-W Yung Placenta 2009; 23; S43). Together ER stress and ROS synthesis are proposed to activate a cascade of pathways leading to cytokine release, prostaglandin synthesis, increased expression of anti-angiogenic factors (e.g. soluble Flt-1) and activation of apoptotic pathways. Other suggested contributors to ROS production include an activating autoantibody to the angiotensin 2 (ATII) receptor which is proposed to lead to ROS synthesis through activation of NADPH oxidase, a key cellular source of superoxide (Siddiqui AH, Irani RA, Blackwell SC, Ramin SM, Kellems RE, Xia Y. Angiotensin receptor agonistic autoantibody is highly prevalent in preeclampsia: correlation with

disease severity. Hypertension. 2010 Feb;55(2):386-93). As summarised in figure 1, it is hypothesised that these disturbances of placental function may be causative of the maternal syndrome.

Antioxidant Supplementation in Pre-eclampsia

The recognition of oxidative stress in the placenta, and also in the maternal circulation, prompted us to evaluate the potential benefit of prophylactic antioxidant supplementation in women with known risk of pre-eclampsia. At first we evaluated the effect of vitamin E and C supplements in women with known risk factors for pre-eclampsia (abnormal uterine artery Doppler waveform or previous pre-eclampsia) (Chappell LC, Seed PT, Briley AL, Kelly FJ, Lee R, Hunt BJ, Parmar K, Bewley SJ, Shennan AH, Steer PJ, Poston L. Effect of antioxidants on the occurrence of pre-eclampsia in women at increased risk: a randomised trial. Lancet. 1999 Sep 4;354(9181):810-6). The study was designed to test the hypothesis that antioxidants would lead to reduction of biomarkers of maternal endothelial dysfunction, the ratio of Plasminogen Activator Inhibitor (PAI)-1: PAI-2 (PAI-1:PAI-2) being chosen as the primary outcome. In this small randomised controlled trial of 283 women, we showed that supplementation with 1gm vitamin C and 400IU of vitamin E daily from around 16 weeks' of gestation until delivery was associated with significant reduction in the PAI-1:PAI-2 ratio. We also reported a reduction in the plasma concentration of 8-epi prostaglandin $F_{2\alpha}$, a marker of lipid peroxidation, in association with elevation of the plasma vitamin C and E concentrations (Chappell LC, Seed PT, Kelly FJ, Briley A, Hunt BJ, Charnock-Jones DS, Mallet A, Poston L. Vitamin C and E supplementation in women at risk of preeclampsia is associated with changes in indices of oxidative

stress and placental function. Am J Obstet Gynecol. 2002 Sep;187(3):777-84). Although not powered for pregnancy outcome the number of women who developed pre-eclampsia was lower in the antioxidant group compared with those women taking the placebo preparation. Encouraged by the evidence that antioxidants could improve oxidative stress and by the suggestion of reduced occurrence of the disease, we performed a randomised controlled trial in 2404 women, adequately powered to detect a difference in the incidence of pre-eclampsia, the primary outcome (Poston L, Briley AL, Seed PT, Kelly FJ, Shennan AH; Vitamins in Pre-eclampsia (VIP) Trial Consortium. Vitamin C and vitamin E in pregnant women at risk for pre-eclampsia (VIP trial): randomised placebo-controlled trial. Lancet. 2006 Apr 8;367(9517):1145-54). Although treatment compliance was good and despite evidence for improved antioxidant capacity in the blood, there was no difference in the number of women who developed pre-eclampsia between intervention (15%) and placebo (16%) arms. There was also a small but statistically significant increase in the incidence of low birthweight in the intervention arm (Risk Ratio 1.15 (1.02 to 1.30). Three other large randomised controlled trials including one undertaken by the WHO in developing countries have also shown a lack of effect, conclusively proving that this antioxidant regime does not prevent pre-eclampsia either in high risk (Villar J, Purwar M, Merialdi M, Zavaleta N, Thi Nhu Ngoc N, Anthony J, De Greeff A, Poston L, Shennan A; WHO Vitamin C and Vitamin E trial group. World Health Organisation multicentre randomised trial of supplementation with vitamins C and E among pregnant women at high risk for pre-eclampsia in populations of low nutritional status from developing countries. BJOG. 2009 May;116(6):780-8) or lower risk (Rumbold AR, Crowther CA, Haslam RR, Dekker GA, Robinson JS; ACTS Study Group. Vitamins C and E and the risks of preeclampsia and perinatal

complications. N Engl J Med. 2006 Apr 27;354(17):1796-806; Roberts JM, Myatt L, Spong CY, Thom EA, Hauth JC, Leveno KJ, Pearson GD, Wapner RJ, Varner MW, Thorp JM Jr, Mercer BM, Peaceman AM, Ramin SM, Carpenter MW, Samuels P, Sciscione A, Harper M, Smith WJ, Saade G, Sorokin Y, Anderson GB; Eunice Kennedy Shriver National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. Vitamins C and E to prevent complications of pregnancy-associated hypertension. N Engl J Med. 2010 Apr 8;362(14):1282-91) women. Two of these have also shown an increase in the incidence of gestational hypertension in the intervention arm (Poston; Roberts), although the observation of low birthweight found in our RCT has not been replicated in the subsequent trials.

