Title: Potential targets for the treatment of preeclampsia

1 Abstract

2.1 Introduction

Preeclampsia is a disorder of pregnancy, typically characterised by hypertension and proteinuria observed after the 20th week of gestation. Preeclampsia has dire consequences for both maternal and neonatal health: it is associated with 50,000 – 100,000 annual deaths globally, as well as serious fetal and neonatal morbidity and mortality, including increased risk of fetal growth restriction and still birth. Despite the severe health, social and economic costs of preeclampsia, currently the only curative therapy is delivery of the baby and placenta, which itself carries the associated risks of premature birth. The lack of treatments for this condition is attributable to a number of causes, including but not limited to, a partial understanding of the complex pathophysiological mechanisms underlying this complex disease, an inability to sensitively predict women who will go on to develop the disease, and a paucity of robust animal models with which to test new treatments.

2.2 Areas covered

Recently, progress has been made in identifying potential new therapeutic targets. This review will discuss in detail the evidence supporting further investigation of these targets, which include angiogenic factors, agents which increase vasodilation, anti-inflammatory drugs, substances which reduce oxidative stress and statins.

2.3 Expert opinion

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004

New therapeutic targets have the potential to make a significant positive impact on maternal and neonatal health. It is exciting that a number of potential therapies are currently being investigated, however it is also vital that basic research continues to identify potential mechanisms and targets, and that any potential therapy is thoroughly tested before progression to clinical trial.

3 Keywords

Angiogenic factors, anti-inflammatory; melatonin, nitric oxide, oxidative stress, preeclampsia, sildenafil, statins, VEGF

4. Introduction

Preeclampsia is a pregnancy specific multisystem disorder characterised by new onset hypertension (systolic blood pressure ≥140 and / or diastolic blood pressure ≥90 mmHg), and involvement of one or more other organ systems after 20 weeks gestation. Proteinuria (> 300 mg / 24hrs) is the most commonly associated additional feature of preeclampsia, but is not mandatory for diagnosis. Other signs or symptoms diagnostic of preeclampsia include fetal growth restriction, neurologic sequelae (convulsions, persistent headache, persistent visual disturbance), renal impairment (oliguria or raised serum creatinine), hematologic sequelae (thrombocytopenia, haemolysis) and liver involvement (raised serum transaminases). [1] The cost of preeclampsia is high, and relates to both the direct costs of monitoring pregnancies and the morbidity and mortality directly associated with preeclampsia, but also to associated conditions such as growth restriction and prematurity. Worldwide, eclampsia (convulsions or seizures occurring as a complication of preeclampsia) alone is estimated to account for 50,000 maternal deaths a year, the majority of which occur in resource poor countries. [2]

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004

The pathophysiology of preeclampsia is complex, and poorly understood (for a recent review see [3]), but it is thought that the disease process occurs in two stages. The first stage is preclinical, and occurs as a result of aberrant placental development resulting in placental hypoxia (with or without reperfusion injury), resulting in oxidative stress, inflammation, and apoptosis of the placental syncytium. Release of placental debris into the maternal circulation triggers the release of anti-angiogenic factors and inflammatory cytokines, the consequence of which is widespread maternal endothelial dysfunction resulting in the clinical signs and symptoms of preeclampsia.

Once a diagnosis of preeclampsia is made, a decline in maternal or fetal condition is inevitable, although the onset of this may be insidious, the course unpredictable, and deterioration rapid. At present the only known cure for preeclampsia is delivery of the baby and placenta; management consists of close observation of the maternal and fetal condition, close control of blood pressure, seizure prophylaxis and timely delivery before maternal or fetal compromise occurs. Currently, there are no treatments in clinical practise that stabilise or reverse the pathology of preeclampsia.

Although incomplete, current knowledge of disease pathophysiology has identified several promising targets which may provide an opportunity for the development of new therapies that could halt or reverse disease progression. These include restoring the balance of pro- vs anti-angiogenic factors, reducing inflammation, reducing oxidative stress, and restoring the balance of vasodilation over vasoconstriction. This review will provide a brief overview of the rationale, strengths and limitations behind such potential new targets for the treatment of preeclampsia.

5. Therapies targeting the balance of pro-angiogenic vs anti-angiogenic factors

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004

All stages of placental development - including vasculogenesis, angiogenesis, cytotrophoblast invasion and spiral artery modification – are regulated by a family of angiogenic growth factors, notably vascular endothelial growth factor (VEGF) and placental growth factor (PlGF).[4] The pro-angiogenic effects of these proteins are antagonised by anti-angiogenic factors, such as soluble endoglin (s-Eng) – which inhibits capillary formation - and soluble fms like tyrosine kinase-1 (sFlt-1) - a soluble variant of the VEGF receptor that binds to free VEGF and PlGF making the factor unavailable for normal signalling activity. [5,6]

A disturbance in the balance of pro- and anti-angiogenic factors is repeatedly demonstrated in preeclamptic pregnancies. Levels of circulating PlGF[7,8] and VEGF[8] are reduced whilst placental and circulating levels of sFLT-1[6-12] or sEng[5,13] are increased. These derangements are evident weeks prior to signs or symptoms of preeclampsia occurring, and at levels correlating with disease severity, suggesting that changes are important in the aetiology of the disease. [5,6,8,9,12,14] Other support for angiogenic factor imbalance being an underlying cause of preeclampsia comes from animal models, where adenovirus mediated increases in sFLT-1 and/or s-Eng induce a preeclampsia-like phenotype in a dose dependent manner. [5,6,15]

Therapies that realign this imbalance between angiogenic and anti-angiogenic factors - by increasing expression or concentration of pro-angiogenic factors, or reducing expression or concentration of anti-angiogenic factors - may be useful treatments for preeclampsia. Adenovirus mediated overexpression of VEGF appears to neutralise the anti-angiogenic effects of excess sFlt-1, and this approach has been studied repeatedly in a range of small animal models of preeclampsia including adenoviral mediated overexpression of sFLT-1,[16,17] administration of angiotensin-1-autoantibodies,[18] reduced uterine perfusion in pregnancy (RUPP),[19] and the BPH/5 mouse strain[20] (characterised by defective trophoblast invasion,

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004

proteinuria and hypertension and a blunted increase in VEGF and PlGF compared to wild-type mice). Without exception, these studies have demonstrated the beneficial effects of VEGF therapy on hypertension and proteinuria. There is a paucity of models of preeclampsia in large animals, however, a study using adenovirus mediated overexpression of VEGF as a treatment for fetal growth restriction (FGR) in over-nourished ewes showed no adverse effect of VEGF and a reduction in severe FGR 43 days after vector injection. [21] These findings are important in the context of preeclampsia, as they demonstrate a lack of adverse effects over a more prolonged exposure than is possible in small animal studies. Beneficial effects on fetal growth are also important, as FGR often coexists with preeclampsia. The next step in developing VEGF as a clinical therapy is to assess safety in phase I clinical trials (which are currently planned). [22] If these studies show VEGF is a safe, effective treatment for FGR, it may provide an opportunity to test this therapy for the prophylaxis or treatment of preeclampsia.

