

Preeclampsia Link to Gestational Hypoxia

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1 Abstract

2 Complications of pregnancy remain key drivers of morbidity and mortality, affecting the health
3 of both the mother and her offspring in the short and long term. There is lack of detailed
4 understanding of the pathways involved in the pathology and pathogenesis of compromised
5 pregnancy, as well as a shortfall of effective prognostic, diagnostic and treatment options. In
6 many complications of pregnancy, such as in preeclampsia, there is an increase in
7 uteroplacental vascular resistance. However, the cause and effect relationship between
8 placental dysfunction and adverse outcomes in the mother and offspring remains uncertain.
9 In this review, we aim to highlight the value of gestational hypoxia-induced complications of
10 pregnancy in elucidating underlying molecular pathways and in assessing candidate therapeutic
11 options for these complex disorders. Chronic maternal hypoxia not only mimics the placental
12 pathology associated with obstetric syndromes like gestational hypertension at morphological,
13 molecular and functional levels, but it also recapitulates key symptoms that occur as maternal
14 and fetal clinical manifestations of these pregnancy disorders. We propose that gestational
15 hypoxia provides a useful model to study the inter-relationship between placental dysfunction
16 and adverse outcomes in the mother and offspring in a wide array of examples of complicated
17 pregnancy, such as in preeclampsia.

18 Key words: gestational hypoxia, oxidative stress, fetal growth restriction, compromised
19 pregnancy, placental dysfunction

20 **The burden of compromised pregnancy**

21 Pregnancy is a highly vulnerable period for both the mother and her unborn child.
22 Complications during this time can adversely affect both maternal health and fetal development.
23 The World Health Organization estimates that 830 women die each day due to preventable
24 causes related to pregnancy and childbirth, while more than 6 million perinatal deaths occur
25 each year^{1,2}. Adverse intrauterine conditions are not only major drivers of short-term neonatal
26 morbidity and mortality, but also impose serious risks for childhood and adult health, such as
27 an increased incidence of various cardiometabolic diseases at adulthood^{3,4,5}. For instance, fetal
28 development in utero can be highly sensitive to external perturbations and environmental
29 stressors, such as parental socioeconomic disadvantages, even more so than to changes in the
30 length of gestation itself^{6,7}. Adverse conditions in utero may then trigger adaptive mechanisms
31 to protect the developing fetus against the suboptimal intrauterine environment at the expense
32 of increasing risk of disease in later life^{5,8}. Even though complications of pregnancy present a
33 substantial burden on public health across the world, there is lack of understanding of the
34 underlying pathways, partly due to the multi-factorial nature of these syndromes and partly due
35 to the plethora of ethnic, social and economic confounding factors involved. Equally, there is
36 a shortfall of effective prognostic, diagnostic and treatment options for some disorders, such as
37 for pre-eclampsia. This calls for the development of improved animal models, in which to
38 carry out more targeted research to isolate mechanisms and thereby design potential therapeutic
39 intervention⁹⁻¹¹.

40 **Increased uteroplacental vascular resistance: Effects on maternal and fetal health**

41 In mammals, advancing gestation is associated with an increase in uteroplacental blood flow
42 to sustain the demands of the growing fetus^{12,13}. Hence, fetal weight is closely linked to the
43 extent of that physiological increase in uteroplacental blood flow¹²⁻¹⁴. This highlights the

44 importance of the well-perfused placenta in determining the appropriate transplacental
45 exchange of nutrients and blood gases in healthy pregnancy¹²⁻¹⁴. Therefore, increased vascular
46 resistance in the uteroplacental vascular bed can directly slow fetal growth and compromise
47 fetal development by impairing uteroplacental blood flow, limiting adequate oxygen and
48 nutrient delivery to the growing young^{15, 16}. In a human study with low-risk participants that
49 were matched for age, socioeconomic background, ethnicity, health and nutritional access,
50 intrauterine growth restriction (IUGR) was associated with indices of low uteroplacental blood
51 flow^{17, 18}. This strongly indicates that altered placental vascular function is more important in
52 determining fetal growth than other factors, such as maternal nutrition *per se*^{17, 18}. The same
53 study also identified reduced placental perfusion as a risk factor for adverse cardiovascular and
54 metabolic conditions in later life, which could not be explained by differences in birth weight^{17,}
55 ¹⁹. This suggests that the placenta is also key in determining developmental origins of disease
56 with lifelong consequences on offspring health, both in conjunction with and independent of
57 its effects on fetal growth^{17, 19}.

