Title: The Role of Aspirin, Heparin and Other Interventions in the Prevention and Treatment of Fetal Growth Restriction.

Authors: Katie M. GROOM and Anna L. DAVID.

Katie M. GROOM, Dr. MBBS BSc (Hons) PhD FRANZCOG CMFM

Department of Obstetrics & Gynaecology, University of Auckland and National Women's Health, Auckland City Hospital, Auckland, New Zealand.

Anna L. DAVID, Professor. BSc (Hons) MB ChB PhD FRCOG.

Institute for Women's Health, University College London, 86-96 Chenies Mews, London WC1E 6HX.

NIHR University College London Hospitals Biomedical Research Centre, Maple House Suite A 1st floor, 149 Tottenham Court Road, London W1T 7DN.

Katholieke Universiteit Leuven, Oude Markt 13, 3000 Leuven, Belgium.

Disclosure statement: KG reports no conflict of interest. ALD is a shareholder in Magnus Growth, a company that is aiming to take a therapy for fetal growth restriction into the clinic.

Corresponding author's contact information:

Dr Katie M. Groom

Department of Obstetrics and Gynaecology, the University of Auckland, Private Bag 92019, Auckland, New Zealand.

Telephone +64 9 373 7599 ext 89823

Email: k.groom@auckland.ac.nz

Word Count:

Abstract = 478

Main Text = 4954

Condensation: A review of the current evidence for preventative and treatment options for fetal growth restriction with some insights into new therapies on the horizon.

Short version of title: Therapeutic Interventions in Fetal Growth Restriction.

Abstract

Fetal growth restriction and related placental pathologies such as pre-eclampsia, stillbirth and placental abruption are believed to arise in early pregnancy when inadequate remodelling of the maternal spiral arteries leads to persistent high-resistance and low-flow uteroplacental circulation. The consequent placental ischaemia, re-perfusion injury and oxidative stress are associated with an imbalance in angiogenic/anti-angiogenic factors. Many interventions have centred on prevention and/or treatment of preeclampsia with results pertaining to fetal growth restriction and small for gestational age pregnancy often included as secondary outcomes because of the common pathophysiology. This renders the study findings less reliable for determining clinical significance.

For prevention of fetal growth restriction, recent large study level meta-analysis and individual patient data meta-analysis confirm that aspirin modestly reduces small for gestational age pregnancy in women at high risk (relative risk 0.90, 95%CI: 0.81-1.00) and that a dose of ≥100mg should be recommended, and to start at or before 16 weeks of gestation. These findings support national clinical practice guidelines. *In vitro* and *in vivo* studies suggest that low molecular weight heparin may prevent fetal growth restriction, however, evidence from randomised control trials is inconsistent. Meta-analysis of multi-centre trial data does not demonstrate any positive preventative effect of low molecular weight heparin on a primary composite outcome of placenta-mediated complications including fetal growth restriction; 18% vs 18%, absolute risk difference 0.6%, 95%CI: 10.4-9.2); use of low molecular weight heparin for the prevention of fetal growth restriction should remain in the research setting.

There are even fewer treatment options once fetal growth restriction is diagnosed. At present the only management option if the risk of hypoxia, acidosis and intrauterine

death is high is iatrogenic preterm birth, with the use of peri-partum maternal administration of magnesium sulphate for neuroprotection and corticosteroids for fetal lung maturity, to prevent adverse neonatal outcomes.

The pipeline of potential therapies employ different strategies, many aiming to increase fetal growth by improving poor placentation and uterine blood flow. Phosphodiesterase type-5 inhibitors that potentiate nitric oxide availability such as sildenafil citrate have been extensively researched both in preclinical and clinical studies; results from the STRIDER consortium of randomised control clinical trials are keenly awaited. Targeting the uteroplacental circulation with novel therapeutics is another approach; the most advanced being maternal VEGF gene therapy which is being translated into the clinic via the EVERREST consortium. Other targeting approaches include nanoparticles and microRNAs to deliver drugs locally to the uterine arterial endothelium or trophoblast. *In vitro* and *in vivo* studies and animal models have demonstrated effects of nitric oxide donors, dietary nitrate, hydrogen sulphide donors, statins and proton pump inhibitors on maternal blood pressure, utero-placental resistance indices and angiogenic/anti-angiogenic factors. Data from human pregnancies, and in particular, pregnancies with fetal growth restriction remains very limited. Early research into melatonin, creatine and N-acetyl cysteine supplementation in pregnancy suggests they may have potential as neuro and cardio-protective agents in fetal growth restriction.

Keywords: Fetal growth restriction, FGR, intrauterine growth restriction, IUGR, small for gestational age, SGA, preeclampsia, low molecular weight heparin, aspirin, sildenafil, VEGF gene therapy, pravastatin, nitric oxide donor, esomeprazole, melatonin, creatine, N-acetylcysteine.

Introduction

Fetal growth restriction (FGR) describes a group of conditions in which a fetus fails to reach its full growth potential. FGR is difficult to define and measure and so small for gestational age (SGA), defined by birthweight percentile, is often used as the most reliable surrogate marker. FGR and SGA may be caused by fetal issues such as chromosomal anomalies, genetic syndromes and fetal infection; maternal disease; environmental toxins including cigarette smoking; and the most common cause, utero-placental insufficiency. This article will focus on preventative and treatment options for FGR due to utero-placental insufficiency.

During early pregnancy trophoblast invasion of the maternal spiral arteries remodels and disrupts their smooth muscle layer creating a low-resistance and high-flow utero-placental circulation capable of efficient gaseous and nutrient exchange for optimal fetal growth.[1] Inadequate or abnormal trophoblast invasion results in incomplete remodelling of the spiral arteries and persistence of a high-resistance and low-flow circulation.[2, 3] It is hypothesized that this results in a sequence of events including reduced placental perfusion, placental ischaemia and re-perfusion injury[4], oxidative stress,[5] an imbalance in angiogenic factors [6-8]; vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), with anti-angiogenic factors; soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin and an increased frequency of atherosis in the placental bed.[9] Clinically these events present as the placenta-mediated complications of pregnancy; FGR, preeclampsia, placental abruption and late pregnancy loss. Placental bed biopsies in pregnancies affected by FGR and pre-eclampsia confirm that there is a major defect in myometrial spiral artery remodeling that is linked to these clinical parameters.[10-12]

The on-going adverse *in utero* environment associated with FGR ultimately may lead to hypoxic damage and stillbirth. With no proven therapeutic interventions available planned early birth must be considered and offered once a fetus reaches a viable gestational age and size. However, preterm birth then adds further morbidity and mortality risk to an already compromised neonate. There is an urgent need to identify early in pregnancy, those women at most risk of developing FGR to investigate and offer preventative therapies. Once FGR is diagnosed other strategies will be required to improve fetal growth and wellbeing, which may allow iatrogenic delivery to be delayed and/or to ameliorate the harm of the hypoxic intrauterine environment.

Prevention of FGR

Aspirin and other anti-platelet agents

The release sFlt-1 and soluble endoglin[6, 7] into the maternal circulation cause endothelial dysfunction, a feature of the placenta-mediated complications of pregnancy and in particular preeclampsia, and an imbalance in vasoactive factors such as endothelin[13], nitric oxide[14] and prostacyclin[15], resulting in reduced vasodilatation and increased vasoconstriction. Aspirin has a number of effects at the vascular level that may prevent FGR (Figure 1). For many years it was understood that aspirin suppresses the production of prostaglandins and thromboxanes through its irreversible inactivation of the cyclooxygenase enzyme. Thromboxane is a powerful vasoconstrictor and pro-thrombotic antiplatelet agent. Low-dose, long-term aspirin use irreversibly blocks the formation of thromboxane A2 in platelets, inhibiting platelet aggregation. More recently, novel cytoprotective and antioxidant mechanisms of aspirin have been observed that are independent of cyclooxygenase inhibition. Aspirin acetylates endothelial nitric oxide synthase leading to nitric oxide release from vascular endothelium.[16] In addition aspirin increases the activity of heme oxygenase-1 in endothelial cells to catabolize heme which leads to a reduction in oxidative stress, injury and inflammation.[17]

Most aspirin studies have centred on preeclampsia as a primary outcome measure with FGR included as a secondary outcome only. The volume and quality of evidence however does allow meaningful interpretation and implementation of findings.

This year there was simultaneous publication of systematic reviews based on study level meta-analysis[18] and individual patient data meta-analysis[19] of randomised trials of aspirin and other antiplatelet agents that included 20 909 and 32 217 women respectively. Both analyses supported pre-existing evidence that aspirin provides a modest risk reduction for FGR and SGA (<5th or <10th percentile) at birth; individual patient data analysis relative risk 0.90, 95% CI 0.81-1.00.[19] The difference in the conclusions of these meta-analyses arose from assessment of gestational age at initiation of therapy, before or after 16 weeks (Table 1). The individual patient data meta-analysis found that low-dose aspirin and other antiplatelet agents had a consistent effect on preeclampsia regardless of whether treatment was started before or after 16 weeks gestation.[19] Data specific to FGR supports earlier initiation of therapy where possible. In the study level meta-analysis there was a dose-response relationship for SGA when treatment was initiated ≤16 weeks, favouring a dose of 100-150mg.[18]

Studies demonstrating circadian effects of aspirin on plasma renin activity[20] and urinary excretion of cortisol, dopamine and norepinephrine[21] as well as clinical trials that show a circadian effect of aspirin to treat pre-hypertension[22] and mild hypertension[23] in non-pregnant adults suggest timing of daily dosing should be considered, particularly with reference to the prevention of preeclampsia. Two small randomised trials in pregnancy have found that evening but not morning administration of aspirin is associated with a reduction in ambulatory blood pressure[24, 25] and in one of these trials a reduction in the incidence of preeclampsia and FGR was also seen.[24] The circadian mechanism of action in the prevention of FGR seems less clear. However, if recommending daily aspirin therapy it seems prudent to recommend evening dosing.