One concern with VEGF treatment is the potential for adverse fetal effects with overexposure: excess VEGF has been shown to result in embryologic demise due to cardiac failure.[23] An alternative option would be to attempt to lower the circulating concentration of anti-angiogenic factors. In a small pilot study, extracorporeal apheresis was used to reduce circulating s-FLT-1 in women with preeclampsia.[24] Single or multiple treatments were used and appeared to be well tolerated, although due to the small size and pilot design of the study it is unclear whether this treatment results in prolongation of pregnancy or reduction in perinatal and maternal morbidity. [24] Other therapeutic options include inhibiting the production of sFLT-1 or the use of neutralising antibodies against s-FLT-1; however these strategies are yet to be assessed.[25]

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004

6. Therapies targeting the balance of vasodilation and vasoconstriction

As the final common pathway linking signs and symptoms of preeclampsia involves systemic endothelial dysfunction, restoring the balance of vascular tone to favour vasodilation over vasoconstriction has been suggested as a means of treating preeclampsia. The most well-studied therapies are those that target the nitric oxide pathway, however, potential new targets include those that work via mechanisms that increase hydrogen sulphide, or attenuate the effect of vasoconstrictors such as endothelin-1.

6.1 Nitric oxide based therapies

Nitric oxide (NO) is an important mediator of endothelium dependent vasodilation and myogenic tone in myometrial arteries of normal human pregnancy,[26-30] and appears to play a critical role in normal placental development. Animal models - such as genetic modification to prevent NO production, or chronic pharmacologic blockade of NO synthase by L-NAME - result in structural and functional impairments in uterine artery adaptation and reduced uterine artery blood flow. [31-34] [35] As the precipitating aetiological event of preeclampsia is thought to be abnormal placentation, and endothelial dysfunction is the hallmark feature of disease, therapies which increase NO availability are attractive possibilities for both the prevention and treatment of preeclampsia. Methods of increasing NO availability include administration of NO precursors (L-arginine), use of NO donors (such as glyceryl trinitrate (GTN)), and inhibition of the clearance of NO downstream messengers (sildenafil citrate).

6.1.1 L-Argnine

L-arginine is the sole precursor to NO[36] and administration has been shown to improve NO dependent endothelial relaxation in patients with cardiovascular risk factors. [37] One

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004

randomised controlled trial of L-arginine, in combination with antioxidant vitamins, has demonstrated a 40% reduction in risk of preeclampsia in women with a personal or close family history of preeclampsia. [38] Treatment initiation in this study varied markedly (from 14-32 weeks gestation), and it is unclear whether this influenced on outcomes. It should also be noted that over half the study participants discontinued their assigned treatment, suggesting that this therapy may not have been well tolerated or acceptable for many women. Interestingly, this study also showed a reduction in preeclampsia with antioxidant vitamins alone which is not consistent with metanalysis of other clinical trials. [39] Further well-designed controlled studies are required to clarify whether these results are reproducible and to test the efficacy of L-arginine alone as a prophylaxis for preeclampsia.

6.1.2 Sildenafil citrate

Sildenafil citrate is a phosphodiesterase-5 inhibitor which inhibits the breakdown of the NO second messenger cyclic guanosine monophosphate (cGMP). Sildenafil has been used predominantly as a treatment for male erectile dysfunction, but has also been used successfully to treat pulmonary hypertension, including its successful use in pregnancy as part of a treatment regimen for Eisenmenger's syndrome. [40,41]

Several animal studies support the efficacy of sildenafil as a treatment for preeclampsia. Catechol-methyl-transferase knockout ($COMT^{-/-}$) mice exhibit pregnancy associated hypertension, proteinuria and fetal growth restriction. [42,43] When treated with sildenafil from mid-gestation, pup weight and abdominal circumference were increased. [43] In rats where L-NAME was used to induce a model of preeclampsia, oral sildenafil from early pregnancy normalized systolic blood pressure and reduced urinary protein excretion. [44] Evidence from clinical trials of sildenafil for pregnancies complicated by preeclampsia is currently too

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004

limited to provide conclusive evidence of benefit in preeclampsia, but suggests that use may be associated with improved fetal growth. A small multicentre study randomized women with preeclampsia at 24-34 weeks gestation to placebo or sildenafil treatment (up to 80mg TDS). There were no withdrawals due to side effects, but no difference in time from randomization to delivery between the groups was observed. Median duration of treatment was 4 days in the sildenafil group compared to 4.5 days in placebo, and it is unlikely that the drug had time to affect fetal growth. [45] However, a pilot study of women with severely growth restricted pregnancies found a significant increase in abdominal circumference growth with Sildenafil treatment (25 mg three times daily), compared with non-treated controls. [46]

Current evidence suggests that sildenafil is well tolerated in pregnancy, with accumulating ex vivo and clinical data suggesting benefit in the treatment of fetal growth restriction, although evidence in favour of its effectiveness as a treatment for preeclampsia remains less robust. Recruitment has started for randomised clinical trials designed to evaluate the efficacy of sildenafil as a treatment for severe early onset FGR. While the primary outcome for these trials is morbidity-free neonatal survival, the incidence of hypertension or preeclampsia will be evaluated as a secondary outcome. These studies will also provide the opportunity to assess long term developmental effects in children exposed to sildenafil in utero.[47]

6.1.3 Nitric oxide donors

Nitric oxide (NO) donors such as glyceryl-trinitrate (GTN) are attractive treatment strategies, as they have been shown to improve uterine[48-50] and umbilical blood flow,[48,51] and appear to protect the syncytiotrophoblast from apoptosis, lipid peroxidation and superoxide formation following hypoxia –reperfusion insults. [52] Of the available routes of administration, transdermal administration appears to be the most feasible as a long-term treatment option;

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004

sublingual administration has a short duration so would potentially require half hourly dosing.[53] One small trial has evaluated the use of GTN patches vs placebo as prophylaxis in women at high risk of preeclampsia based on mid-trimester uterine artery Doppler screening. There was no significant difference in maternal blood pressure, Doppler indices, gestational age at delivery, birthweight, or incidence of small for gestational age infants between groups.[54] As GTN was started after 24-26 weeks gestation, this may have been too late to provide prophylaxis at an appropriate stage of disease development. The side effect profile of GTN - a consequence of its potent venodilatory action - may greatly limit its use as a treatment. A randomised controlled trial using a GTN patch (10mg/24hr) in women with gestational hypertension was abandoned after recruiting 16 women, as all women randomised to active patches developed severe headache within 6 hours and withdrew from the study. [55] Furthermore, treatment with GTN has been associated with falls in maternal arterial pressure and/or a rise in maternal heart rate in most studies of pregnancy. [48,49,51,53] While chronic control of blood pressure is important in preventing complications of preeclampsia, abrupt or precipitous falls in blood pressure may be harmful to an already compromised fetus.

6.2 Hydrogen sulphide

Hydrogen suphide (H_2S) is a small water soluble molecule derived from both endothelial and vascular smooth muscle cells which shares structural and functional properties with NO. H_2S causes vasorelaxation[56] including profound vasodilation of the feto-placental vasculature,[57] and has pro-angiogenic effects, as demonstrated both in vitro, and in vivo. [58] The enzymes involved in the production of H_2S - cystathionine-γ-lyase (CSE) and cystathionine-β-synthase (CBS) – are expressed in the endothelium of the fetal-placental vasculature,[59] and perturbation of RNA and protein levels have been found in association with preeclampsia.