58 In parallel with human clinical studies, abnormal uteroplacental vascularization has been found
59 in many animal models of compromised pregnancy and uteroplacental perfusion is reduced in
60 many ovine models of suboptimal pregnancy^{16, 20, 21}. Similarly, interventions that restored
61 uteroplacental blood flow could improve the severity of fetal growth restriction (FGR)^{16, 20, 21}.
62 Consistent results have been found in rodent models of IUGR, which have been exploited
63 significantly in this field of research due their rapid generational turnover and the haemochorial
64 nature of their placentation that resembles the human situation²¹⁻²⁵.

65 An increase in vascular resistance in the uteroplacental vascular bed can also have profound
66 adverse effects on the maternal physiology. Most directly, this is by perturbing the high

67 capacitance-low resistance utero-placental vascular bed, promoting an increase in maternal
68 total peripheral vascular resistance, raising maternal cardiac afterload and thereby contributing
69 to an increase in maternal arterial blood pressure with advancing gestational age²⁶. In addition,
70 increased vascular tone in the uteroplacental vascular bed can trigger the placenta to enter into
71 a state of oxidative stress, aggravating placental malperfusion and dysfunction^{27,28} (Fig.1). This
72 can cause the placenta to become a source of circulating vasoactive factors that eventually
73 cause widespread systemic maternal vascular dysfunction, as occurs in pre-eclampsia²⁹⁻³²
74 (Fig.1). Pathways involved in this pathogenesis include the activation of the renin-angiotensin
75 system, stimulation of prostaglandins, release of various anti-angiogenic factors, and altered
76 synthesis of, and reactivity to, several gasotransmitters and vasoconstrictors^{24,32-35}. For example,
77 in an interesting study, Woods et al. demonstrated that reduced uteroplacental perfusion
78 increased blood pressure in pregnant dogs, and that the causative factor was thromboxane.
79 Highlighting this study is important, because it was among the first to demonstrate that release
80 of a substance by the uteroplacental vascular bed could increase maternal blood pressure in
81 complicated pregnancy³².

82 **Maternal hypoxia: Effects on the fetoplacental unit and maternal health**

83 The term 'hypoxia' describes a lack of oxygen supply at the tissue and cellular level. It can be
84 induced experimentally in animals, for instance by limiting the oxygen content of inspired air,
85 which leads to 'systemic hypoxia' affecting the entire body. Alternatively, hypoxia can be
86 induced by reducing the blood supply to the tissue of interest. Impaired uteroplacental blood
87 flow would then promote 'uteroplacental hypoxia'³⁶. In addition to different terms to describe
88 different regions suffering hypoxia, authors have used several terms to describe the duration of
89 hypoxia. These terms include 'chronic hypoxia', 'sustained hypoxia', 'long-term hypoxia' and
90 'gestational hypoxia', which have sometimes been used interchangeably to describe hypoxic

91 exposure lasting from hours to months³⁷⁻⁴⁰. In this review, we use the term ‘chronic hypoxia’
92 to describe oxygen deprivation for a significant part of gestation, like a third to a half, and we
93 use ‘gestational hypoxia’ to describe impaired oxygenation for most of gestation.

94 *Effects of maternal hypoxia on the fetoplacental unit*

95 The suitability of gestational hypoxia induced by sustained reductions in the maternal inspired
96 fraction of oxygen as a model of placental insufficiency has been questioned, because maternal
97 compensatory cardiorespiratory responses may buffer the impaired placental oxygenation.
98 However, both human studies and animal models of maternal hypoxia now confirm that even
99 exposure to chronic hypoxia for a third of gestation can lead to changes in the placental
100 structure and function, indicative of increased uteroplacental vascular resistance and
101 uteroplacental hypoxia^{27, 41-43} (Fig.2). For instance, *in vivo* and *in vitro* evidence shows that
102 proliferation patterns of the uteroplacental vasculature are altered in response to chronic
103 maternal hypoxia⁴⁴⁻⁴⁶. These alterations in the placental vascular phenotype, may underlie the
104 diminished dilator and enhanced constrictor reactivity measured in the uteroplacental vascular
105 bed of the hypoxic pregnant mother⁴⁴⁻⁴⁶ (Fig.2). Such changes will oppose the physiological
106 increase in uteroplacental perfusion with advancing gestation, further compromising oxygen
107 delivery to the fetoplacental unit and promoting placental oxidative stress, triggering a vicious
108 cycle^{42, 46-51} (Fig.2). Of interest, the placental response to the chronic hypobaric hypoxia of
109 pregnancy at high altitude resembles many of these complications, not only in terms of
110 symptoms and pregnancy outcome, but also in terms of global gene expression at the level of
111 the placenta²⁷ (Fig.2). This may, at least partly, explain the increased incidence of pregnancy
112 complications at high altitude, such as the markedly increased prevalence of preeclampsia^{52, 53}
113 (Fig.2). Studies of human pregnancy at high altitude and in several animal models of maternal
114 exposure to chronic hypoxia have confirmed that gestational hypoxia leads to significant