Most national and international guidelines recommend 100-150mg aspirin dose to prevent FGR and SGA pregnancy in women at ‘high risk’.[26] However, patient selection and accurate identification of those at most risk of FGR is not clear as, like most studies of therapies for the prevention of placenta-mediated complications of pregnancy, prediction studies have been more focussed on preeclampsia rather than FGR. This is highlighted by a recent large multicentre randomised trial of aspirin to prevent preterm preeclampsia. The Aspirin for evidence-based preeclampsia prevention (ASPRE) trial used a complex algorithm including maternal factors, mean arterial pressure, uterine artery Doppler pulsatility index, and maternal serum biomarkers (maternal serum pregnancy-associated plasma protein A and placental growth factor) to identify women at high risk. Although aspirin use was associated with a reduction in preterm preeclampsia, rates of SGA <10^{th}, <5^{th} or <3^{rd} percentile were unchanged[27] suggesting alternative prediction models are required before being able to truly assess the effect of aspirin on those at highest risk.

Heparin and Low Molecular Weight Heparin

Unfractionated heparin and low molecular weight heparin (LMWH) are commonly used in pregnancy for thrombo-prophylaxis and the treatment of venous thromboembolism. More recently LMWH is preferred to unfractionated heparin and appears safe and effective for these indications.[28] Unfractionated heparin and LMWH do not cross the placenta[29] and thus pose little direct risk to the fetus. Initial interest in heparins to prevent placental pathology centred on their anticoagulant properties and presumed ability to prevent placental thrombosis and subsequent infarction leading to miscarriage. *In vitro* and *in vivo* data suggest heparins have a variety of other biological properties including anti-inflammatory[30], complement inhibition[31] and anti-tumour[32] actions as well as being pro-angiogenic[33-37] (Figure 1). These additional effects may positively influence trophoblast development and invasion making them potential candidates for the prevention of placenta-mediated complications of pregnancy including FGR.

Preclinical studies of unfractionated heparin and LMWH on angiogenesis

In vitro studies using placental villous explants found that both unfractionated heparin and LMWH promote angiogenesis.[33, 34, 36] The mechanism of action is unclear, but enhanced expression of matrix metalloproteinases may be contributory.[38] However, there are inconsistencies in *in vitro* study results with some demonstrating suppression of trophoblast invasion[39] particularly when heparin is used at therapeutic levels.[40] Further caution is raised by the finding of elevated sFlt-1 concentration and impaired VEGF signalling in endothelial cells when placental villi are exposed to LMWH at therapeutic doses,[41] although this was most significant in healthy early and term pregnancy placentae but not in placentae from pregnancies with preeclampsia and/or FGR.

Clinical studies of LMWH

In vivo use of LMWH appears to have a more positive effect on markers of angiogenesis. When used in pregnancy for anticoagulation, serum PlGF concentration is increased and there is a lower sFlt-1/PlGF ratio compared with gestation matched controls[37] and in a small randomised trial of women at high risk of preeclampsia plasma levels of PlGF were elevated one and three hours after LWMH administration, not seen in women at similar risk receiving placebo.[35]

The effect of heparin therapy on utero-placental circulation is less clear. In a small open label study of women with gestational hypertension, treatment with LMWH reduced uterine artery resistance index.[42] However, more sustained use of LMWH in a randomised control trial of LMWH and aspirin versus aspirin alone found no differences in uterine artery Doppler resistance index at 22-24 weeks or in umbilical artery Doppler pulsatility index at 22-24 weeks and later gestational ages.[43]

As early evidence suggested a relatively strong association between inherited thrombophilias and preeclampsia and FGR, initial randomized trials of heparin focused specifically on populations of women with or without thrombophilia.[44-46] More recent evidence from prospective cohort studies suggests any association of thrombophilia and placenta-mediated complications, if present, is only weak[47] and so more recent trials have included women regardless of thrombophilia status. Many trials have diverse inclusion criteria identifying women not only at high risk of FGR and preeclampsia but also earlier pregnancy complications such as recurrent miscarriage and non-placenta related conditions such as venous thromboembolism.

Results of early randomized trials were encouraging and suggested that heparin could reduce the risk of preeclampsia and FGR.[45,44] But a positive effect of LMWH was not been seen consistently across all published trials [44-46, 48-52] possibly reflecting the heterogeneity of the populations being examined, the type of LMWH being used, prolonged trial recruitment phases [44, 46] and early trial discontinuations.[45, 48]

A study level meta-analysis of six trials (848 women) demonstrated LMWH (included trials used enoxaparin, dalteparin and nadroparin) was associated with a reduction in a composite outcome (preeclampsia, birthweight <10th percentile, placental abruption or pregnancy loss >20 weeks) 18.7% vs 42.9% (relative risk 0.52, 95%CI 0.32–0.86) with similar risk reductions for a number of secondary outcomes including SGA <10th percentile and <5th percentile.[53] However, there were high levels of heterogeneity across trials and trials of higher-quality suggested no treatment effect. The same authors have subsequently completed an individual patient data meta-analysis including five trials from the study level meta-analysis and three additional trials (963 women).[54] Again a composite primary outcome (early-onset or severe preeclampsia, SGA < 5th percentile, placental abruption, and late pregnancy loss after 20 weeks).was used but with no difference seen between those treated and those untreated, 14% vs 22% (relative risk 0.64, 95%CI 0.36-1.11). Reviewing all trials data LMWH therapy was associated with a reduction in SGA <10th percentile and <5th percentile but not <3rd percentile. However, trial quality also impacted on these results with heterogeneity seen between single-centre and multicentre trials; there was no effect of LMWH seen when only considering data from multicentre trials (Table 2). In subgroup analysis, including only women with a history of a SGA infant, LWMH was not associated with any reduction in the composite primary outcome. These meta-analyses did not include sub-group analysis by type of LMWH used but a further study level meta-analysis including fewer participants (403 women in five heterogeneous trials) has compared dalteparin and enoxaparin use. Both types of LMWH were associated with a reduction in preeclampsia but only dalteparin was effective in reducing the incidence of FGR.[55]

Since the publication of the 2016 individual patient data meta-analysis[54] two further multicentre trials have been published. The Heparin-Preeclampsia (HEPEPE)[49] and Enoxaparin for Preeclampsia and Intrauterine Growth Restriction (EPPI)[52] trials included only women at high risk of placenta-mediated pregnancy complications, with or without inherited thrombophilia. The EPPI trial included a higher proportion of women with a prior history of a SGA infant than most other trials.[52] Both trials reported no difference in rates of composite primary outcomes (maternal death, perinatal death, preeclampsia, placental abruption and/or SGA < 10th percentile in the HEPEPE trial and preeclampsia and/or SGA <5th percentile in the EPPI trial).or of any secondary outcomes specific to fetal growth. These recent trials add significant participant numbers (n=406) and show consistent results with the conclusion of the published individual patient data meta-analysis, that LMWH does not reduce the risk of recurrent placenta-mediated pregnancy complications in at-risk women. If LMWH therapy is protective for the recurrence of placenta-mediated pregnancy complications, then the effect is likely to be modest and, if present, possibly confined to certain subgroups only or specific types of LMWH. Currently LMWH therapy for the prevention of FGR should be limited to the research setting. Before any future trials are undertaken further research is required to accurately phenotype women deemed to be at the highest risk to better identify those who may benefit from treatment.

Treatment of FGR

Phosphodiesterase type-5 inhibitors

Phosphodiesterase type-5 inhibitors block the phosphodiesterase enzyme preventing the inactivation of the intracellular second messenger cyclic guanosine monophosphate within vascular smooth muscle cells which potentiates the action of nitric oxide leading to vasodilatation. Maternal spiral arteries that have not undertaken complete remodelling early in pregnancy have intact or partially intact muscular layers and so potentially remain responsive to nitric oxide and amenable to vasodilatation. The majority of work investigating phosphodiesterase type-5 inhibitors and FGR has used sildenafil but more recently other agents, including the longer acting tadalafil, have been studied.

Preclinical studies

In vitro studies show that when compared to healthy control vessels, myometrial small arteries from pregnancies affected by FGR have increased vasoconstriction and reduced vasorelaxation; pre-incubation with sildenafil ameliorates this difference.[56] Work in animal models predominantly support the theory of improved fetal growth with maternal sildenafil use, however, interestingly raises some questions over the mechanism of action. In the catechol-O-methyl transferase (COMT-/-) knockout mouse model of preeclampsia and FGR[57], sildenafil in maternal drinking water in late pregnancy normalises pup growth measures and abnormal umbilical artery Doppler flow indices when compared to untreated COMT-/- controls.[58] However, this beneficial effect on feto-placental blood flow and fetal growth was not associated with increased uterine artery blood flow. Sildenafil use also increased pup weight in an alternative mouse model of FGR that has a normal vascular phenotype.[59] Alterations in placental weight may be an alternative to vasodilatation as the mechanism of action, a theory that is further supported by studies in ovine models of FGR. In maternal nutrient restricted FGR sheep pregnancy, sildenafil increased fetal growth and amnio acid availability. In addition, when FGR was created in sheep using uterine artery embolization, sildenafil improved placental and lamb weight and ameliorated the increased umbilical artery resistance but with no effect on maternal myometrial vessel resistance.[60] Not all preclinical studies however have demonstrated positive effects of sildenafil treatment on FGR with some animal models showing no effect and others showing negative and potentially harmful effects.[61, 62]

Clinical studies

Several case reports and a small randomised trial of sildenafil to selectively reduce pulmonary vascular resistance in pregnant women with pulmonary arterial hypertension demonstrate improved maternal cardiorespiratory performance and echocardiography status with better neonatal outcomes.[63-66] It also appears to be a useful adjunctive therapy for persistent pulmonary hypertension of the newborn.[67, 68] Use in pregnancy and the early neonatal period for these indications have not raised safety concerns.