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004

[57,59] One study has assessed the effect of an H_2S donor in adenovirus mediated sFLT-1 overexpression in (non-pregnant) rats, and observed reduced hypertension, proteinuria and a reduction in glomerular endotheliosis with treatment. [60] Further studies in animal models of preeclampsia will be important in determining if this treatment will play a useful role in the treatment of pregnancy disorders.

6.3 Inhibition of Endothelin-1

Endothelin-1 is a potent vasoconstrictor, with levels reported to be higher in women with preeclampsia than women whose pregnancies are uncomplicated. [61-64] There is some evidence to suggest that endothelin-1 may be activated by inflammatory and anti-angiogenic factors, and as levels are reported to be higher once a diagnosis of preeclampsia is established modulation of endothelin-1 may play a role in a final common pathway or progression of disease rather than in disease initiation. [65] Limited studies have assessed the effects of endothelin receptor blockade in models of preeclampsia, with some evidence of benefit. In an L-NAME model of preeclampsia, endothelin receptor antagonist (BQ123) resulted in attenuated hypertension. [66] Blockade of endothelin type A receptors in a RUPP model of preeclampsia resulted in attenuated hypertension as well as a small increase in pup weight. Interestingly, no change in arterial pressure was seen in non-pregnant animals. [67] Further animal studies are warranted to investigate the therapeutic potential and long term effects of this treatment.

7. Therapies targeting inflammation and / or immune - modulation

Preeclampsia has been associated with short and long-term systemic inflammatory changes, including increased circulating levels of cytokines, pro-inflammatory molecules and

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004

leukocyte activation. [68-71] Further support for a role of inflammation in the pathophysiology of preeclampsia can be found in animal models: inoculation with inflammatory cytokines[72,73] or endotoxin,[74] leads to hypertension and renal changes in pregnancy similar to those seen in preeclampsia; fetal biometric growth, however, appears to be conserved in these models, unlike human preeclampsia. It seems unlikely that inflammation is a primary initiating event in the pathogenesis of preeclampsia, as a temporal relationship of increased inflammatory molecules preceding the onset of the clinical disease is lacking. [68,75,76] However, a heightened inflammatory state may be triggered by initiating events (for example reduced uterine perfusion is associated with a rise in TNF-α in rats[77]) and may exacerbate disease progression by contributing to metabolic disturbance or endothelial dysfunction – as inflammatory cytokines have been shown to stimulate the release of anti-angiogenic factors. [11,73] Therefore, targeting inflammation may prove a useful therapeutic intervention once a diagnosis has been made, while potential as prophylaxis may be more limited.

In vitro data suggests that sulfasalazine - an anti-inflammatory agent– reduces the inflammatory mediated release of anti-angiogenic factors s-Eng and s-Flt-1, thereby potentially improving endothelial dysfunction. [78] A limited number of animal studies have investigated anti-inflammatory treatments in models of preeclampsia. In a rat model, where signs of preeclampsia are induced by treatment with low dose mineralocorticoids and saline, immunosuppressant therapy with azathioprine or mycophenolate mofetil attenuated hypertension, proteinuria and endothelial dysfunction in pregnancy, as well as the pro-inflammatory cytokine profile. [79] In the rat RUPP model, treatment with TNF-α inhibitor ameliorated hypertension but did not correct the associated growth restriction. [77] Further

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004

research into the effects of anti-inflammatories – on both anti-angiogenic profiles and clinically important outcomes - is needed. Some of these therapies - such as sulphasalazine - have already been used in pregnancy for the treatment of immune mediated conditions unrelated to preeclampsia, without evidence of harmful consequence. Should significant benefit be demonstrated in animal trials, the safety profile should provide an advantage in translating these studies into clinical pilot studies.

8. Therapies targeting oxidative stress

Inadequate adaptation of maternal vessels may result in fluctuations in maternal-placental blood flow, resulting not only in hypoxia but also in reperfusion injury with associated increases in oxidative stress. It is hypothesised that oxidative stress leads to apoptotic damage of the syncytium and subsequent release of pro-inflammatory cytokines and stimulation of anti-angiogenic factors, culminating in endothelial dysfunction and the signs and symptoms of preeclampsia. [80,8182] This hypothesis is supported by findings from placentae of preeclamptic women, which are characterised by increased lipid peroxides, excessive free radicals and elevated markers oxidative stress, [83-85] and suggests that anti-oxidants may prove useful in both prevention and treatment of preeclampsia.

8.1 Anti-oxidant vitamins

Epidemiologic studies have linked dietary deficiencies of antioxidant vitamins to an increased risk of preeclampsia, and anti-oxidant vitamins have been extensively studied as possible prophylaxis against preeclampsia. Antioxidant vitamins C and E have been shown to supress most effects of hypoxia –re-oxygenation *in vitro*, and small randomised trials

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004

suggested that supplementation may be beneficial at reducing the risk of preeclampsia in women at high risk of developing the condition. [86] However, more comprehensive randomised clinical trials as well as large systematic reviews have shown no benefit of antioxidant vitamins in preeclampsia prevention. [87-89] Moreover, two randomised clinical trials have treated women with severe preeclampsia with antioxidants such as Vitamin C, E, and allopurinol [90] and N acetyl-cysteine.[91] Both studies found no prolongation of pregnancy with treatment, although they were underpowered to detect a significant change in perinatal outcomes. Further combined analysis of these small studies is not possible due to differences in definitions of severe preeclampsia and the type and dose of antioxidants used, and further research – in both animals and humans - is required to identify whether the benefits suggested *in vitro* will translate to clinically meaningful outcomes.

8.2 Melatonin

Melatonin, a neuro-hormone produced by the pineal gland, is involved in regulation of circadian and seasonal physiological rhythms. In addition to its neurobiological effects, it is a potent antioxidant with properties that both reduce and prevent oxygen free radical production and damage. [92,93] The placenta expresses receptors for melatonin, and concentration of placental receptor shave been reported to be reduced in in preeclampsia. [94] These findings have led to the hypothesis that treatment with melatonin may prevent or minimise placental free-radical production and damage associated with this condition.