115 FGR⁵⁴⁻⁵⁷ (Fig.2). In addition, there is evidence that placental oxidative stress may expose the
116 fetus to potential oxidative injury, including protein or nucleic acid oxidation, translational
117 inhibition or cell death, which in turn worsens pregnancy outcome and promotes IUGR⁵⁸⁻⁶⁰.

118 Exposure to maternal hypoxia during early gestation may have a profound influence on
119 placentation by altering the characteristics of trophoblast proliferation^{61, 62}. Considering the
120 importance of local oxygen tension in determining the onset of spiral artery conversion and
121 development of the uteroplacental circulation at the end of the first trimester, it is not surprising
122 that a suboptimal oxygen environment early in pregnancy will have significant adverse
123 consequences on the morphological and functional maturation of the uteroplacental vascular
124 bed, leading to reduced placental and fetal weights at term⁶²⁻⁶⁶ (Fig.2). Gestational hypoxia and
125 placental oxidative stress in the first trimester have also been linked to adverse effects on fetal
126 brain development and are associated with several psychological disorders in later life, such as
127 schizophrenia^{67, 68}. Similarly, exposure of experimental animals to hypoxia during early
128 gestation is associated with defects in cardiac development, impairing morphogenesis and
129 ventricular function⁶⁹⁻⁷². On the other hand, many experimental studies on early-onset hypoxia
130 during gestation show evidence of placental adaptation to adverse intrauterine conditions.
131 These include an increase in placental weight, vascularization and capillary surface area for
132 exchange, which depend on the severity and duration of the hypoxic insult and may not
133 necessarily lead to significant effects on fetal body weight⁷³⁻⁷⁷. In this context, studies in rodent
134 pregnancy by our own laboratory have shown that late-onset hypoxia for the last third of
135 gestation leads to significant fetal growth restriction⁷⁸. In contrast, early-onset hypoxic
136 pregnancy increases placental weight, cushioning the adverse effects on fetal development,
137 leading to maintained birth weight⁷⁷. It is also important to acknowledge that before 10 weeks
138 of gestation in humans, intrauterine development occurs under relatively hypoxic conditions

139 until the haemochorial placenta is fully established. Accordingly, at least in vitro,
140 cytotrophoblast cells are insensitive to hypoxic conditions before 7 weeks of gestation, while
141 between 10 to 12 weeks of gestation the same degree of hypoxia will significantly affect the
142 invasion profile of the cytotrophoblast^{61,66}. Thus, in human pregnancy, maternal hypoxia before
143 10 weeks of gestation may not significantly affect fetal organogenesis. On the contrary,
144 premature onset of intervillous blood flow and oxygenation within the intrauterine environment
145 may have adverse consequences on placental and fetal development through the development
146 of placental oxidative stress⁷⁹⁻⁸².

147 Many molecular mechanisms have been proposed to mediate the adverse effects of maternal
148 hypoxia on the fetoplacental unit, most of which impact on uteroplacental vascular function.

149 *Nitric oxide*. The important gasotransmitter nitric oxide (NO) has been identified as a key
150 vasodilator in the uteroplacental circulation, in which pregnancy induces an increase in
151 endothelial NO^{41,83,84}. NO signalling may be one of the mechanisms underlying the pregnancy-
152 induced uterine artery vasodilatation, allowing the crucial increase in uteroplacental blood flow
153 to support the growing fetus^{83,85-87}. Furthermore, eNOS knockout mice show significant FGR
154 associated with a substantial increase in resistance in the uteroplacental vascular bed, impaired
155 uteroplacental perfusion, placental hypoxia, oxidative stress and reduced trans-placental
156 nutrient transport^{25,41,88} (Fig.1). This was coupled with maternal proteinuria and alterations in
157 maternal cardiovascular function, including a reduction in endothelium-dependent
158 vasorelaxation, increased uterine artery vasoconstriction and hypertension^{57, 59}. Under
159 gestational hypoxia the effects of NO on uterine artery vasodilatation are decreased^{46,89} (Fig.2).
160 This may occur due to decreased expression of NO synthesizing enzymes, or due to free radical
161 scavenging of NO⁸⁹⁻⁹¹. Further, the sequestration of NO by superoxide forms peroxynitrite,

162 which is thought to accumulate in placental tissues and itself has pro-oxidant effects capable
163 of disrupting placental cellular proliferation and vascular function^{89,92,93}.