Two small randomised trials have studied sildenafil treatment of preeclampsia in which 30-60% of participants had co-existing FGR.[69, 70] Both trials demonstrated positive effects on maternal blood pressure and in one trial sildenafil was associated with an increase in mean prolongation of pregnancy (14.4 days vs 10.4 days, p=0.008). No differences were seen in measures of fetal growth but compared to placebo, uterine and umbilical artery Doppler pulsatility index was reduced 24 hours after commencing sildenafil.[70]

More specific to FGR pregnancies, a single dose randomised placebo controlled trial showed that two hours after ingestion of 50mg sildenafil there was reduced resistance in the umbilical artery and increased resistance in the fetal middle cerebral artery, showing it can influence the feto-placental circulation.[71] To date, more prolonged use of sildenafil to treat FGR has only been reported in case reports[72, 73] and a small case-control study.[74] In this open study, 10 women with early-onset FGR received 25mg TDS sildenafil and were compared to 17 matched untreated control women. A higher proportion of women taking sildenafil had an increased post-eligibility fetal abdominal circumference growth velocity (90% vs 41%, odds ratio 12.9, 95% CI 1.3-126) with a tendency towards improved survival and intact survival to hospital discharge. However, it should be noted that the sildenafil treated group were eligible for the study an average of 10 days later and delivered an average of nine days after those untreated, delivering at a time (<28 weeks) when gestational age is likely to be the most significant predictor of outcome.

These limited human pregnancy studies to date have not raised specific concerns of maternal and/or fetal side effects. However, sildenafil does have a side effect profile including most commonly headache, flushing, dyspepsia, nasal congestion and impaired vision and blurred vision.[75] Fetal effects are less well known. Sildenafil is likely to cross the placenta and so effects, in particular, on pulmonary vasculature and cerebral blood flow[71] must be considered. In addition some animal studies suggest a detrimental rather positive effect on uterine blood flow and fetal wellbeing[61] and although any delay in delivery is hoped to improve long–term outcome, on-going exposure to a hostile *in-utero* environment has potential to cause greater harm than that caused by preterm delivery. The results of randomised trials of sildenafil and other phosphodiesterase type-5 inhibitors are keenly awaited. The international Sildenafil Therapy In Dismal Prognosis Early-Onset Intrauterine Growth Restriction (STRIDER) Consortium includes five placebo-controlled randomised trials in the United Kingdom[76], New Zealand and Australia[77], the Netherlands[78], Canada[79] and Ireland.[80] These trials have been conceived and designed through international collaboration and include women with early onset-FGR. Although independently funded and executed, shared data management systems and outcomes will allow assessment in prospectively planned systematic reviews including individual patient data meta-analyses.[81] Trials in the United Kingdom and New Zealand and Australia have completed participant recruitment and results are expected soon. Both these trials have childhood outcome studies underway to asses surviving children at the age of 2-3 years and provide important data on longer-term neurological and cardio-metabolic outcomes.

Maternal VEGF Gene Therapy

An alternative approach to treating FGR is to increase the levels of VEGF in the maternal uterine arteries, thus improving local vasodilatation and angiogenesis (Figure 1). This can be achieved with an adenoviral (Ad) gene therapy vector, either injected into the uterine arteries or applied to the outside of the vessels, which produces short-term VEGF expression (Ad.VEGF). This technique, called therapeutic angiogenesis has been trialled extensively for coronary artery ischaemia and is now reaching phase 3 trials.[82] Studies in large and small FGR animal models have confirmed the efficacy of this approach for improving fetal growth before birth. In normal sheep pregnancy, injection of Ad.VEGF (1×10^{11} particles), compared with injection of a control non-vasoactive vector, increased uterine artery volume blood flow within 7 days of injection, and long term, this increase in flow persisted for at least 4 weeks until the end of gestation.[83-85] The mechanism is mediated via short term VEGF expression detectable in the perivascular adventitia of the treated vessels. This is associated with increased endothelial nitric oxide synthase expression, which results in reduces vascular constriction. Long term there is vascular remodelling with a reduced intima to media ratio, increased endothelial cell proliferation in the perivascular adventitia of injected vessels, and reduced uterine artery contractile response. Importantly, there was no evidence of vector spread or expression in fetal tissues and no effect of the vector on maternal or fetal haemodynamic measures. In FGR sheep and guinea pig models, fetal growth velocity is increased, and fewer fetuses are affected by severe FGR at birth.[86-89] There appears to be amelioration of the "brain sparing effect" in FGR fetuses of treated pregnancies, with a lower brain to liver weight ratio by ultrasound measurement and at birth. Offspring born after treated FGR pregnancies have higher postnatal lean tissue mass, a faster growth rate and improved cardiovascular phenotype. In the clinical context, vector delivery into the uterine arteries could be achieved through interventional radiology, which is used as a prophylactic measure before delivery in women at high risk of postpartum haemorrhage.[90] While this is more invasive than administering oral medication, it has the potential advantage of targeting vasoactive changes to the maternal uteroplacental circulation.

The EVERREST (doEs Vascular endothelial growth factor gene therapy safEly impRove outcome in seveRe Early-onset fetal growth reSTriction) Project, which started in 2013, aims to carry out a phase I/IIa clinical trial to assess the safety and efficacy of maternal uterine artery Ad.VEGF gene therapy for severe early-onset

FGR.[91] The project, funded by the European Union, involves a multinational, multidisciplinary consortium, including experts in bioethics, fetal medicine, fetal therapy, obstetrics, and neonatology. A bioethical study found no absolute ethical, regulatory or legal objections to the use of maternal gene therapy in pregnancy, with patients welcoming the development of new drugs for this untreatable disease.[92] The consortium is performing a prospective observational study of pregnancies with severe early onset FGR to define their trial inclusion criteria, which is likely to recruit those women who are most at risk of an intrauterine death or neonatal death between 22 and 27 weeks of gestation. [93]

Nanotechnology and other uteroplacental targeting strategies to treat FGR

There are a number of other novel strategies emerging that could target drugs or particles to the uteroplacental circulation and/or the trophoblast with the aim of improving uterine blood flow, placental function or both. Tumor-homing peptide sequences CGKRK and iRGD bind selectively to the placental surface of humans and mice and do not interfere with normal development. By coating nanoparticles with these sequences, cargoes of proteins such as insulin-like growth factor 2 can be delivered specifically to the placenta.[94] Insulin-like growth factors promote placental cell proliferation and survival, and facilitate the placental uptake of glucose and amino acids. In the placenta-specific insulin-like growth factor 2 knockout mouse model of late-onset FGR[95] such nanoparticle insulin-like growth factor 2 treatment improved fetal weight.[96] Recently a novel nitric oxide donor (SE175) encapsulated into targeted liposomes has been delivered systemically to the endothelial nitric oxide synthase knockout ($eNOS^{-/-}$) mouse which exhibits impaired uteroplacental blood flow and FGR[97] leading to increased fetal weight and mean spiral artery diameter, and decreased placental weight, indicative of improved placental efficiency.[98]

Another approach has used mitochondria-targeted antioxidant MitoQ bound to nanoparticles, to localise and prevent oxidative stress in the placenta.[99] Finally, targeted micro-RNA treatment to the placenta may enhance intrinsic placental growth signaling. miR-145 and miR675 have previously been identified as negative regulators of placental growth. When applied to human first trimester trophoblast explants, conjugates of the placental homing placental homing peptide CCGKRK with these peptide-microRNAs enhanced cytotrophoblast proliferation.[100] These approaches will need careful study from a safety and efficacy perspective but they look promising for a targeted FGR treatment.

Potential drug therapies for FGR

Investigation of new drug therapies remains at the preclinical or very early clinical phases and has focussed on treatment of preeclampsia rather than FGR. Statins are lipid lowering medications with anti-inflammatory, antioxidant and angiogenic properties (Figure 1). Within small animal models of preeclampsia pravastatin reduces levels of sFlt-1 and maternal hypertension and increases VEGF and fetal weight.[101, 102] In a single non-randomised study including 21 women with antiphospholipid syndrome and treated with aspirin and LMWH the addition of pravastatin in 11 women after the onset of preeclampsia and/or FGR, appeared to delay delivery and improve pregnancy outcomes compared with 10 women who did not receive pravastatin[103]. In the Statins to Ameliorate early onset Pre-eclampsia (STAMP) randomised trial which completed recruitment in 2014 [104]; birthweight is included as a secondary outcome but results are still awaited. A further multicentre pilot study in the United States is expected to completed recruitment at the end of 2018 with rate of SGA included as a secondary outcome.[105]

Nitric oxide relaxes vascular smooth muscle cells resulting in vasodilatation (Figure 1). In women with preeclampsia short term treatment with a nitric oxide donor, isosorbide dinitrate, reduces maternal blood pressure[106, 107] and lowers resistance in umbilical artery[107, 108] and uterine artery[107] Doppler waveforms. No randomised trials of nitric oxide donors have included long term therapy or been sufficiently powered to assess any effect on pregnancy outcomes.