The benefits of melatonin in reducing oxidative stress have been demonstrated in small animal studies, where melatonin treatment reduced ischemia-reperfusion induced oxidative damage in the rat placenta,[95] and up-regulated placental antioxidants in pregnant undernourished rats. [96] Melatonin has been shown to have fetal neuro-protective effects,[97] and

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004

appears to improve umbilical artery blood flow. [98] However, conclusive evidence of an effect of melatonin on the clinical sequelae of preeclampsia – such as hypertension, proteinuria, or fetal growth – has yet to be obtained. Although melatonin treatment restored fetal growth in an undernourished rat model of growth restriction,[96] there was no beneficial effect on fetal growth in lambs of undernourished pregnant ewes. [98] Melatonin has been used to treat neonates with sepsis or distress with a good safety profile for short term use,[99,100] and animal toxicology studies have yielded good safety profiles [101] these add to the appeal of melatonin as a treatment to study in human pregnancy. Pilot studies of the use of melatonin for pregnancy complications are now under way, including a study aimed at evaluating whether melatonin can increase the time between preeclampsia diagnosis and delivery, as well as the effect of melatonin on oxidative stress, and on markers of preeclampsia.[101,102]

9. Statins

Statins are a class of pharmacologic agents that inhibit the enzyme HMG-CoA reductase, lowering low density lipoprotein production in the liver. They have been used successfully in individuals with cardiovascular disease and those with cardiovascular risk factors to improve outcomes,[103,104] and as cardiovascular disease has etiologic characteristics of inflammation and endothelial dysfunction in common with preeclampsia, statins have been proposed as a potential therapy for treatment. Statins display promising characteristics that could potentially disrupt the pathophysiology of preeclampsia at many different stages. They been shown to increase Heme-oxygenase expression (which has vasodilator and anti-inflammatory consequences[105]), inhibit sFLT-1 release both in vivo and *in vitro*,[106107-109] reduced sENG concentrations,[107] and up-regulate *in vivo* eNOS expression. [110]

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004

Early concerns regarding the teratogenicity of statins have not been supported by subsequent meta-analysis or cohort studies. [111-113] However, because of limited safety information (particularly in regards to long term effects of fetal exposure), pravastatin - the most hydrophilic and therefore least able to cross the placental barrier - is still the statin of choice for study in pregnancy complications. In different rodent studies, treatment with pravastatin has ameliorated hypertension,[109,114] restored pup growth and improved vascular reactivity. [108] Based on these findings, a pilot study of maternal and fetal safety and the pharmacokinetic profile of pravastatin as a daily prophylaxis is underway,[113] as well as a proof-of-principle-double blind randomised controlled trial designed to evaluate whether pravastatin can reduce circulating anti-angiogenic factors in women with early-onset preeclampsia.

10. Conclusion

Preeclampsia is a major complication of pregnancy, and is associated with significant maternal and perinatal morbidity and mortality. Currently there are no therapies in clinical practice which are proven to prevent, slow or reverse disease progression. There are several potential new therapeutic drugs in development which may prove beneficial in the treatment or prevention of preeclampsia; these include VEGF treatment, sildenafil citrate, melatonin and pravastatin. Ongoing research - both in animals and in clinical trials - is required to determine whether these treatments are clinically useful.

11. Expert Opinion

The area of greatest clinical challenge – and the area most likely to benefit from novel drug development - is that of early onset preeclampsia where preeclampsia develops prior to 30 weeks of gestation. In these cases, expedited delivery is often required in order to minimise

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004

risks of maternal morbidity and mortality. However, preterm delivery is associated with increased perinatal short-term morbidity and mortality, chronic complications of prematurity (such as lung-disease and cerebral palsy) and increased lifelong cardiovascular risk. Therefore, while the ultimate goal of novel therapies would be to entirely prevent the development of preeclampsia, perhaps a more realistic short-term goal is to develop therapies that ameliorate or stabilise the maternal condition (whilst facilitating normal fetal growth – see below), for a period long enough that the fetus can gain maturity *in utero* so that perinatal outcomes at delivery are improved. The length of time by which pregnancy would need to be extended to gain clinical benefit varies dependent on the gestation of onset of preeclampsia and the presence of other comorbid conditions, but at very early gestations (for example 26-27 weeks), gaining as little as a further two weeks maturity in utero may dramatically improve outcomes for these neonates.

Fetal growth restriction invariably coexists with early onset or severe preeclampsia, and shares a common aetiology of impaired placental development. Furthermore, fetal growth restriction increases the vulnerability of the neonate to all complications of prematurity. As fetal growth is such an important predictor of outcome, it is imperative that it be evaluated – both in terms of *in utero* trajectory as well as birthweight - in both pre-clinical and clinical studies of emerging therapies for preeclampsia. Furthermore, it is our opinion that the therapies that show the most promise as effective therapies for treatment of preeclampsia are those that are thought to have beneficial effect on angiogenesis and/or those been associated with improvements in fetal growth in animal studies or clinical trials, such as sildenafil citrate, or VEGF therapy.

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004

Efforts to develop novel and effective therapies for preeclampsia are hampered by deficits in our knowledge of disease pathophysiology, an absence of robust animal models of preeclampsia, and the inability to sensitively predict women and babies that will be affected by the disease (and therefore benefit most from prophylaxis). It is essential that basic science research into each of these areas continues if we are to advance in the quest to develop new and effective therapies for preeclampsia. Even if a new therapy proves effective in improving maternal signs and symptoms of preeclampsia, deferring preterm delivery and improving fetal growth, there are still questions to be answered in regards to the long-term effects of new therapies on the fetus. Planning for follow-up studies of offspring should be an early consideration in the development of new therapies, and the importance of animal studies (and the need for good models of preeclampsia in the animals studied) cannot be overstated.

In summary, there are several new and exciting possibilities for the treatment or prevention of preeclampsia which are approaching the stage of or currently undergoing clinical testing, and include adenovirus mediated VEGF treatment, sildenafil citrate, Melatonin and Pravastatin. Even for those drugs where clinical studies are already underway, conclusive evidence of benefit or no effect is still likely to be years away. In the meantime, it is imperative that basic research continue to further elucidate underlying triggers and mechanisms for this disease, and to develop robust and appropriate models to test the efficacy and safety of therapies before they are moved to clinical studies.

12. Article highlights

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004

Preeclampsia is an important cause of maternal and perinatal morbidity and mortality, but no therapies exist which can prevent, stabilise or cure this condition.

Potential therapeutic targets for preeclampsia include increasing pro-angiogenic factors, increasing vasodilation, reducing inflammation, and reducing oxidative stress.

Further research is required to determine whether new therapies are effective as well at their long term effects on offspring.

Basic scientific research into the underlying aetiology of, predictive biomarkers for, and the development of robust animal models of preeclampsia are essential in advancing the quest to develop new therapies for this condition.

13. References

1. Lowe SA, Bowyer L, Lust K, et al. The SOMANZ guideline for the management of hypertensive disorders of pregnancy. . 2014.

2. DULEY L. Maternal mortality associated with hypertensive disorders of pregnancy in africa, asia, latin america and the caribbean. *BJOG: An International Journal of Obstetrics & Gynaecology*. 1992;99(7):547-553.

3. Sircar M, Thadhani R, Karumanchi SA. Pathogenesis of preeclampsia. *Curr Opin Nephrol Hypertens*. 2015.

4. Andraweera PH, Dekker GA, Roberts CT. The vascular endothelial growth factor family in adverse pregnancy outcomes. *Human Reproduction Update*. 2012;18(4):436-457.

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004

5. Shivalingappa Venkatesha, Mourad Toporsian, Chun Lam, et al. Soluble endoglin contributes to the pathogenesis of preeclampsia. *Nat Med*. 2006;12(6):642.

6. Maynard SE, Min JY, Merchan J, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest*. 2003;111(5):649-658.

7. Shibata E, Rajakumar A, Powers RW, et al. Soluble fms-like tyrosine kinase 1 is increased in preeclampsia but not in normotensive pregnancies with small-for-gestational-age neonates: Relationship to circulating placental growth factor. *J Clin Endocrinol Metab*. 2005;90(8):4895-4903.