164 *Reactive oxygen species*. This reduction in NO-induced increase in placental perfusion may be
165 explained by the excess generation of reactive oxygen species (ROS) during chronic hypoxia;
166 they act to sequester the available NO within the oxidatively-stressed uteroplacental bed⁹⁴
167 (Fig.2). In an ovine model of gestational hypoxia, increased ROS production by NADPH
168 oxidase 2 (NOX2) was responsible for increasing uterine artery myogenic tone, which was not
169 observed when NOX2 was inhibited using apomycin³⁸. Hypoxia-induced oxidative stress has
170 also been found to provide a strong stimulus for endoplasmic reticulum (ER) stress, which is
171 associated with protein synthesis inhibition and impaired trophoblast survival and proliferation,
172 further contributing to the increased prevalence of FGR and pregnancy complications at high
173 altitude^{60,95,96}. Many studies in animal models have also reported antioxidant protection against
174 fetal growth restriction in pregnancy complicated by gestational hypoxia^{41,57,97,98}. Out of these
175 agents, the mitochondrial antioxidant MitoQ has recently gained special interest due to its
176 ability to specifically target mitochondrial oxidative stress, which is a major source of ROS in
177 the placenta by nature of electron transport during oxidative phosphorylation^{99,100}. This may
178 explain the hypoxia-induced decrease in mitochondrial oxygen consumption and decrease in
179 mitochondrial complex I activity in particular, which is the main site of electron leakage and
180 mitochondrial ROS production^{99,101-103}. Maternal treatment with MitoQ in animal models of
181 chronic hypoxia has led to the improvement of both placental mitochondrial stress and fetal
182 outcomes, including birth weight and developmental programming of cardiovascular and
183 psychiatric diseases, highlighting the importance of mitochondrial stress in mediating hypoxia-
184 induced pathology^{67,77,99}.

185 *Calcium activated potassium channels.* During pregnancy, Ca²⁺-activated K⁺ (BK_{Ca}) channels
186 in vascular smooth muscle cells have gained traction in being important mediators of uterine
187 artery vasodilatation, and their inhibition reduces uteroplacental blood flow, contributing to
188 IUGR¹⁰⁴ (Fig.1). Interestingly, hypoxia and oxidative stress suppress BK_{Ca} channel activity
189 expression, which may explain the maladaptive myogenic response of the uteroplacental
190 circulation in response to chronic hypoxia^{38, 105, 106} (Fig.2). In addition, ROS, have been
191 identified as key inhibitors of BK_{Ca} channel activity, an effect not seen in the presence of the
192 antioxidant N-acetylcysteine¹⁰⁷. Therefore, ROS may affect the vasculature of the
193 uteroplacental bed in at least two ways, leading to dysfunction of both the endothelial layer via
194 impairing NO-mediated mechanisms and of the smooth muscle cell layers by interfering with
195 the function of Ca²⁺ channels.

196 *Hypoxia-inducible factor 1-alpha.* One of the key regulators of cellular responses to hypoxia
197 are the hypoxia-inducible factors (HIFs), which are rapidly stabilized upon the onset of oxygen
198 deprivation, and interact with a variety of cellular enzymes and transcription factors to control
199 cellular oxygen homeostasis¹⁰⁸. HIF-1 α expression, along with expression of HIF-regulated
200 proteins, such as transforming growth factor beta-3 and vascular endothelial growth factor
201 (VEGF), negatively correlates with fetal to placental weight ratio and positively relates with
202 the adverse clinical outcome of chronic gestational hypoxia at high altitude²⁷ (Fig.2). HIF-1 α
203 and HIF-1 α -regulated genes are similarly dysregulated in placentas during pre-eclampsia, with
204 increased circulating levels of HIF-1 α measured in pre-eclamptic mothers¹⁰⁹⁻¹¹¹ (Fig.1). Levels
205 of HIF-1 α only decline following delivery of the placenta, indicating that it may be useful as a
206 predictive biomarker of failed placentation in pre-eclampsia, and further supporting the theory
207 that the pathogenesis of pre-eclampsia is at least partly driven by hypoxia-mediated
208 signalling^{109, 111, 112}.