Hydrogen sulphide, like nitric oxide, is a gas that produces vasodilatation by acting on smooth muscle cell adenosine triphosphate-sensitive potassium channels, while its angiogenic effects appear to be mediated by VEGF and the VEGF receptor 2 (Figure 1).[109] In a sFlt-1 induced hypertensive, proteinuric rat model sodium hydrosulfide treatment resulted in elevated VEGF levels and reduced sFlt-1 levels.[110] Further work is now needed to investigate the therapeutic potential of hydrogen sulphide donors in poor placentation.

Repurposing drugs for FGR, proton pump inhibitors

As the development of new drugs or the testing of unused drugs for treatment of FGR pregnancy is difficult and costly, repurposing of existing drugs that have a known safety profile in pregnancy is an exciting area. Proton pump inhibitors such as esomeprazole have long term safety data about treatment of gastric reflux in pregnancy. *In vitro* studies show proton pump inhibitors decrease sFlt-1, soluble endoglin and improve markers of endothelial dysfunction (Figure 1),[111] while esomeprazole reduces blood pressure in a pre-eclampsia transgenic mouse model that overexpresses sFlt-1.[111] The randomised control Preeclampsia Intervention with Esomeprazole (PIE) trial will assess esomeprazole to treat early-onset preeclampsia, however, limited secondary neonatal outcomes do not include measures of fetal growth.[112]

Preventing the adverse outcomes of FGR

Amelioration of the adverse effects of FGR before delivery is an important therapeutic option. When the risks of hypoxia, acidosis and intrauterine death are deemed high and the fetus is considered to have reached a viable gestational age and size, iatrogenic preterm birth should be offered. Timely antenatal administration of corticosteroids for fetal lung maturation[113-115] and magnesium sulphate for neuroprotection[113, 116] is required to prepare for birth with careful consideration of the most appropriate mode of delivery.[117] FGR is associated with long term neurodevelopmental and cardiac impairment, likely due to oxidative stress.[118-122] Interventions are now being developed to ameliorate this antenatal insult.

Melatonin

Melatonin, an endogenous lipid soluble hormone produced by the pineal gland, exerts its powerful antioxidant effect directly by scavenging reactive oxygen species and indirectly by increasing expression of antioxidant enzymes such as glutathione peroxidase and glutathione reductase. Melatonin crosses the placenta[123] and the fetal blood brain barrier[124] and hence has potential to protect the developing fetal brain and heart from damage by oxidative stress.

In an ovine model of FGR maternal administration of melatonin protects against cardiac infarct and coronary artery stiffness, cerebral white- and grey-matter injury, abnormal cerebrovascular development with improvement in some early neurological outcomes in the offspring. A safety study of melatonin in six women with early-onset FGR (4mg BD for duration of pregnancy) found no fetal[125-127] or maternal safety concerns. Cord blood levels of melatonin were higher and placental malondialdehyde concentrations, a marker of oxidative stress, were lower in the melatonin treated group compared to control untreated women.[126] Trials of efficacy to support melatonin as a neuro- and cardio-protective agent [128] are awaited. A single on-going study in women at risk of imminent preterm delivery (not specific to FGR)[129] may provide additional information.

Creatine

Creatine is a naturally produced amino acid derivative that facilitates recycling of adenosine triphosphate and is essential for cellular energy production. As creatine can cross the placenta, maternal supplementation may increase fetal intracellular creatine and prolong cellular energy homeostasis during hypoxia, potentially providing protection for the brain and other organs in FGR pregnancies.

Maternal dietary creatine supplementation in a spiny mouse model with late gestation hypoxic injury increases neonatal survival after birth hypoxia and prevents hypoxic damage to the brain, kidney and skeletal muscle.[130-132] Studies in larger animal models with more prolonged hypoxic injury are on-going. Low maternal serum and urine creatine levels have been associated with poor fetal growth[133], but no randomised trials of maternal dietary creatine supplementation in humans have been undertaken.[134]

N-acetylcysteine

N-acetylcysteine scavenges reactive oxygen species and forms the antioxidant glutathione, thereby counteracting oxidative stress and increasing the bioavailability of nitric oxide.[135] Studies in a rat model of pre-eclampsia and FGR found that N-acetylcysteine alleviated a rise in maternal blood pressure and increased pup brain weight.[136] In a guinea pig model of maternal chronic hypoxia, administration of N-acetylcysteine did not affect pup weight but did ameliorate oxidative stress responses to hypoxia in the fetal liver.[137] However a small double-blind randomised controlled trial found that oral N-acetylcysteine did not stabilise the process of established severe preeclampsia, or improve neonatal outcome.[138] Further studies are needed to investigate whether N-acetylcysteine may prevent fetal complications of FGR.

Implications for practice

Currently clinicians have limited ability to enhance placentation and prevent FGR, partly due to the paucity of proven therapeutic options but also our inability to accurately identify those at highest risk. A 100-150mg evening dose of aspirin commenced prior to 16 weeks gestation provides a modest risk reduction in women at risk using conventional obstetric history based risk factors.

There are no proven treatments of FGR that will improve fetal growth or outcome once it is diagnosed. The only intervention clinicians can offer is iatrogenic preterm birth with timely administration of maternal corticosteroids and magnesium sulphate to improve neonatal outcome after early preterm birth. Several potential new therapies are on the horizon but many of these are being primarily investigated for preeclampsia therapy with FGR as a secondary outcome only. It is important that clinicians wait for the results of appropriately designed and powered randomised control trials specific to FGR which include information on meaningful longer-term outcomes before extrapolating positive preclinical and early clinical study findings into clinical practice.

Glossary

Ad.: Adenovirus

COMT: catechol-O-methyl transferase

eNOS: endothelial nitric oxide synthase knockout

FGR: fetal growth restriction

IPD: individual patient data

SGA: small for gestational age

sFlt-1: soluble fms-like tyrosine kinase 1

PlGF: Placental Growth Factor

VEGF: Vascular Endothelial Growth Factor

References

1. Pijnenborg R, Bland JM, Robertson WB, Brosens I. Uteroplacental arterial changes related to interstitial trophoblast migration in early human pregnancy. Placenta. 1983;4(4):397-413.
2. Brosens JJ, Pijnenborg R, Brosens IA. The myometrial junctional zone spiral arteries in normal and abnormal pregnancies: a review of the literature. Am J Obstet Gynecol. 2002;187(5):1416-23.
3. Brosens IA, Robertson WB, Dixon HG. The role of the spiral arteries in the pathogenesis of preeclampsia. Obstet Gynecol Annu. 1972;1:177-91.
4. 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):1031-43.
5. Poston L, Raijmakers MT. Trophoblast oxidative stress, antioxidants and pregnancy outcome--a review. Placenta. 2004;25 Suppl A:S72-8.
6. Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP, et al. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. N Engl J Med. 2006;355(10):992-1005.
7. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, et al. Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med. 2004;350(7):672-83.
8. Benton SJ, McCowan LM, Heazell AE, Grynspan D, Hutcheon JA, Senger C, et al. Placental growth factor as a marker of fetal growth restriction caused by placental dysfunction. Placenta. 2016;42:1-8.
9. Labarrere CA, DiCarlo HL, Bammerlin E, Hardin JW, Kim YM, Chaemsaithong P, et al. Failure of physiologic transformation of spiral arteries, endothelial and trophoblast cell activation, and acute atherosis in the basal plate of the placenta. Am J Obstet Gynecol. 2017;216(3):287 e1- e16.
10. Lyall F, Robson SC, Bulmer JN. Spiral artery remodeling and trophoblast invasion in preeclampsia and fetal growth restriction: relationship to clinical outcome. Hypertension. 2013;62(6):1046-54.
11. Khong TY, De Wolf F, Robertson WB, Brosens I. Inadequate maternal vascular response to placentation in pregnancies complicated by pre-eclampsia and by small-for-gestational age infants. Br J Obstet Gynaecol. 1986;93(10):1049-59.
12. Barber A, Robson SC, Myatt L, Bulmer JN, Lyall F. Heme oxygenase expression in human placenta and placental bed: reduced expression of placenta endothelial HO-2 in preeclampsia and fetal growth restriction. FASEB J. 2001;15(7):1158-68.
13. Nova A, Sibai BM, Barton JR, Mercer BM, Mitchell MD. Maternal plasma level of endothelin is increased in preeclampsia. Am J Obstet Gynecol. 1991;165(3):724-7.
14. Sandrim VC, Palei AC, Metzger IF, Gomes VA, Cavalli RC, Tanus-Santos JE. Nitric oxide formation is inversely related to serum levels of antiangiogenic factors soluble fms-like tyrosine kinase-1 and soluble endogline in preeclampsia. Hypertension. 2008;52(2):402-7.
15. Fitzgerald DJ, Entman SS, Mulloy K, FitzGerald GA. Decreased prostacyclin biosynthesis preceding the clinical manifestation of pregnancy-induced hypertension. Circulation. 1987;75(5):956-63.
16. Taubert D, Berkels R, Grosser N, Schroder H, Grundemann D, Schomig E. Aspirin induces nitric oxide release from vascular endothelium: a novel mechanism of action. Br J Pharmacol. 2004;143(1):159-65.
17. Grosser N, Abate A, Oberle S, Vreman HJ, Dennery PA, Becker JC, et al. Heme oxygenase-1 induction may explain the antioxidant profile of aspirin. Biochem Biophys Res Commun. 2003;308(4):956-60.
18. Roberge S, Nicolaides K, Demers S, Hyett J, Chaillet N, Bujold E. The role of aspirin dose on the prevention of preeclampsia and fetal growth restriction: systematic review and meta-analysis. Am J Obstet Gynecol. 2017;216(2):110-20 e6.
19. Meher S, Duley L, Hunter K, Askie L. Antiplatelet therapy before or after 16 weeks' gestation for preventing preeclampsia: an individual participant data meta-analysis. Am J Obstet Gynecol. 2017;216(2):121-8 e2.