8. Levine RJ, Maynard SE, Qian C, et al. Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med*. 2004;350(7):672-683.

9. Rana S, Karumanchi SA, Levine RJ, et al. Sequential changes in antiangiogenic factors in early pregnancy and risk of developing preeclampsia. *Hypertension*. 2007;50(1):137-142.

10. Tsatsaris V, Goffin F, Munaut C, et al. Overexpression of the soluble vascular endothelial growth factor receptor in preeclamptic patients: Pathophysiological consequences. *J Clin Endocrinol Metab*. 2003;88(11):5555-5563.

11. Ahmad S, Ahmed A. Elevated placental soluble vascular endothelial growth factor receptor- 1 inhibits angiogenesis in preeclampsia. *Circ Res*. 2004;95(9):884-891.

12. Chaiworapongsa T, Romero R, Kim YM, et al. Plasma soluble vascular endothelial growth factor receptor-1 concentration is elevated prior to the clinical diagnosis of pre-eclampsia. *J Matern Fetal Neonatal Med*. 2005;17(1):3-18.

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004

13. Levine RJ, Lam C, Qian C, et al. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. *N Engl J Med*. 2006;355(10):992-1005.

14. Baker PN, Krasnow J, Roberts JM, Yeo KT. Elevated serum levels of vascular endothelial growth factor in patients with preeclampsia. *Obstet Gynecol*. 1995;86(5):815-821.

15. Lu F, Longo M, Tamayo E, et al. The effect of over-expression of sFlt-1 on blood pressure and the occurrence of other manifestations of preeclampsia in unrestrained conscious pregnant mice. *Obstet Gynecol*. 2007;196(4):396.e1-396.e7.

16. Bergmann A, Ahmad S, Cudmore M, et al. Reduction of circulating soluble flt- 1 alleviates preeclampsia- like symptoms in a mouse model. *J Cell Mol Med*. 2010;14(6):1857-1867.

17. Li Z, Zhang Y, Ying Ma J, et al. Recombinant vascular endothelial growth factor 121 attenuates hypertension and improves kidney damage in a rat model of preeclampsia. *Hypertension*. 2007;50(4):686-692.

18. Siddiqui AH, Irani RA, Zhang Y, et al. Recombinant vascular endothelial growth factor 121 attenuates autoantibody- induced features of pre- eclampsia in pregnant mice. *American Journal of Hypertension*. 2011;24(5):606-612.

19. Gilbert JS, Verzwyvelt J, Colson D, Arany M, Karumanchi SA, Granger JP. Recombinant vascular endothelial growth factor 121 infusion lowers blood pressure and improves renal function in rats with PlacentalIschemia-induced hypertension. *Hypertension*. 2010;55(2):380-385.

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004

20. Woods AK, Hoffmann DS, Weydert CJ, et al. Adenoviral delivery of VEGF121 early in pregnancy prevents spontaneous development of preeclampsia in BPH/5 mice. *Hypertension*. 2011;57(1):94.

21. Carr DJ, Wallace JM, Aitken RP, et al. Uteroplacental adenovirus vascular endothelial growth factor gene therapy increases fetal growth velocity in growth- restricted sheep pregnancies. *Hum Gene Ther*. 2014;25(4):375.

22. Spencer RN, C. Treatment of poor placentation and the prevention of associated adverse outcomes – what does the future hold? *Prenat Diagn*. 2014;34(7):677-684.

23. Miquerol L, L. Embryonic development is disrupted by modest increases in vascular endothelial growth factor gene expression. *Development*. 2000;127(18):3941.

24. Thadhani R, FAU - Kisner T, Kisner T, et al. Pilot study of extracorporeal removal of soluble fms-like tyrosine kinase 1 in preeclampsia. - *Circulation.2011 Aug 23;124(8):940-50.doi: 10.1161/CIRCULATIONAHA.111.034793.Epub 2011 Aug 1.* (1524-4539 (Electronic); 0009-7322 (Linking)).

25. Thadhani R. Inching towards A targeted therapy for preeclampsia. *Hypertension*. 2010;55(2):238-240.

26. Nelson SH, FAU - Steinsland OS, Steinsland OS, et al. Pregnancy augments nitric oxide-dependent dilator response to acetylcholine in the human uterine artery. - *Hum Reprod.1998 May;13(5):1361-7.* (0268-1161 (Print); 0268-1161 (Linking)).

27. Wimalasundera RC, Thom SAM, Regan L, Hughes AD. Effects of vasoactive agents on intracellular calcium and force in myometrial and subcutaneous resistance arteries isolated

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004

from preeclamptic, pregnant, and nonpregnant woman. *Obstet Gynecol*. 2005;192(2):625-632.

28. Ashworth JR, Baker PN, Warren AY, Phil M, Johnson IR. Mechanisms of endothelium-dependent relaxation in myometrial resistance vessels and their alteration in preeclampsia. *Hypertens Pregnancy*. 1999;18(1):57-71.

29. Kublickiene KR, FAU - Nisell H, Nisell H, et al. Modulation of vascular tone by nitric oxide and endothelin 1 in myometrial resistance arteries from pregnant women at term. - *Am J Obstet Gynecol.2000 Jan;182(1 Pt 1):87-93.* (0002-9378 (Print); 0002-9378 (Linking)).

30. Eckman DM, FAU - Gupta R, Gupta R, et al. Pregnancy increases myometrial artery myogenic tone via NOS- or COX-independent mechanisms. - *Am J Physiol Regul Integr Comp Physiol.2012 Aug 15;303(4):R368-75.doi: 10.1152/ajpregu.00490.2011.Epub 2012 Jun 27.* (1522-1490 (Electronic); 0363-6119 (Linking)).

31. Kulandavelu S, Qu D, Adamson SL. Cardiovascular function in mice during normal pregnancy and in the absence of endothelial NO synthase. *Hypertension*. 2006;47(6):1175-1182.

32. Kulandavelu S, FAU - Whiteley KJ, Whiteley KJ, et al. Endothelial NO synthase augments fetoplacental blood flow, placental vascularization, and fetal growth in mice. - *Hypertension.2013 Jan;61(1):259-66.doi: 10.1161/HYPERTENSIONAHA.112.201996.Epub 2012 Nov 12.* (1524-4563 (Electronic); 0194-911X (Linking)).

33. Kulandavelu S, Whiteley KJ, Qu D, Mu J, Bainbridge SA, Adamson SL. Endothelial nitric oxide synthase deficiency reduces uterine blood flow, spiral artery elongation, and placental oxygenation in pregnant mice. *Hypertension*. 2012;60(1):231-238.

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004

34. Kusinski LC, FAU - Stanley JL, Stanley JL, et al. eNOS knockout mouse as a model of fetal growth restriction with an impaired uterine artery function and placental transport phenotype. - *Am J Physiol Regul Integr Comp Physiol.2012 Jul 1;303(1):R86-93.doi: 10.1152/ajpregu.00600.2011.Epub 2012 May 2.* (1522-1490 (Electronic); 0363-6119 (Linking)).