Why do antioxidants Vitamin C and E not prevent Pre-eclampsia?

Despite overwhelming evidence for oxidative stress in pre-eclampsia, a regime of a vitamin C and E supplementation does not prevent pre-eclampsia. Amongst the several potential explanations, the first must be that oxidative stress, whilst undoubtedly present, plays no causative role in the aetiology of the disorder. This counters all the observational studies and extensive *in vitro* analyses detailing the responsible signalling pathways potentially involved. Until evidence to the contrary is presented, this explanation cannot be discounted. The second is that this antioxidant regime is inappropriate. Longitudinal blood sampling was performed in a sub-group of participants in the second trial of vitamins C and E from our group; supplementation leads to a significant fall in the plasma concentrations of γ-tocopherol (Figure 2). The vitamin E preparation was given as natural source RRR α tocopherol, the stereoisomer that is preferentially absorbed in humans. The fall in γ-

tocopherol might be anticipated since dietary α and γ tocopherol compete in the liver for the tocopherol transfer protein (TTP) which facilitates uptake into the circulation (Hosomi A, Arita M, Sato Y, Kiyose C, Ueda T, Igarashi O, Arai H, Inoue K. Affinity for alpha-tocopherol transfer protein as a determinant of the biological activities of vitamin E analogs. FEBS Lett. 1997 Jun 2;409(1):105-8). However γ-tocopherol has biological activity, including anti-inflammatory properties; lowering of the blood concentration may impair the capacity to combat the inflammatory response of pre-eclampsia (Devaraj S, Jialal I. Failure of vitamin E in clinical trials: is gamma-tocopherol the answer? Nutr Rev. 2005 Aug;63(8):290-3; Reiter E, Jiang Q, Christen S. Anti-inflammatory properties of alpha- and gamma-tocopherol. Mol Aspects Med. 2007 Oct-Dec;28(5-6):668-91). It was of interest that antioxidant supplementation also led to a significant fall in the plasma concentration of sflt-1, the anti-angiogenic soluble receptor for VEGF-1 which has been strongly implicated in development of pre-eclampsia, and a rise in placenta growth factor (PlGF) (Figure 3), but had no effect on endoglin. Studies *in vitro* have also shown that vitamins C and E prevent elevation of sFlt-1 in response to hypoxia- reperfusion in isolated placental trophoblast from normal pregnancies (Cindrova-Davies et al, Gabor Lecture Award Placenta 2009).

It is also possible that the randomised controlled trials of antioxidants have provided the antioxidant supplements at too late a stage in gestation. Although the recently reported study from the USA started antioxidants earlier in pregnancy than the other trials (9-16 weeks' gestation) (Roberts et al 2010), there has been no study to address prophylaxis over the periconceptual period. It is of interest that in a large

observational study in USA women, Bodnar et al have observed that regular use of multivitamin preparations in the peri-conceptual period was associated with a 45% reduction in pre-eclampsia risk compared with non-use (odds ratio 0.32-0.95) (Bodnar L et al. Am J Epidemiol 2006).

Alternative Strategies?

Vitamins C and E have little influence on the development of the peroxynitrite radical ($ONOO^{\bullet}$) formed from the interaction of nitric oxide ($NO^{\bullet}$, a nitrogen radical) with superoxide ($O2^{\bullet}$). In a recent study, Davidge's group showed evidence for up-regulation of the LOX- 1 receptor in the endothelium of small arteries dissected from omental biopsies obtained during Caesarean section from women with pre-eclampsia (LOX-1 is the receptor for oxidised LDL), which on gaining access to the endothelium leads to an inflammatory response, stimulating macrophage transudation across the endothelial barrier; LOX-1 expression is stimulated by peroxynitrite and these authors showed *in vitro* that a peroxynitrite scavenger prevented upregulation of LOX-1 by pre-eclamptic serum in arteries from normal pregnant women (Sankaralingam S et al, Hypertension 2009). Thus anti-peroxynitrite strategies may be an alternative evidence-based strategy for reducing oxidative stress and improving endothelial function in affected women. Here, melatonin may be a potential candidate as it is a recognised scavenger of $ONOO^{\bullet}$,