209 *Endothelin-1 signalling.* One of the many downstream effectors of HIF-1 α is endothelin-1 (ET-
210 1), which is an important antagonist of NO-mediated vasodilatation within a complex network
211 of mediators acting on the vascular endothelium¹¹³. ET-1 interacts with NO by altering gene
212 expression and ligand-receptor interactions, providing a close link between NO and ET-1
213 signalling, and generating a powerful vasoconstrictor effect¹¹³⁻¹¹⁵. Many investigators have
214 proposed that an imbalance between these two essential endothelial agonists is implicated in
215 various vascular pathologies, notably in several different forms of hypertension^{113, 116}. This
216 interplay also seems to play an important role in the impairment of uteroplacental perfusion in
217 hypoxia-induced FGR, which is one of the major complications of intrauterine exposure to
218 hypoxia^{51, 117} (Fig.1). While ET-1 shows little effect on uteroplacental vascular tone under
219 physiological conditions, ET-1 and its receptors are markedly upregulated under conditions of
220 chronic hypoxia through HIF-mediated signalling^{118, 119} (Fig.2). ET-1 binding to endothelin
221 receptor A appears to be causative of impaired uteroplacental blood flow during chronic
222 hypoxia and pregnancy at high altitude; a higher ET-1 to NO ratio shows a clear association
223 with FGR at high altitude^{51, 117}. The importance of the effects of ET and of chronic maternal
224 hypoxia on the uteroplacental vascular bed is further supported by the presence of different
225 single nucleotide polymorphisms in the ET-1 gene in Andeans compared with Europeans¹¹⁹.
226 While Andeans show a pregnancy-related fall in plasma ET-1 levels with advancing gestation,
227 this does not occur in Europeans at high altitude. This may explain the relative protection
228 against high altitude-induced FGR in highland native populations, such as the Andeans and
229 Tibetans¹¹⁹⁻¹²³.

230 *Placental hydrogen sulfide biology.* Initially simply regarded as a toxic gas, H₂S has come into
231 physiological focus due to its role as an antioxidant, second messenger and regulator of

232 vascular function¹²⁴⁻¹²⁶. Specifically, H₂S is vasoactive and an important modulator of
233 angiogenesis, thereby involved in the maintenance of optimal placental vascular function
234 during healthy pregnancy¹²⁷⁻¹³⁰. H₂S is also cytoprotective, being involved in ischaemic
235 preconditioning and in the enhancement of the mitochondrial redox balance¹³⁰. The rate-
236 limiting enzyme for H₂S production, cystathionine γ -lyase (CSE), is localized in the smooth
237 muscle cells of placental stem villi and its expression and activity are reduced in placentas
238 under conditions of hypoxia and oxidative stress¹³¹⁻¹³⁴. Both women with hypertension and pre-
239 eclampsia also present with lower circulating levels of H₂S, indicating that H₂S has important
240 antihypertensive properties¹³¹⁻¹³⁴ (Fig.1). A growing body of evidence suggests that maternal
241 chronic hypoxia suppresses placental levels of CSE via miR-21-mediated mechanisms, and
242 that this is associated with mitochondrial depolarization, increased apoptosis and villous
243 remodelling^{129, 131, 132, 135-138} (Fig.2). These changes are further associated with evidence of
244 impaired uteroplacental blood flow, uteroplacental hypoxia, IUGR, and maternal vascular
245 dysfunction, making H₂S an agent of increasing interest in the inter-relationship between
246 uteroplacental dysfunction and adverse fetal and maternal outcome in complicated
247 pregnancy^{129, 131, 135-138}. Different mechanisms of action have been proposed for the protective
248 effects of H₂S in adverse pregnancy, including the sequestration of ROS, the regulation of
249 potassium channels, the modulation of the renin-angiotensin system and the inhibition
250 antiangiogenic factors^{129, 130, 139}. Therefore, decreased levels of placental CSE expression and
251 activity following gestational hypoxia may have widespread adverse effects^{139, 140}. These may
252 include direct adverse effects on systemic and utero-placental vascular tone through impaired
253 vasodilator actions on the endothelium and/or smooth muscle cells, or indirect adverse effects,
254 for instance by exacerbating oxidative stress due to loss of its antioxidant properties^{139, 140}.