35. Baylis C, Engels K. Adverse interactions between pregnancy and a new model of systemic hypertension produced by chronic blockade of endothelial derived relaxing factor (EDRF) in the rat. *Hypertens Pregnancy*. 1992;b11(2-3):117-129.

36. Lowe DT. Nitric oxide dysfunction in the pathophysiology of preeclampsia. - *Nitric Oxide.2000 Aug;4(4):441-58.* (1089-8603 (Print); 1089-8603 (Linking)).

37. Wu G, FAU - Meininger CJ, Meininger CJ. Arginine nutrition and cardiovascular function. - *J Nutr.2000 Nov;130(11):2626-9.* (0022-3166 (Print); 0022-3166 (Linking)).

38. Vadillo-Ortega F, Perichart-Perera O, Espino S, et al. Effect of supplementation during pregnancy with L-arginine and antioxidant vitamins in medical food on pre-eclampsia in high risk population: Randomised controlled trial. . ;342.

39. Rumbold A, FAU - Duley L, Duley L, et al. Antioxidants for preventing pre-eclampsia. - *Cochrane Database Syst Rev.2008 Jan 23;(1):CD004227.doi: 10.1002/14651858.CD004227.pub3.* (1469-493X (Electronic); 1361-6137 (Linking)).

40. Molelekwa V, FAU - Akhter P, Akhter P, et al. Eisenmenger's syndrome in a 27 week pregnancy--management with bosentan and sildenafil. - *Ir Med J.2005 Mar;98(3):87-8.* (0332-3102 (Print); 0332-3102 (Linking)).

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004

41. Lacassie HJ, FAU - Germain AM, Germain AM, et al. Management of eisenmenger syndrome in pregnancy with sildenafil and L-arginine. - *Obstet Gynecol.2004 May;103(5 Pt 2):1118-20.* (0029-7844 (Print); 0029-7844 (Linking)).

42. Kanasaki K, Palmsten K, Sugimoto H, et al. Deficiency in catechol-O-methyltransferase and 2-methoxyoestradiol is associated with pre-eclampsia. *Nature*. 2008;453(7198):1117-1121.

43. Stanley JL, FAU - Andersson IJ, Andersson IJ, et al. Sildenafil citrate rescues fetal growth in the catechol-O-methyl transferase knockout mouse model. - *Hypertension.2012 May;59(5):1021-8.doi: 10.1161/HYPERTENSIONAHA.111.186270.Epub 2012 Mar 5.* (1524-4563 (Electronic); 0194-911X (Linking)).

44. Herraiz S, FAU - Pellicer B, Pellicer B, et al. Sildenafil citrate improves perinatal outcome in fetuses from pre-eclamptic rats. - *BJOG.2012 Oct;119(11):1394-402.doi: 10.1111/j.1471-0528.2012.03430.x.Epub 2012 Jul 25.* (1471-0528 (Electronic); 1470-0328 (Linking)).

45. Samangaya RA, FAU - Mires G, Mires G, et al. A randomised, double-blinded, placebo-controlled study of the phosphodiesterase type 5 inhibitor sildenafil for the treatment of preeclampsia. - *Hypertens Pregnancy.2009 Aug;28(4):369-82.doi: 10.3109/10641950802601278.* (1525-6065 (Electronic); 1064-1955 (Linking)).

46. von Dadelszen P, FAU - Dwinnell S, Dwinnell S, et al. Sildenafil citrate therapy for severe early-onset intrauterine growth restriction. - *BJOG.2011 Apr;118(5):624-8.doi: 10.1111/j.1471-0528.2010.02879.x.* (1471-0528 (Electronic); 1470-0328 (Linking)).

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004

47. Ganzevoort W, Alfirevic Z, von Dadelszen P, et al. STRIDER: Sildenafil therapy in dismal prognosis early-onset intrauterine growth restriction--a protocol for a systematic review with individual participant data and aggregate data meta-analysis and trial sequential analysis. *Syst Rev*. 2014;3:23-4053-3-23.

48. Trapani A Jr, FAU - Goncalves LF, Goncalves LF, FAU - Pires MMdS, Pires MM. Transdermal nitroglycerin in patients with severe pre-eclampsia with placental insufficiency: Effect on uterine, umbilical and fetal middle cerebral artery resistance indices. - *Ultrasound Obstet Gynecol.2011 Oct;38(4):389-94.doi: 10.1002/uog.8983.* (1469-0705 (Electronic); 0960-7692 (Linking)).

49. Cacciatore B, FAU - Halmesmaki E, Halmesmaki E, et al. Effects of transdermal nitroglycerin on impedance to flow in the uterine, umbilical, and fetal middle cerebral arteries in pregnancies complicated by preeclampsia and intrauterine growth retardation. - *Am J Obstet Gynecol.1998 Jul;179(1):140-5.* (0002-9378 (Print); 0002-9378 (Linking)).

50. Ramsay B, FAU - De Belder A, De Belder A, et al. A nitric oxide donor improves uterine artery diastolic blood flow in normal early pregnancy and in women at high risk of pre-eclampsia. - *Eur J Clin Invest.1994 Jan;24(1):76-8.* (0014-2972 (Print); 0014-2972 (Linking)).

51. Grunewald C, FAU - Kublickas M, Kublickas M, et al. Effects of nitroglycerin on the uterine and umbilical circulation in severe preeclampsia. - *Obstet Gynecol.1995 Oct;86(4 Pt 1):600-4.* (0029-7844 (Print); 0029-7844 (Linking)).

52. Belkacemi L, FAU - Bainbridge SA, Bainbridge SA, et al. Glyceryl trinitrate inhibits hypoxia/reoxygenation-induced apoptosis in the syncytiotrophoblast of the human placenta:

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004

Therapeutic implications for preeclampsia. - *Am J Pathol.2007 Mar;170(3):909-20.* (0002-9440 (Print); 0002-9440 (Linking)).

53. Giles W, FAU - O'Callaghan S, O'Callaghan S, et al. Reduction in human fetal umbilical-placental vascular resistance by glyceryl trinitrate. - *Lancet.1992 Oct 3;340(8823):856.* (0140-6736 (Print); 0140-6736 (Linking)).

54. Lees C, FAU - Valensise H, Valensise H, et al. The efficacy and fetal-maternal cardiovascular effects of transdermal glyceryl trinitrate in the prophylaxis of pre-eclampsia and its complications: A randomized double-blind placebo-controlled trial. - *Ultrasound Obstet Gynecol.1998 Nov;12(5):334-8.* (0960-7692 (Print); 0960-7692 (Linking)).

55. Davis DG, FAU - Brown PM, Brown PM. Re: Glyceryl trinitrate (GTN) patches are unsuitable in hypertensive pregnancy. - *Aust N Z J Obstet Gynaecol.2001 Nov;41(4):474.* (0004-8666 (Print); 0004-8666 (Linking)).

56. Holwerda KM, Karumanchi SA, Lely AT. Hydrogen sulfide: Role in vascular physiology and pathology. *Curr Opin Nephrol Hypertens*. 2015;24(2):170-176.