There is also a recognised association between essential micronutrient selenium status and pre-eclampsia, which may have implications for a different approach to

prevention of oxidative stress. Several selenoproteins, notable the glutathione peroxidases, play an important role in cellular antioxidant defence by reducing lipid hydroperoxides to their corresponding un-reactive alcohols and reducing free hydrogen peroxide to water (Oster & Prellwitz, 1990; Rayman, 2000). Geographically, there are wide variations in dietary selenium intake depending on the selenium content of the soil. Pre-eclampsia has been linked to lower placental tissue, blood and toenail (long term status) selenium status and to reduced activity of glutathione peroxidases (Mistry et al 2008; Atamer et al, 2005; Rayman et al., 2003). Our group recently reported increased plasma concentrations of thiobarbituric acid reactive substances (TBARS; a marker for lipid peroxidation) in maternal and cord plasma in women with pre-eclampsia compared to controls; moreover, total glutathione peroxidase activity in both maternal and cord plasma and in placental tissue was significantly reduced and plasma activity positively related to the plasma selenium concentration (Mistry, et al. 2008). Selenium supplementation therefore offers another potential strategy for pre-eclampsia prevention particularly in geographical regions such as Europe with low soil selenium content (Thomson, 2004). Further prospective, longitudinal studies are required to elucidate a 'cause or effect' relationship. A small randomised control trail of selenium supplementation (selenium in pregnancy; SPRINT) conducted in the UK is underway to asses the impact of selenium supplements on pre-eclampsia related biomarkers. Perhaps unsurprisingly, dietary selenium insufficiency has been linked to infertility through oxidative stress.

Theoretically, statins could play a role in prevention of pre-eclampsia, although safety in pregnancy must first be established. Statins offer a multi-pronged rationale as aside from improving the HDL:LDL cholesterol ratio statins have anti-inflammatory properties, as well as antioxidant effects through inhibition of NADPH oxidase (Montecucco F, Mach F. Update on statin-mediated anti-inflammatory activities in atherosclerosis. Semin Immunopathol. 2009 Jun;31(1):127-42).

In summary, oxidative stress is associated with infertility, miscarriage and pre-eclampsia, but there is at present little convincing evidence that antioxidant supplements can improve fertility, or prevent miscarriage or pre-eclampsia. The breadth of strategies attempted has not been extensive, and there is a good evidence base to indicate well conducted, adequately powered trials which utilise other approaches. Safety and ethical considerations must however remain a predominant issue in any move towards other approaches.

Figure XX: Plasma g-tocopherol concentration across gestation in 89 women on placebo and 95 women taking vitamin C and E supplementation; effect of supplementation: 0.49 (CI 0.43 – 0.57).

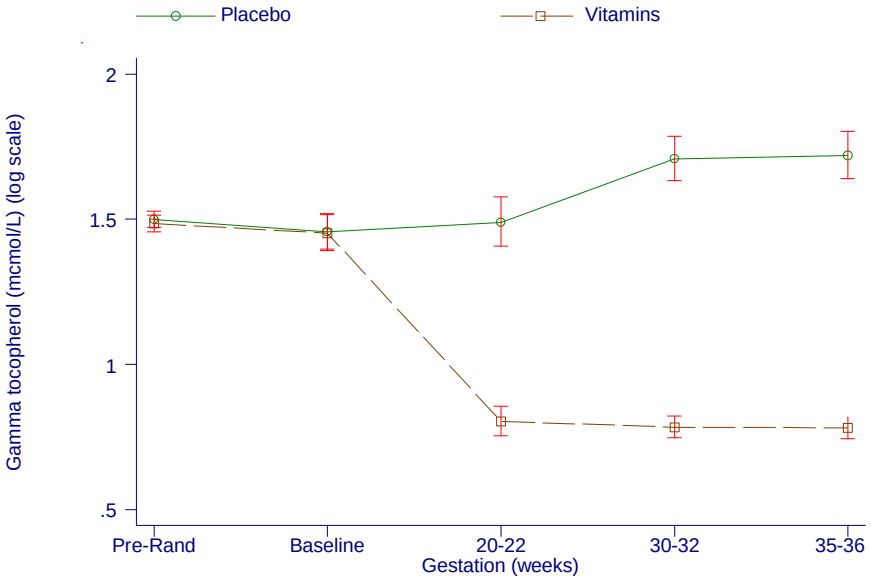

Figure XX: Plasma s-flt-1 concentration (logarithmic scale) across gestation in 45 women on placebo and 54 women taking vitamin C and E supplementation; effect of supplementation: 0.48 (CI 0.19 to 0.67 over 16 weeks), p=0.037, compared to the placebo group.

Figure XX: Plasma PlGF concentration (logarithmic scale) across gestation in 45 women on placebo and 54 women taking vitamin C and E supplementation; effect of supplementation: 2.1 (CI 1.03 to 4.40 over 16 weeks), p=0.037, compared to the placebo group.