255 *Effects of maternal hypoxia on maternal health*

256 In parallel with the plethora of evidence indicating adverse effects of gestational hypoxia on
257 the fetoplacental unit, the same applies to the promotion of adverse effects of gestational
258 hypoxia on the maternal circulation^{41, 48, 62} (Fig.2). Thus, dysregulation of many signalling
259 pathways affecting the fetoplacental unit also seem to adversely affect the maternal
260 cardiovascular system in gestational hypoxia, such as those involving ROS, NO, ET-1 and
261 H₂S^{38, 48, 62, 133, 141, 142} (Fig.1). For example, in a human case control study, treatment of women
262 suffering gestational hypertension with antihypertensive drugs supplemented with NO donors
263 and plasma volume expansion improved the uteroplacental resistance index and reduced both
264 maternal hypertension and FGR compared with pregnant women treated with antihypertensive
265 agents alone¹⁴³. Altered NO bioavailability is thought to be closely linked to oxidative stress
266 and to ROS mediated oxidative damage, which is increased in both the ischaemic placenta and
267 the systemic vasculature of women suffering from PE, for example by activated immunocytes
268 in the maternal endothelium⁸⁹. These immunocytes are also the source inflammatory cytokines,
269 such as TNF- α , which are at least partly responsible for the increase in ET-1 concentrations in
270 maternal serum in response to placental ischaemia^{144, 145}. ET-1 has potent vasoactive effects on
271 the maternal endothelium and is an important mediator of maternal hypertension during pre-
272 eclampsia, whilst endothelin receptor A antagonism has been shown to prevent the ET-1
273 mediated rise in blood pressure in many animal models¹⁴⁴⁻¹⁴⁷. Women suffering from pre-
274 eclampsia also show decreased plasma levels the vasoactive agent H₂S, which contributes to
275 the maternal hypertension and renal damage of the pre-eclamptic phenotype^{133, 148}.

276 In addition to the contribution of signalling pathways involving ROS, NO, ET-1 and H₂S, the
277 maternal vascular function also relies on the complex interaction between an exhaustive list of
278 angiogenic and vasoactive factors, as well as cytokines and growth factors^{30, 31, 149} (Fig.2). Any
279 disruption of this intricate balance of circulating factors by the stressed placenta, as occurs in

280 gestational hypoxia, may promote widespread endothelial dysfunction and vascular
281 inflammation with detrimental effects on the maternal vasculature^{30,31,149}.

282 *Angiogenic imbalance.* The placenta-derived placental growth and the vascular endothelial
283 growth factors (VEGF and PlGF) are crucial for maternal endothelial health, promoting
284 trophoblast survival and placental angiogenesis in the uteroplacental vascular bed¹³⁵⁻¹³⁸. The
285 soluble fms-like tyrosine kinase-1 (sFlt-1) acts as a soluble receptor and antagonist of VEGF
286 and PlGF^{150,151}. Chronic hypoxia, both in early and late gestation, placental oxidative stress and
287 pre-eclampsia are all associated with an increased ratio of sFlt-1 to PlGF and VEGF in both
288 the trophoblast and the maternal circulation, and administration of sFlt-1 itself has been found
289 to further increased tissue ROS^{137,152-157} (Fig.1; Fig.2). While early in pregnancy the effect of
290 sFlt-1 is mostly mediated via direct effects on placentation by inhibiting cytotrophoblast
291 invasion and differentiation, the inhibitory effect of sFlt-1 on PlGF and VEGF has been
292 suggested to be causative in the development of proteinuria and maternal endothelial
293 dysfunction in pre-eclampsia in the later stages of gestation^{138,152,156,158,159}. Interestingly, at least
294 part of the suggested beneficial effects of H₂S supplementation on maternal vascular function
295 in pregnant women with pre-eclampsia is thought to occur via H₂S-mediated upregulation of
296 miR-133b, which in turn downregulates sFlt-1 release¹⁶⁰. Such findings have sparked off
297 interest in the suitability of measuring the sFlt-1 to PlGF ratio as a biomarker for obstetric
298 disorders related to placental insufficiency, such as pre-eclampsia¹⁶¹⁻¹⁶³. This has led to the
299 development of commercial bioassays to be used as additional diagnostic tools for pre-
300 eclampsia, the efficacy of which has been validated^{164,165}. An aspect of this story less well
301 investigated is the potential of manipulating these angiogenic pathways in the treatment of pre-
302 eclampsia, for example through the administration of VEGF or PlGF to increase their
303 bioavailability. While increased VEGF levels may be associated with adverse side effects, such

304 as oedema, PIGF administration has been found to abolish the maternal hypertension in a rats
305 model of pre-eclampsia with no adverse effects on the maternal extracellular water content¹⁶⁶⁻
306 ¹⁶⁸. Reports on PIGF administration are limited and details on its mechanisms of protective
307 action in adverse pregnancy are not completely understood. The beneficial effects of PIGF in
308 compromised pregnancy have been attributed thus far to be partly mediated by a reduction in
309 placental oxidative stress and an improvement in maternal endothelial function via NO and
310 cyclic guanosine monophosphate derived vasorelaxation¹⁶⁶⁻¹⁶⁹.