57. Cindrova-Davies T. Reduced cystathionine γ- lyase and increased miR- 21 expression are associated with increased vascular resistance in growth- restricted pregnancies: Hydrogen sulfide as a placental vasodilator. *The American journal of pathology*. 2013;182(4):1448.

58. Cai W, Wang M, Moore PK, Jin H, Yao T, Zhu Y. *Cardiovasc Res*. 2008;76(1):29-40. doi: 10.1016/j.cardiores.2007.05.026.

59. Holwerda KM, B. Hydrogen sulfide producing enzymes in pregnancy and preeclampsia. *Placenta*. 2012;33(6):518-521.

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004

60. Holwerda KM, Burke SD, Faas MM, et al. Hydrogen sulfide attenuates sFlt1-induced hypertension and renal damage by upregulating vascular endothelial growth factor. *J Am Soc Nephrol*. 2014;25(4):717-725.

61. Sharma D, Singh A, Trivedi SS, Bhattacharjee J. Role of endothelin and inflammatory cytokines in pre-eclampsia - A pilot north indian study. *Am J Reprod Immunol*. 2011;65(4):428-432.

62. Taylor RN, Varma M, Teng NN, Roberts JM. Women with preeclampsia have higher plasma endothelin levels than women with normal pregnancies. *J Clin Endocrinol Metab*. 1990;71(6):1675-1677.

63. Dekker GA, Kraayenbrink AA, Zeeman GG, van Kamp GJ. Increased plasma levels of the novel vasoconstrictor peptide endothelin in severe pre-eclampsia. *Eur J Obstet Gynecol Reprod Biol*. 1991;40(3):215-220.

64. Clark BA, Halvorson L, Sachs B, Epstein FH. Plasma endothelin levels in preeclampsia: Elevation and correlation with uric acid levels and renal impairment. *Am J Obstet Gynecol*. 1992;166(3):962-968.

65. George EM, Palei AC, Granger JP. Endothelin as a final common pathway in the pathophysiology of preeclampsia: Therapeutic implications. *Curr Opin Nephrol Hypertens*. 2012;21(2):157-162.

66. Olson GL, Saade GR, Buhimschi I, Chwalisz K, Garfield RE. The effect of an endothelin antagonist on blood pressure in a rat model of preeclampsia. *Am J Obstet Gynecol*. 1999;181(3):638-641.

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004

67. Alexander BT, Rinewalt AN, Cockrell KL, Massey MB, Bennett WA, Granger JP. Endothelin type a receptor blockade attenuates the hypertension in response to chronic reductions in uterine perfusion pressure. *Hypertension*. 2001;37(2 Pt 2):485-489.

68. Freeman DJ, McManus F, Brown EA, et al. Short- and long-term changes in plasma inflammatory markers associated with preeclampsia. *Hypertension*. 2004;44(5):708-714.

69. Greer I,A., Lyall F, Perera T, Boswell F, Macara L,M. Increased concentrations of cytokines interleukin- 6 and interleukin- 1 receptor antagonist in plasma of women with preeclampsia: A mechanism for endothelial dysfunction? *Obstetrics & Gynecology*. 1994;84(6):937-940.

70. Conrad KP, Miles TM, Benyo DF. Circulating levels of immunoreactive cytokines in women with preeclampsia. *American Journal of Reproductive Immunology*. 1998;40(2):102-111.

71. Peracoli JC, Rudge MV, Peracoli MT. Tumor necrosis factor-alpha in gestation and puerperium of women with gestational hypertension and pre-eclampsia. *Am J Reprod Immunol*. 2007;57(3):177-185.

72. Hayakawa S, Fujikawa T, Fukuoka H, et al. Murine fetal resorption and experimental pre-eclampsia are induced by both excessive Th1 and Th2 activation. *J Reprod Immunol*. 2000;47(2):121-138.

73. Sunderland NS, Thomson SE, Heffernan SJ, et al. Tumor necrosis factor alpha induces a model of preeclampsia in pregnant baboons (papio hamadryas). *Cytokine*. 2011;56(2):192-199.

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004

74. Faas MM, Schuiling GA, Baller JF, Visscher CA, Bakker WW. A new animal model for human preeclampsia: Ultra-low-dose endotoxin infusion in pregnant rats. *Am J Obstet Gynecol*. 1994;171(1):158-164.

75. Kronborg CS, Gjedsted J, Vittinghus E, Hansen TK, Allen J, Knudsen UB. Longitudinal measurement of cytokines in pre-eclamptic and normotensive pregnancies. *Acta Obstet Gynecol Scand*. 2011;90(7):791-796.

76. Ramma W, A. Is inflammation the cause of pre-eclampsia? *Biochem Soc Trans*. 2011;39:1619-1627.

77. LaMarca B, Speed J, Fournier L, et al. Hypertension in response to chronic reductions in uterine perfusion in pregnant rats: Effect of tumor necrosis factor-Î± blockade. *Hypertension*. 2008;52(6):1161-1167.

78. Brownfoot FC, Tong S, Hannan N, Hastie R, Cannon P, Kaitu'u-Lino TJ. 273-POS]: Sulfasalazine reduces the toxins of preeclampsia soluble Flt1 and soluble endoglin and quenches endothelial dysfunction in primary human tissues: A novel potential therapeutic. *Pregnancy Hypertens*. 2015;5(1):136-137.

79. Tinsley JH, Chiasson VL, South S, Mahajan A, Mitchell BM. Immunosuppression improves blood pressure and endothelial function in a rat model of pregnancy-induced hypertension. *Am J Hypertens*. 2009;22(10):1107-1114.

80. Burton GJ, Jauniaux E. Placental oxidative stress: From miscarriage to preeclampsia. *J Soc Gynecol Investig*. 2004;11(6):342-352.

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004

81. Redman CW, Sargent IL. Latest advances in understanding preeclampsia. *Science*. 2005;308(5728):1592-1594.

82. Hung TH, Skepper JN, Burton GJ. In vitro ischemia-reperfusion injury in term human placenta as a model for oxidative stress in pathological pregnancies. *Am J Pathol*. 2001;159(3):1031-1043.

83. Hubel CA, FAU - Roberts JM, Roberts JM, et al. Lipid peroxidation in pregnancy: New perspectives on preeclampsia. - *Am J Obstet Gynecol.1989 Oct;161(4):1025-34.* (0002-9378 (Print); 0002-9378 (Linking)).

84. Siddiqui IA, FAU - Jaleel A, Jaleel A, et al. Role of oxidative stress in the pathogenesis of preeclampsia. - *Arch Gynecol Obstet.2010 Nov;282(5):469-74.doi: 10.1007/s00404-010-1538-6.Epub 2010 Jun 15.* (1432-0711 (Electronic); 0932-0067 (Linking)).

85. Patil SB, FAU - Kodliwadmath MV, Kodliwadmath MV, FAU - Kodliwadmath M, Kodliwadmath M. Lipid peroxidation and antioxidant activity in complicated pregnancies. - *Clin Exp Obstet Gynecol.2009;36(2):110-2.* (0390-6663 (Print); 0390-6663 (Linking)).

86. Chappell LC, Briley AL, Shennan AH, et al. Effect of antioxidants on the occurrence of pre- eclampsia in women at increased risk: A randomised trial. *Lancet*. 1999;354(9181):810-816.