20. Wang JL, Cheng HF, Harris RC. Cyclooxygenase-2 inhibition decreases renin content and lowers blood pressure in a model of renovascular hypertension. Hypertension. 1999;34(1):96-101.
21. Snoep JD, Hovens MM, Pasha SM, Frolich M, Pijl H, Tamsma JT, et al. Time-dependent effects of low-dose aspirin on plasma renin activity, aldosterone, cortisol, and catecholamines. Hypertension. 2009;54(5):1136-42.
22. Hermida RC, Ayala DE, Mojon A, Fernandez JR. Ambulatory blood pressure control with bedtime aspirin administration in subjects with prehypertension. Am J Hypertens. 2009;22(8):896-903.
23. Ayala DE, Hermida RC. Sex differences in the administration-time-dependent effects of low-dose aspirin on ambulatory blood pressure in hypertensive subjects. Chronobiol Int. 2010;27(2):345-62.
24. Ayala DE, Ucieda R, Hermida RC. Chronotherapy with low-dose aspirin for prevention of complications in pregnancy. Chronobiol Int. 2013;30(1-2):260-79.
25. Hermida RC, Ayala DE, Iglesias M, Mojon A, Silva I, Ucieda R, et al. Time-dependent effects of low-dose aspirin administration on blood pressure in pregnant women. Hypertension. 1997;30(3 Pt 2):589-95.
26. McCowan LM. Evidence-based guidelines for the management of suspected fetal growth restriction: comparison and convergence. American Journal of Obstetrics and Gynecology. 2017.
27. Rolnik DL, Wright D, Poon LC, O'Gorman N, Syngelaki A, de Paco Matallana C, et al. Aspirin versus Placebo in Pregnancies at High Risk for Preterm Preeclampsia. N Engl J Med. 2017;377(7):613-22.
28. Greer IA, Nelson-Piercy C. Low-molecular-weight heparins for thromboprophylaxis and treatment of venous thromboembolism in pregnancy: a systematic review of safety and efficacy. Blood. 2005;106(2):401-7.
29. Omri A, Delaloye JF, Andersen H, Bachmann F. Low molecular weight heparin Novo (LHN-1) does not cross the placenta during the second trimester of pregnancy. Thromb Haemost. 1989;61(1):55-6.
30. Mousavi S, Moradi M, Khorshidahmad T, Motamedi M. Anti-Inflammatory Effects of Heparin and Its Derivatives: A Systematic Review. Adv Pharmacol Sci. 2015;2015:507151.
31. Oberkersch R, Attorresi AI, Calabrese GC. Low-molecular-weight heparin inhibition in classical complement activation pathway during pregnancy. Thromb Res. 2010;125(5):e240-5.
32. Mousa SA, Petersen LJ. Anti-cancer properties of low-molecular-weight heparin: preclinical evidence. Thromb Haemost. 2009;102(2):258-67.
33. Bose P, Black S, Kadyrov M, Bartz C, Shlebak A, Regan L, et al. Adverse effects of lupus anticoagulant positive blood sera on placental viability can be prevented by heparin in vitro. Am J Obstet Gynecol. 2004;191(6):2125-31.
34. Bose P, Black S, Kadyrov M, Weissenborn U, Neulen J, Regan L, et al. Heparin and aspirin attenuate placental apoptosis in vitro: implications for early pregnancy failure. Am J Obstet Gynecol. 2005;192(1):23-30.
35. McLaughlin K, Baczyk D, Potts A, Hladunewich M, Parker JD, Kingdom JC. Low Molecular Weight Heparin Improves Endothelial Function in Pregnant Women at High Risk of Preeclampsia. Hypertension. 2017;69(1):180-8.
36. Sobel ML, Kingdom J, Drewlo S. Angiogenic response of placental villi to heparin. Obstet Gynecol. 2011;117(6):1375-83.
37. Yinon Y, Ben Meir E, Margolis L, Lipitz S, Schiff E, Mazaki-Tovi S, et al. Low molecular weight heparin therapy during pregnancy is associated with elevated circulatory levels of placental growth factor. Placenta. 2015;36(2):121-4.
38. Di Simone N, Di Nicuolo F, Sanguinetti M, Ferrazzani S, D'Alessio MC, Castellani R, et al. Low-molecular weight heparin induces in vitro trophoblast invasiveness: role of matrix metalloproteinases and tissue inhibitors. Placenta. 2007;28(4):298-304.

39. Ganapathy R, Whitley GS, Cartwright JE, Dash PR, Thilaganathan B. Effect of heparin and fractionated heparin on trophoblast invasion. Hum Reprod. 2007;22(9):2523-7.
40. Quenby S, Mountfield S, Cartwright JE, Whitley GS, Vince G. Effects of low-molecular-weight and unfractionated heparin on trophoblast function. Obstet Gynecol. 2004;104(2):354-61.
41. Drewlo S, Levytska K, Sobel M, Baczyk D, Lye SJ, Kingdom JC. Heparin promotes soluble VEGF receptor expression in human placental villi to impair endothelial VEGF signaling. J Thromb Haemost. 2011;9(12):2486-97.
42. Torricelli M, Reis FM, Florio P, Severi FM, Bocchi C, Picciolini E, et al. Low-molecular-weight heparin improves the performance of uterine artery Doppler velocimetry to predict preeclampsia and small-for-gestational age infant in women with gestational hypertension. Ultrasound Med Biol. 2006;32(9):1431-5.
43. Abheiden C, Van Hoorn ME, Hague WM, Kostense PJ, van Pampus MG, de Vries J. Does low-molecular-weight heparin influence fetal growth or uterine and umbilical arterial Doppler in women with a history of early-onset uteroplacental insufficiency and an inheritable thrombophilia? Secondary randomised controlled trial results. BJOG. 2016;123(5):797-805.
44. de Vries JI, van Pampus MG, Hague WM, Bezemer PD, Joosten JH, Investigators F. Low-molecular-weight heparin added to aspirin in the prevention of recurrent early-onset pre-eclampsia in women with inheritable thrombophilia: the FRUIT-RCT. J Thromb Haemost. 2012;10(1):64-72.
45. Rey E, Garneau P, David M, Gauthier R, Leduc L, Michon N, et al. Dalteparin for the prevention of recurrence of placental-mediated complications of pregnancy in women without thrombophilia: a pilot randomized controlled trial. J Thromb Haemost. 2009;7(1):58-64.
46. Rodger MA, Hague WM, Kingdom J, Kahn SR, Karovitch A, Sermer M, et al. Antepartum dalteparin versus no antepartum dalteparin for the prevention of pregnancy complications in pregnant women with thrombophilia (TIPPS): a multinational open-label randomised trial. Lancet. 2014;384(9955):1673-83.
47. Duffett L, Rodger M. LMWH to prevent placenta-mediated pregnancy complications: an update. Br J Haematol. 2015;168(5):619-38.
48. Martinelli I, Ruggenenti P, Cetin I, Pardi G, Perna A, Vergani P, et al. Heparin in pregnant women with previous placenta-mediated pregnancy complications: a prospective, randomized, multicenter, controlled clinical trial. Blood. 2012;119(14):3269-75.
49. Haddad B, Winer N, Chitrit Y, Houfflin-Debarge V, Chauleur C, Bages K, et al. Enoxaparin and Aspirin Compared With Aspirin Alone to Prevent Placenta-Mediated Pregnancy Complications: A Randomized Controlled Trial. Obstet Gynecol. 2016;128(5):1053-63.
50. Visser J, Ulander VM, Helmerhorst FM, Lampinen K, Morin-Papunen L, Bloemenkamp KW, et al. Thromboprophylaxis for recurrent miscarriage in women with or without thrombophilia. HABENOX: a randomised multicentre trial. Thromb Haemost. 2011;105(2):295-301.
51. Kaandorp SP, Goddijn M, van der Post JA, Hutten BA, Verhoeve HR, Hamulyak K, et al. Aspirin plus heparin or aspirin alone in women with recurrent miscarriage. N Engl J Med. 2010;362(17):1586-96.
52. Groom KM, McCowan LM, Mackay LK, Lee AC, Said JM, Kane SC, et al. Enoxaparin for the prevention of preeclampsia and intrauterine growth restriction in women with a history: a randomized trial. Am J Obstet Gynecol. 2017;216(3):296 e1- e14.
53. Rodger MA, Carrier M, Le Gal G, Martinelli I, Perna A, Rey E, et al. Meta-analysis of low-molecular-weight heparin to prevent recurrent placenta-mediated pregnancy complications. Blood. 2014;123(6):822-8.
54. Rodger MA, Gris JC, de Vries JIP, Martinelli I, Rey E, Schleussner E, et al. Low-molecular-weight heparin and recurrent placenta-mediated pregnancy complications: a meta-analysis of individual patient data from randomised controlled trials. Lancet. 2016;388(10060):2629-41.
55. Mastrolia SA, Novack L, Thachil J, Rabinovich A, Pikovsky O, Klaitman V, et al. LMWH in the prevention of preeclampsia and fetal growth restriction in women without thrombophilia. A systematic review and meta-analysis. Thromb Haemost. 2016;116(5):868-78.