311 *Inflammatory cytokine signalling.* One of the downstream consequences of placental hypoxia,
312 excess ROS availability and oxidative damage is cellular inflammation, triggering the release
313 of inflammatory stress markers¹⁷⁰⁻¹⁷² (Fig.2). These may be of either placental or endothelial
314 origin and they act in synergy to generate a systemic endovascular inflammatory state, which
315 contributes to the development of hypertension and kidney disease in pre-eclampsia^{28, 173-175}
316 (Fig.1). Of interest, the hypoxic placenta undergoes a clear shift in its inflammatory cytokine
317 profile, showing reduced expression of anti-inflammatory cytokines, such as interleukin-10,
318 and up-regulation of pro-inflammatory cytokines, such as tumor necrosis factor- α (TNF- α),
319 interleukin-6, interleukin-8 and interleukin-1 β . However, the role of IL-6 in pregnancy is
320 controversial and it may be involved in a variety of functions in the female reproductive tract^{149,}
321 ¹⁷⁶⁻¹⁸². The onset of this altered inflammatory cytokine profile in response to hypoxic exposure
322 is thought to occur after 11 weeks of gestation in humans and becomes more prominent with
323 advancing gestation, but is detectable in the maternal circulation and in the amniotic fluid prior
324 to the manifestation of pre-eclampsia-related symptoms^{172, 180, 183, 184}. In addition, TNF- α is a
325 potent stimulus for ET-1 signalling, which may at least partly underlie TNF- α -mediated
326 maternal hypertension as well as provide a link between placental, maternal and fetal pathology
327 in inflammatory conditions during gestation^{145, 185}.

328 *Extracellular vesicles*. Another consequence of hypoxic damage to the placenta may be the
329 increased release of placental debris or so-called syncytiotrophoblast microparticles (STBM)
330 alongside placental exosomes into the maternal circulation (Fig.2). This process is exacerbated
331 in pathological pregnancies, such as in preeclampsia and hypoxia as a result of poor
332 placentation¹⁸⁶⁻¹⁹³. This causes necrotic trophoblast damage following placental ischaemia and
333 it has been proposed to trigger widespread maternal endothelial dysfunction, possibly due to
334 release of inflammatory cytokines and endothelial phagocytosis of microparticles¹⁸⁶⁻¹⁹³ (Fig.1).
335 STBMs negatively impact endothelial proliferation, while promoting the secretion of anti-
336 angiogenic factors and inflammatory cytokines¹⁸⁷. This was confirmed *in vitro* in isolated
337 perfused maternal resistance vessels, in which the presence of STBM vesicles in the perfusate
338 reduced acetylcholine-mediated vasodilatation in subcutaneous fat arteries. This suggests that
339 the mechanism of action of STBM-related maternal hypertension may be mediated via adverse
340 effects on peripheral vasodilatation¹⁹⁴. The presence of increased necrotic trophoblast debris in
341 the maternal circulation is found to be a characteristic of pregnancy-induced hypertension, but
342 not IUGR in the absence of hypertension, thereby appearing to be central to the maternal
343 component of the pre-eclamptic syndrome¹⁹².

344 Exosomes are, like STMBs, constituents of cell-derived extracellular vesicles released into the
345 extracellular environment, containing agents destined for paracrine and endocrine signalling,
346 such as miRs and growth factors^{195,196}. The placenta is an active source of exosomes during
347 pregnancy and maternal plasma concentration of exosomes increases with advancing gestation,
348 potentially responsible for maternal physiological adaptation to pregnancy and maternal-fetal
349 immune regulation¹⁹⁷⁻¹⁹⁹. This process occurs under physiological conditions during pregnancy,
350 but is increased under conditions of hypoxia and during some complications of pregnancy, such

351 as pre-eclampsia as a result of placental apoptosis and necrosis, which also alters the exosomal
352 content²⁰⁰⁻²⁰³. These have been shown to affect maternal endothelial and vascular function,
353 possibly through dysregulation of pro-inflammatory cytokines or endothelial-related miRs,
354 such as miR-126, miR-17, miR-155 and miR-210, with potential roles in endothelial
355 dysfunction in pre-eclampsia^{204, 205, 206}.