87. Poston L, FAU - Briley AL, Briley AL, et al. 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.* (1474-547X (Electronic); 0140-6736 (Linking)).

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004

88. Rossi AC, Mullin PM. Prevention of pre-eclampsia with low-dose aspirin or vitamins C and E in women at high or low risk: A systematic review with meta-analysis. *Eur J Obstet Gynecol Reprod Biol*. 2011;158(1):9-16.

89. Rumbold A, D. Antioxidants for preventing pre- eclampsia. *The Cochrane database of systematic reviews*. 2008(1):CD004227.

90. Gulmezoglu AM, FAU - Hofmeyr GJ, Hofmeyr GJ, FAU - Oosthuisen MM, Oosthuisen MM. Antioxidants in the treatment of severe pre-eclampsia: An explanatory randomised controlled trial. - *Br J Obstet Gynaecol.1997 Jun;104(6):689-96.* (0306-5456 (Print); 0306-5456 (Linking)).

91. Roes EM, FAU - Raijmakers MT, Raijmakers MT, et al. Oral N-acetylcysteine administration does not stabilise the process of established severe preeclampsia. - *Eur J Obstet Gynecol Reprod Biol.2006 Jul;127(1):61-7.Epub 2005 Oct 21.* (0301-2115 (Print); 0301-2115 (Linking)).

92. Reiter RJ, Tan DX, Osuna C, Gitto E. Actions of melatonin in the reduction of oxidative stress. A review. *J Biomed Sci*. 2000;7(6):444-458.

93. Reiter RJ, Tan D, Tamura H, Cruz MHC, Fuentes-Broto L. Clinical relevance of melatonin in ovarian and placental physiology: A review. *Gynecological Endocrinology, 2014,*. 2014;30; Vol.30(2; 2):83; 83-89; 89.

94. Lanoix D, Guerin P, Vaillancourt C. Placental melatonin production and melatonin receptor expression are altered in preeclampsia: New insights into the role of this hormone in pregnancy. *J Pineal Res*. 2012;53(4):417-425.

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004

95. Okatani Y, W. Melatonin protects against oxidative mitochondrial damage induced in rat placenta by ischemia and reperfusion. *J Pineal Res*. 2001;31(2):173-178.

96. Richter HG, Hansell JA, Raut S, Giussani DA. Melatonin improves placental efficiency and birth weight and increases the placental expression of antioxidant enzymes in undernourished pregnancy. *J Pineal Res*. 2009;46(4):357-364.

97. Miller SL, Yan EB, Castillo-Meléndez M, Jenkin G, Walker DW. Melatonin provides neuroprotection in the late- gestation fetal sheep brain in response to umbilical cord occlusion. *Dev Neurosci*. 2005;27(2):200-210.

98. Lemley CO, Meyer AM, Camacho LE, et al. Melatonin supplementation alters uteroplacental hemodynamics and fetal development in an ovine model of intrauterine growth restriction. *American journal of physiology.Regulatory, integrative and comparative physiology*. 2012;302(4):R454.

99. Fulia F, Gitto E, Cuzzocrea S, et al. Increased levels of malondialdehyde and nitrite/ nitrate in the blood of asphyxiated newborns: Reduction by melatonin. *J Pineal Res*. 2001;31(4):343-349.

100. Gitto E, Pellegrino S, Gitto P, Barberi I, Reiter RJ. Oxidative stress of the newborn in the pre- and postnatal period and the clinical utility of melatonin. *J Pineal Res*. 2009;46(2):128-139.

101. Hobson SR, Lim R, Gardiner EE, Alers NO, Wallace EM. Phase I pilot clinical trial of antenatal maternally administered melatonin to decrease the level of oxidative stress in human pregnancies affected by pre- eclampsia (PAMPR): Study protocol. *BMJ open*. 2013;3(9):e003788.

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004

102. Alers NO, Jenkin G, Miller SL, Wallace EM. Antenatal melatonin as an antioxidant in human pregnancies complicated by fetal growth restriction--a phase I pilot clinical trial: Study protocol. *BMJ open*. 2013;3(12):e004141.

103. Brugts JJ, Yetgin T, Hoeks SE, et al. The benefits of statins in people without established cardiovascular disease but with cardiovascular risk factors: Meta-analysis of randomised controlled trials. *BMJ*. 2009;338:b2376.

104. Mills EJ, Rachlis B, Wu P, Devereaux PJ, Arora P, Perri D. Primary prevention of cardiovascular mortality and events with statin treatments: A network meta-analysis involving more than 65,000 patients. *J Am Coll Cardiol*. 2008;52(22):1769-1781.

105. Ramma W, Ahmed A. Therapeutic potential of statins and the induction of heme oxygenase-1 in preeclampsia. *J Reprod Immunol*. 2014;101–102(0):153-160.

106. Cudmore M, Ahmad S, Al-Ani B, et al. Negative regulation of soluble flt-1 and soluble endoglin release by heme oxygenase-1. *Circulation*. 2007;115(13):1789-1797.

107. Saad AF, Kechichian T, Yin H, et al. Effects of pravastatin on angiogenic and placental hypoxic imbalance in a mouse model of preeclampsia. *Reprod Sci*. 2014;21(1):138-145.

108. Costantine MM, Tamayo E, Lu F, et al. Using pravastatin to improve the vascular reactivity in a mouse model of soluble fms-like tyrosine kinase-1-induced preeclampsia. *Obstet Gynecol*. 2010;116(1):114-120.

109. Kumasawa K, Ikawa M, Kidoya H, et al. Pravastatin induces placental growth factor (PGF) and ameliorates preeclampsia in a mouse model. *Proc Natl Acad Sci U S A*. 2011;108(4):1451-1455.

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004

110. Fox KA, Longo M, Tamayo E, et al. Effects of pravastatin on mediators of vascular function in a mouse model of soluble fms-like tyrosine kinase-1-induced preeclampsia. *Am J Obstet Gynecol*. 2011;205(4):366.e1-366.e5.

111. Bateman BT, Hernandez-Diaz S, Fischer MA, et al. Statins and congenital malformations: Cohort study. *BMJ*. 2015;350:h1035.

112. Zarek J, Koren G. The fetal safety of statins: A systematic review and meta- analysis. *Journal of obstetrics and gynaecology Canada : JOGC = Journal d'obstétrique et gynécologie du Canada : JOGC*. 2014;36(6):506.

113. Costantine MM, Cleary K, Eunice Kennedy Shriver National Institute of Child Health and Human Development Obstetric--Fetal Pharmacology Research Units Network. Pravastatin for the prevention of preeclampsia in high-risk pregnant women. *Obstet Gynecol*. 2013;121(2 Pt 1):349-353.

114. Bauer AJ, Banek CT, Needham K, et al. Pravastatin attenuates hypertension, oxidative stress, and angiogenic imbalance in rat model of placental ischemia-induced hypertension. *Hypertension*. 2013;61(5):1103-1110.

This is the authors' final accepted manuscript, post peer review.
The publisher's version of record may be found at
http://dx.doi.org/10.1517/14728222.2015.1088004