56. Wareing M, Myers JE, O'Hara M, Baker PN. Sildenafil citrate (Viagra) enhances vasodilatation in fetal growth restriction. J Clin Endocrinol Metab. 2005;90(5):2550-5.
57. Kanasaki K, Palmsten K, Sugimoto H, Ahmad S, Hamano Y, Xie L, et al. Deficiency in catechol-O-methyltransferase and 2-methoxyoestradiol is associated with pre-eclampsia. Nature. 2008;453(7198):1117-21.
58. Stanley JL, Andersson IJ, Poudel R, Rueda-Clausen CF, Sibley CP, Davidge ST, et al. Sildenafil citrate rescues fetal growth in the catechol-O-methyl transferase knockout mouse model. Hypertension. 2012;59(5):1021-8.
59. Dilworth MR, Andersson I, Renshall LJ, Cowley E, Baker P, Greenwood S, et al. Sildenafil citrate increases fetal weight in a mouse model of fetal growth restriction with a normal vascular phenotype. PLoS One. 2013;8(10):e77748.
60. Oyston C, Stanley JL, Oliver MH, Bloomfield FH, Baker PN. Maternal Administration of Sildenafil Citrate Alters Fetal and Placental Growth and Fetal-Placental Vascular Resistance in the Growth-Restricted Ovine Fetus. Hypertension. 2016;68(3):760-7.
61. Miller SL, Loose JM, Jenkin G, Wallace EM. The effects of sildenafil citrate (Viagra) on uterine blood flow and well being in the intrauterine growth-restricted fetus. Am J Obstet Gynecol. 2009;200(1):102 e1-7.
62. Nassar AH, Masrouha KZ, Itani H, Nader KA, Usta IM. Effects of sildenafil in Nomega-nitro-L-arginine methyl ester-induced intrauterine growth restriction in a rat model. Am J Perinatol. 2012;29(6):429-34.
63. Lacassie HJ, Germain AM, Valdes G, Fernandez MS, Allamand F, Lopez H. Management of Eisenmenger syndrome in pregnancy with sildenafil and L-arginine. Obstet Gynecol. 2004;103(5 Pt 2):1118-20.
64. Molelekwa V, Akhter P, McKenna P, Bowen M, Walsh K. Eisenmenger's syndrome in a 27 week pregnancy--management with bosentan and sildenafil. Ir Med J. 2005;98(3):87-8.
65. Streit M, Speich R, Fischler M, Ulrich S. Successful pregnancy in pulmonary arterial hypertension associated with systemic lupus erythematosus: a case report. J Med Case Rep. 2009;3:7255.
66. Sun X, Wang K, Wang W, Li B. [Clinical study on sildenafil in treatment of pregnant women with pulmonary arterial hypertension]. Zhonghua Fu Chan Ke Za Zhi. 2014;49(6):414-8.
67. Iacovidou N, Syggelou A, Fanos V, Xanthos T. The use of sildenafil in the treatment of persistent pulmonary hypertension of the newborn: a review of the literature. Curr Pharm Des. 2012;18(21):3034-45.
68. Shah PS, Ohlsson A. Sildenafil for pulmonary hypertension in neonates. Cochrane Database Syst Rev. 2011(8):CD005494.
69. Samangaya RA, Mires G, Shennan A, Skillern L, Howe D, McLeod A, et al. A randomised, double-blinded, placebo-controlled study of the phosphodiesterase type 5 inhibitor sildenafil for the treatment of preeclampsia. Hypertens Pregnancy. 2009;28(4):369-82.
70. Trapani A, Jr., Goncalves LF, Trapani TF, Vieira S, Pires M, Pires MM. Perinatal and Hemodynamic Evaluation of Sildenafil Citrate for Preeclampsia Treatment: A Randomized Controlled Trial. Obstet Gynecol. 2016;128(2):253-9.
71. Dastjerdi MV, Hosseini S, Bayani L. Sildenafil citrate and uteroplacental perfusion in fetal growth restriction. J Res Med Sci. 2012;17(7):632-6.
72. Lin TH, Su YN, Shih JC, Hsu HC, Lee CN. Resolution of high uterine artery pulsatility index and notching following sildenafil citrate treatment in a growth-restricted pregnancy. Ultrasound Obstet Gynecol. 2012;40(5):609-10.
73. Panda S, Das A, Md Nowroz H. Sildenafil citrate in fetal growth restriction. J Reprod Infertil. 2014;15(3):168-9.
74. von Dadelszen P, Dwinnell S, Magee LA, Carleton BC, Gruslin A, Lee B, et al. Sildenafil citrate therapy for severe early-onset intrauterine growth restriction. BJOG. 2011;118(5):624-8.

75. Datasheet M. Data Sheet Silvasta 2016 [Available from: http://www.medsafe.govt.nz/profs/Datasheet/s/silvastatab.pdf.
76. Alfirevic Z, Baker P, Papageorghiou A, Johnstone E, Kenny L. STRIDER UK: A Randomized Controlled Trial of Sildenafil Therapy In Dismal Prognosis Early-Onset Intrauterine Growth Restriction 2016 [
77. Groom KM, Baker P, McCowan LM, Stone PR, Lee AC. STRIDER (NZAus): A Randomised Controlled Trial of Sildenafil Therapy In Dismal Prognosis Early-Onset Intrauterine Growth Restriction (New Zealand and Australia) 2016 [
78. Ganzevoort W, Bloemenkamp KW, Von Dadelszen P, de Groot C, de Laat M, Mol BW, et al. The Dutch STRIDER (Sildenafil TheRapy In Dismal prognosis Early-onset fetal growth Restriction) 2016 [
79. Von Dadelszen P, Lim KI, Magee LA, Lalji S. STRIDER Canada: A Randomized Controlled Trial of Sildenafil Therapy In Dismal Prognosis Early-Onset Intrauterine Growth Restriction 2016 [
80. Kenny L. The STRIDER Ireland Protocol Version 5.0 2017 [
81. Ganzevoort W, Alfirevic Z, von Dadelszen P, Kenny L, Papageorghiou A, van Wassenaer-Leemhuis A, 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.
82. Laakkonen JP, Yla-Herttuala S. Recent Advancements in Cardiovascular Gene Therapy and Vascular Biology. Hum Gene Ther. 2015;26(8):518-24.
83. David AL, Torondel B, Zachary I, Wigley V, Abi-Nader K, Mehta V, et al. Local delivery of VEGF adenovirus to the uterine artery increases vasorelaxation and uterine blood flow in the pregnant sheep. Gene Ther. 2008;15(19):1344-50.
84. Mehta V, Abi-Nader KN, Peebles DM, Benjamin E, Wigley V, Torondel B, et al. Long-term increase in uterine blood flow is achieved by local overexpression of VEGF-A(165) in the uterine arteries of pregnant sheep. Gene Ther. 2012;19(9):925-35.
85. Mehta V, Abi-Nader KN, Shangaris P, Shaw SW, Filippi E, Benjamin E, et al. Local over-expression of VEGF-DDeltaNDeltaC in the uterine arteries of pregnant sheep results in long-term changes in uterine artery contractility and angiogenesis. PLoS One. 2014;9(6):e100021.
86. Carr DJ, Wallace JM, Aitken RP, Milne JS, Martin JF, Zachary IC, et al. Peri- and Postnatal Effects of Prenatal Adenoviral VEGF Gene Therapy in Growth-Restricted Sheep. Biol Reprod. 2016;94(6):142.
87. Carr DJ, Wallace JM, Aitken RP, Milne JS, Mehta V, Martin JF, 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):375-84.
88. Mehta V, Ofir K, Swanson A, Kloczko E, Boyd M, Barker H, et al. Gene Targeting to the Uteroplacental Circulation of Pregnant Guinea Pigs. Reprod Sci. 2016;23(8):1087-95.
89. Swanson AM, Rossi CA, Ofir K, Mehta V, Boyd M, Barker H, et al. Maternal Therapy with Ad.VEGF-A165 Increases Fetal Weight at Term in a Guinea-Pig Model of Fetal Growth Restriction. Hum Gene Ther. 2016;27(12):997-1007.
90. Royal College of Obstetrics and Gynaecology RCoR. The Role of Emergency and Elective Interventional Radiology in Postpartum Haemorrhage. 2007.
91. Gancberg D, Hoeveler A, Draghia-Akli R. Introduction: Gene Therapy and Gene Transfer Projects of the 7th Framework Programme for Research and Technological Development of the European Union (Second Part). Hum Gene Ther Clin Dev. 2015;26(2):77.
92. Sheppard M, Spencer RN, Ashcroft R, David AL, Consortium E. Ethics and social acceptability of a proposed clinical trial using maternal gene therapy to treat severe early-onset fetal growth restriction. Ultrasound Obstet Gynecol. 2016;47(4):484-91.
93. Spencer R, Ambler G, Brodszki J, Diemert A, Figueras F, Gratacos E, et al. EVERREST prospective study: a 6-year prospective study to define the clinical and biological characteristics of

pregnancies affected by severe early onset fetal growth restriction. BMC Pregnancy Childbirth. 2017;17(1):43.
94. Harris LK. Could peptide-decorated nanoparticles provide an improved approach for treating pregnancy complications? Nanomedicine (Lond). 2016;11(17):2235-8.
95. Constancia M, Hemberger M, Hughes J, Dean W, Ferguson-Smith A, Fundele R, et al. Placental-specific IGF-II is a major modulator of placental and fetal growth. Nature. 2002;417(6892):945-8.
96. King A, Ndifon C, Lui S, Widdows K, Kotamraju VR, Agemy L, et al. Tumor-homing peptides as tools for targeted delivery of payloads to the placenta. Sci Adv. 2016;2(5):e1600349.
97. van der Heijden OW, Essers YP, Fazzi G, Peeters LL, De Mey JG, van Eys GJ. Uterine artery remodeling and reproductive performance are impaired in endothelial nitric oxide synthase-deficient mice. Biol Reprod. 2005;72(5):1161-8.
98. Cureton N, Korotkova I, Baker B, Greenwood S, Wareing M, Kotamraju V, et al. Selective Targeting Of A Novel Vasodilator To The Uterine Vasculature To Treat Impaired Uteroplacental Perfusion In Pregnancy. Theranostics. 2017 (in press).
99. Phillips TJ, Scott H, Menassa DA, Bignell AL, Sood A, Morton JS, et al. Treating the placenta to prevent adverse effects of gestational hypoxia on fetal brain development. Sci Rep. 2017;7(1):9079.
100. Beards F, Jones LE, Charnock J, Forbes K, Harris LK. Placental Homing Peptide-microRNA Inhibitor Conjugates for Targeted Enhancement of Intrinsic Placental Growth Signaling. Theranostics. 2017;7(11):2940-55.
101. Bauer AJ, Banek CT, Needham K, Gillham H, Capoccia S, Regal JF, et al. Pravastatin attenuates hypertension, oxidative stress, and angiogenic imbalance in rat model of placental ischemia-induced hypertension. Hypertension. 2013;61(5):1103-10.
102. Costantine MM, Tamayo E, Lu F, Bytautiene E, Longo M, Hankins GD, 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):114-20.
103. Lefkou E, Mamopoulos A, Dagklis T, Vosnakis C, Rousso D, Girardi G. Pravastatin improves pregnancy outcomes in obstetric antiphospholipid syndrome refractory to antithrombotic therapy. J Clin Invest. 2016;126(8):2933-40.
104. Statins to Ameliorate early onset Pre-eclampsia ISRCTN23410175 [
105. Pravastatin for Prevention of Preeclampsia NCT01717586 [
106. Martinez-Abundis E, Gonzalez-Ortiz M, Hernandez-Salazar F, Huerta JLMT. Sublingual isosorbide dinitrate in the acute control of hypertension in patients with severe preeclampsia. Gynecol Obstet Invest. 2000;50(1):39-42.
107. Thaler I, Amit A, Kamil D, Itskovitz-Eldor J. The effect of isosorbide dinitrate on placental blood flow and maternal blood pressure in women with pregnancy induced hypertension. Am J Hypertens. 1999;12(4 Pt 1):341-7.
108. Makino Y, Izumi H, Makino I, Shirakawa K. The effect of nitric oxide on uterine and umbilical artery flow velocity waveform in pre-eclampsia. Eur J Obstet Gynecol Reprod Biol. 1997;73(2):139-43.
109. Wang MJ, Cai WJ, Li N, Ding YJ, Chen Y, Zhu YC. The hydrogen sulfide donor NaHS promotes angiogenesis in a rat model of hind limb ischemia. Antioxid Redox Signal. 2010;12(9):1065-77.
110. Holwerda KM, Burke SD, Faas MM, Zsengeller Z, Stillman IE, Kang PM, et al. Hydrogen sulfide attenuates sFlt1-induced hypertension and renal damage by upregulating vascular endothelial growth factor. J Am Soc Nephrol. 2014;25(4):717-25.
111. Onda K, Tong S, Beard S, Binder N, Muto M, Senadheera SN, et al. Proton Pump Inhibitors Decrease Soluble fms-Like Tyrosine Kinase-1 and Soluble Endoglin Secretion, Decrease Hypertension, and Rescue Endothelial Dysfunction. Hypertension. 2017;69(3):457-68.
112. Cluver CA, Walker SP, Mol BW, Theron GB, Hall DR, Hiscock R, et al. Double blind, randomised, placebo-controlled trial to evaluate the efficacy of esomeprazole to treat early onset pre-eclampsia (PIE Trial): a study protocol. BMJ Open. 2015;5(10):e008211.

113. Preterm Labour and Birth. National Institute for Health and Care Excellence: Clinical Guidelines. London2015.

114. American College of O, Gynecologists' Committee on Obstetric P, Society for Maternal- Fetal M. Committee Opinion No.677: Antenatal Corticosteroid Therapy for Fetal Maturation. Obstet Gynecol. 2016;128(4):e187-94.

115. Panel ACCPG. Antenatal corticosteroids given to women prior to birth to improve fetal, infant, child and adult health: Clinical practice guidelines. Auckland: Liggins Institute, The University of Auckland2015.

116. Panel TAMSfNGD. Antenatal magnesium sulphate prior to preterm birth for neuroprotection of the fetus, infant and child: National clinical practice guidelines. Adelaide: The University of Adelaide2010.

117. Shah PS, Kingdom J. Antenatal Steroids, Magnesium Sulfate, and Mode of Delivery in Fetal Growth Restriction Amercian Journal of Obstetrics and Gynecology. 2017.

118. Barker DJ, Larsen G, Osmond C, Thornburg KL, Kajantie E, Eriksson JG. The placental origins of sudden cardiac death. Int J Epidemiol. 2012;41(5):1394-9.

119. Crispi F, Hernandez-Andrade E, Pelsers MM, Plasencia W, Benavides-Serralde JA, Eixarch E, et al. Cardiac dysfunction and cell damage across clinical stages of severity in growth-restricted fetuses. Am J Obstet Gynecol. 2008;199(3):254 e1-8.

120. Kallankari H, Kaukola T, Olsen P, Ojaniemi M, Hallman M. Very preterm birth and foetal growth restriction are associated with specific cognitive deficits in children attending mainstream school. Acta Paediatr. 2015;104(1):84-90.

121. Lees CC, Marlow N, van Wassenaer-Leemhuis A, Arabin B, Bilardo CM, Brezinka C, et al. 2 year neurodevelopmental and intermediate perinatal outcomes in infants with very preterm fetal growth restriction (TRUFFLE): a randomised trial. Lancet. 2015;385(9983):2162-72.

122. McCarton CM, Wallace IF, Divon M, Vaughan HG, Jr. Cognitive and neurologic development of the premature, small for gestational age infant through age 6: comparison by birth weight and gestational age. Pediatrics. 1996;98(6 Pt 1):1167-78.

123. Miller SL, Yan EB, Castillo-Melendez 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(2-4):200-10.

124. Welin AK, Svedin P, Lapatto R, Sultan B, Hagberg H, Gressens P, et al. Melatonin reduces inflammation and cell death in white matter in the mid-gestation fetal sheep following umbilical cord occlusion. Pediatr Res. 2007;61(2):153-8.

125. Castillo-Melendez M, Yawno T, Sutherland A, Jenkin G, Wallace EM, Miller SL. Effects of Antenatal Melatonin Treatment on the Cerebral Vasculature in an Ovine Model of Fetal Growth Restriction. Dev Neurosci. 2017;39(1-4):323-37.

126. Miller SL, Yawno T, Alers NO, Castillo-Melendez M, Supramaniam VG, VanZyl N, et al. Antenatal antioxidant treatment with melatonin to decrease newborn neurodevelopmental deficits and brain injury caused by fetal growth restriction. J Pineal Res. 2014;56(3):283-94.

127. Tare M, Parkington HC, Wallace EM, Sutherland AE, Lim R, Yawno T, et al. Maternal melatonin administration mitigates coronary stiffness and endothelial dysfunction, and improves heart resilience to insult in growth restricted lambs. J Physiol. 2014;592(12):2695-709.

128. Wilkinson D, Shepherd E, Wallace EM. Melatonin for women in pregnancy for neuroprotection of the fetus. Cochrane Database Syst Rev. 2016;3:CD010527.

129. Therapeutic Effects of Maternal Melatonin Administration on Brain Injury and White Matter Disease (PREMELIP): ClinicalTrials.gov; 2015 [

130. Ellery SJ, Ireland Z, Kett MM, Snow R, Walker DW, Dickinson H. Creatine pretreatment prevents birth asphyxia-induced injury of the newborn spiny mouse kidney. Pediatr Res. 2013;73(2):201-8.

131. Ireland Z, Dickinson H, Snow R, Walker DW. Maternal creatine: does it reach the fetus and improve survival after an acute hypoxic episode in the spiny mouse (Acomys cahirinus)? Am J Obstet Gynecol. 2008;198(4):431 e1-6.
132. LaRosa DA, Ellery SJ, Snow RJ, Walker DW, Dickinson H. Maternal creatine supplementation during pregnancy prevents acute and long-term deficits in skeletal muscle after birth asphyxia: a study of structure and function of hind limb muscle in the spiny mouse. Pediatr Res. 2016;80(6):852-60.
133. Dickinson H, Davies-Tuck M, Ellery SJ, Grieger JA, Wallace EM, Snow RJ, et al. Maternal creatine in pregnancy: a retrospective cohort study. BJOG. 2016;123(11):1830-8.
134. Dickinson H, Bain E, Wilkinson D, Middleton P, Crowther CA, Walker DW. Creatine for women in pregnancy for neuroprotection of the fetus. Cochrane Database Syst Rev. 2014(12):CD010846.
135. Bisseling TM, Maria Roes E, Raijmakers MT, Steegers EA, Peters WH, Smits P. N-acetylcysteine restores nitric oxide-mediated effects in the fetoplacental circulation of preeclamptic patients. Am J Obstet Gynecol. 2004;191(1):328-33.
136. Chang EY, Barbosa E, Paintlia MK, Singh A, Singh I. The use of N-acetylcysteine for the prevention of hypertension in the reduced uterine perfusion pressure model for preeclampsia in Sprague-Dawley rats. Am J Obstet Gynecol. 2005;193(3 Pt 2):952-6.
137. Hashimoto K, Pinkas G, Evans L, Liu H, Al-Hasan Y, Thompson LP. Protective effect of N-acetylcysteine on liver damage during chronic intrauterine hypoxia in fetal guinea pig. Reprod Sci. 2012;19(9):1001-9.
138. Roes EM, Raijmakers MT, Boo TM, Zusterzeel PL, Merkus HM, Peters WH, et al. Oral N-acetylcysteine administration does not stabilise the process of established severe preeclampsia. Eur J Obstet Gynecol Reprod Biol. 2006;127(1):61-7.

Tables

Table 1. Effect of gestational age at initiation of aspirin therapy for prevention of FGR or SGA at birth.

	Relative Risk	**95% CI**
Study level meta-analysis [53] (FGR)		
≤16 weeks	0.56	0.44-0.70
>16 weeks	0.95	0.86-1.05
IPD meta-analysis [54] (SGA)		
<16 weeks	0.76	0.61-0.94
≥16 weeks	0.95	0.84-1.08

Study level meta-analysis [53] used FGR as outcome to assess fetal size, defined as birthweight <10th or <5th percentile for gestational age or similar definition

IPD meta-analysis [54] used SGA as outcome to assess fetal size; SGA at birth was as defined by individual triallists, including centile charts and cut-off point used

FGR – fetal growth restriction

SGA – small for gestational age

IPD – individual patient data

Table 2. Primary and fetal growth outcomes from individual patient data meta-analysis of LMWH trials for the prevention of recurrence of placenta-mediated pregnancy complications.

	All trials			**Multicentre trials**			**Single-centre trials**		
	LMWH	*No LMWH*	*Absolute difference (95%CI), p value*	*LMWH*	*No LMWH*	*Absolute difference (95%CI), p value*	*LMWH*	*No LMWH*	*Absolute difference (95%CI) p value*
Primary composite outcome†	62/444 (14%)	95/433 (22%)	-8.0% (-17.3 to 1.4) p=0.09	47/263 (18%)	47/255 (18%)	-0.6% (-10.4 to 9.2) p=0.91	15/181 (8%)	48/178 (27%)	-18.7% (-21.6 to -15.7) p<0.0001
SGA <10th percentile	61/444 (14%)	94/429 (22%)	-8.2% (-5.4 to -0.1) p=0.009	47/263 (18%)	53/251 (21%)	-3.2% (-9.6 to 3.1) p=0.32	14/181 (8%)	41/178 (23%)	-15.3% (-19.1 to -11.5) p<0.0001
SGA <5th percentile	27/443 (6%)	38/429 (9%)	-2.8% (-5.4 to -0.1) p=0.042	22/262 (8%)	23/251 (9%)	-0.8% (-3.7 to 0.2) p=0.61	5/181 (3%)	15/178 (8%)	-5.7% (-6.1 to -5.2) p<0.0001
SGA <3rd percentile	13/443 (3%)	12/249 (3%)	0.1% (-1.9 to 2.2) p=0.89	13/262 (5%)	9/251 (4%)	1.4% (-1.3 to 4.1) p=0.32	0/181	3/178 (2%)	*

Data extracted from Rodger et al 2016 [54]

Data expressed as number (percentage)

LMWH - low molecular weight heparin

SGA - small for gestational age

†Primary composite outcome includes early-onset or severe preeclampsia, or SGA $<5^{th}$ percentile or placental abruption, or pregnancy loss ≥20 weeks gestation.

*Expected counts less than five and so no formal testing performed.

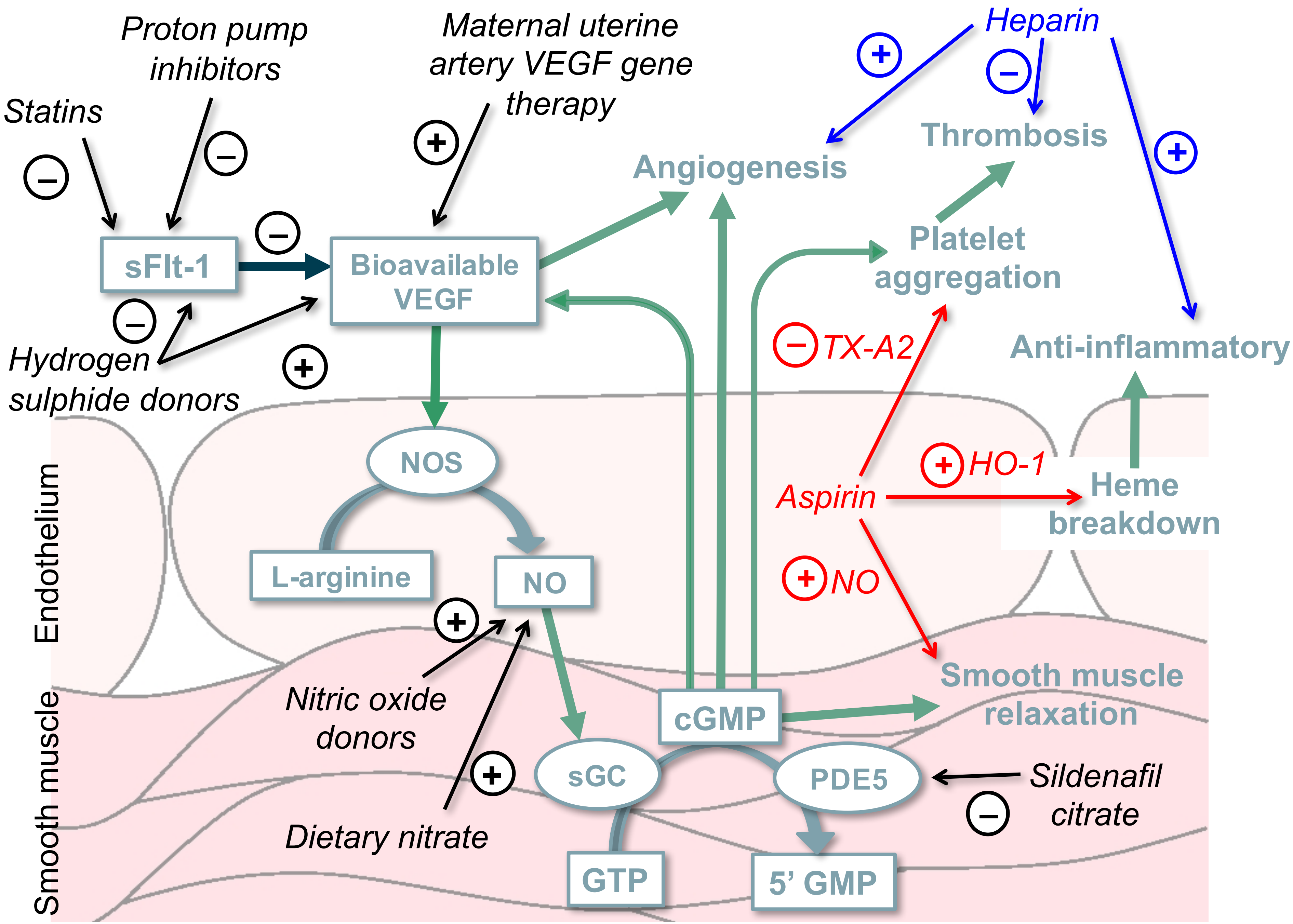
Table 3. Summary of progress of experimental treatments for fetal growth restriction.

Experimental Treatment	Method of Administration	Potential Mechanisms of Action	Current Stage of Investigation
Phosphodiesterase type-5 inhibitors	Oral	Selective vascular smooth muscle relaxation and vasodilatation	Phase II/III clinical trials
Maternal VEGF Gene Therapy	Injected into uterine arteries or applied to outside of vessels	Local vasodilatation and angiogenesis	Phase I/IIa clinical trial
Nanoparticles	Intravenous injection	Uterine blood flow, placental function	Preclinical
microRNAs	Intravenous injection	Uterine blood flow, placental function	Preclinical
Statins	Oral	Anti-inflammatory, antioxidant and angiogenesis	Phase II/III clinical trials (for preeclampsia only)
Nitric oxide donors	Oral	Selective vascular smooth muscle relaxation and vasodilatation	Phase II non-randomised (for preeclampsia only)

Hydrogen sulphide	Oral	Selective vascular smooth muscle relaxation and vasodilatation	Preclinical
Proton pump inhibitors	Oral	Angiogenesis	Phase II/III clinical trials (for preeclampsia only)
Melatonin	Oral	Antioxidant	Phase II non-randomised
Creatine	Oral	Cellular energy homeostasis	Preclinical
N-acetylcysteine	Oral	Selective vascular smooth muscle relaxation and vasodilatation	Phase II randomised (for preeclampsia only)

Figure 1 Sites of action at vascular smooth muscle and endothelium of the interventions under investigation to treat FGR. TX-A2, thromboxane A2; sFlt-1, soluble fms-like tyrosine kinase 1; VEGF, vascular endothelial growth factor; NOS, nitric oxide synthase; NO, nitric oxide; HO-1, heme oxygenase-1; sGC, soluble guanylate cyclase; GTP, guanosine-5′-triphosphate; cGMP, cyclic guanosine monophosphate; 5′ GMP, guanosine monophosphate; PDE5, phosphodiesterase type 5 inhibitor.

Figure(s)



Heparin
Proton pump inhibitors
Maternal uterine artery VEGF gene therapy
Statins
Thrombosis
Angiogenesis
sFlt-1
Bioavailable VEGF
Platelet aggregation
Hydrogen sulphide donors
TX-A2
Anti-inflammatory
NOS
HO-1
Endothelium
Aspirin
Heme breakdown
L-arginine
NO
NO
Nitric oxide donors
cGMP
Smooth muscle relaxation
sGC
PDE5
Sildenafil citrate
Smooth muscle
Dietary nitrate
GTP
5' GMP