356 Maternal hypertension itself may further compound the formation of STBMs and propagate
357 placental dysfunction and FGR. For example, an increased maternal myogenic tone and
358 decreased diameter of maternal resistance vessels increases the velocity of blood entering the
359 uteroplacental bed. This promotes turbulent flow in the intervillous blood spaces, which in
360 turn may exacerbate mechanical and necrotic damage to the placental vasculature^{186, 207, 208}. This
361 triggers the dislocation of microparticulate debris and necrotic trophoblast into the maternal
362 circulation^{186, 207, 208}. Significant damage to the spiral arteries can cause occlusion of the villous
363 blood spaces, resulting in placental infarction, further fueling placental dysfunction²⁰⁹⁻²¹¹.

364 **Direct effects of chronic hypoxia on the fetus**

365 A plethora of research interest has focused on the combined effects of maternal hypoxia on the
366 fetoplacental unit as a whole, most of which address effects on uteroplacental blood flow with
367 indirect effects on fetal development. This is due to the fact that intrauterine hypoxia usually
368 occurs as a result of pre-placental or uteroplacental hypoxia. Occasionally, post-placental
369 hypoxia can occur due to villous defects preventing sufficient oxygen uptake in the placental
370 vascular bed or due to fetal cardiovascular dysfunction, such as that induced by umbilical cord
371 occlusion or thrombosis^{65, 212}. This is associated with IUGR with absent or reversed end-
372 diastolic blood flow in the umbilical artery, giving some insight into the direct effects of
373 hypoxia on fetal development^{65, 213}. However, it is important to note that even post-placental

374 hypoxia can be associated with significant alterations in placental and villous morphology,
375 typical of non-branching angiogenesis resulting in villous hypoplasia, which in itself is
376 associated with an increase in uteroplacental vascular resistance. This makes the distinction
377 between direct and indirect effects of hypoxia on the fetus less straightforward²¹³. Another line
378 of research has focused on incubation of the chick embryo under hypoxic conditions, in which
379 developmental complications occur in the absence of any maternal or placental influence, such
380 as maternal hypertension or placental insufficiency. Such studies have reported that both
381 hypobaric and isobaric hypoxic incubation of the chick embryo lead to significant FGR with a
382 similar “brainsparing” redistribution of the cardiac output compared to the mammalian fetus²¹⁴⁻
383 ²¹⁸. Hypoxic incubation also resulted in embryonic cardiovascular dysfunction, which was
384 absent in normoxic chick embryos from sea level incubation or from high altitude incubation
385 with oxygen supplementation²¹⁹. In addition, development under hypoxic conditions had long
386 term adverse effects on systemic and pulmonary blood pressure regulation and presented with
387 altered baroreflex sensitivity in the adult chicken^{220, 221}. The development of cardiovascular
388 dysfunction in the hypoxic chick embryo could be effectively prevented using antioxidant
389 therapies, such as sildenafil or melatonin^{222, 223}. This provides evidence that fetal development
390 can be influenced directly by both hypoxic conditions and antioxidant treatment, highlighting
391 the importance of considering fetal and uteroplacental hypoxia as separate complications.
392 However, it can be argued that the chorioallantoic membrane may be considered the avian
393 homologue to the mammalian placenta, which may itself be influenced by hypoxic
394 incubation^{214, 215, 224}.

395 **Conclusions**

396 In summary, uteroplacental hypoxia may link many of the effects of placental dysfunction with
397 adverse effects on the mother and the fetus, which occur in many complications of pregnancy,

398 such as in preeclampsia. Chronic hypoxia induces morphological, molecular and functional
399 changes in the placenta that closely resemble those observed in placentae from women
400 suffering from preeclampsia. In addition, chronic hypoxia recapitulates maternal and fetal
401 adverse outcomes associated with the preeclamptic syndrome. Thus, pregnancy compromised
402 by maternal exposure to hypoxia is not only a major risk factor for FGR but it also promotes
403 adverse changes in the placenta, with potential consequent adverse effects on the physiology
404 of the mother and the offspring. Therefore, gestational hypoxia provides a useful model to
405 study the inter-relationship between placental dysfunction and adverse outcomes in the mother
406 and offspring in a wide array of examples of complicated pregnancy, such as in preeclampsia.

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415 **Conflicts of Interest**

416 None

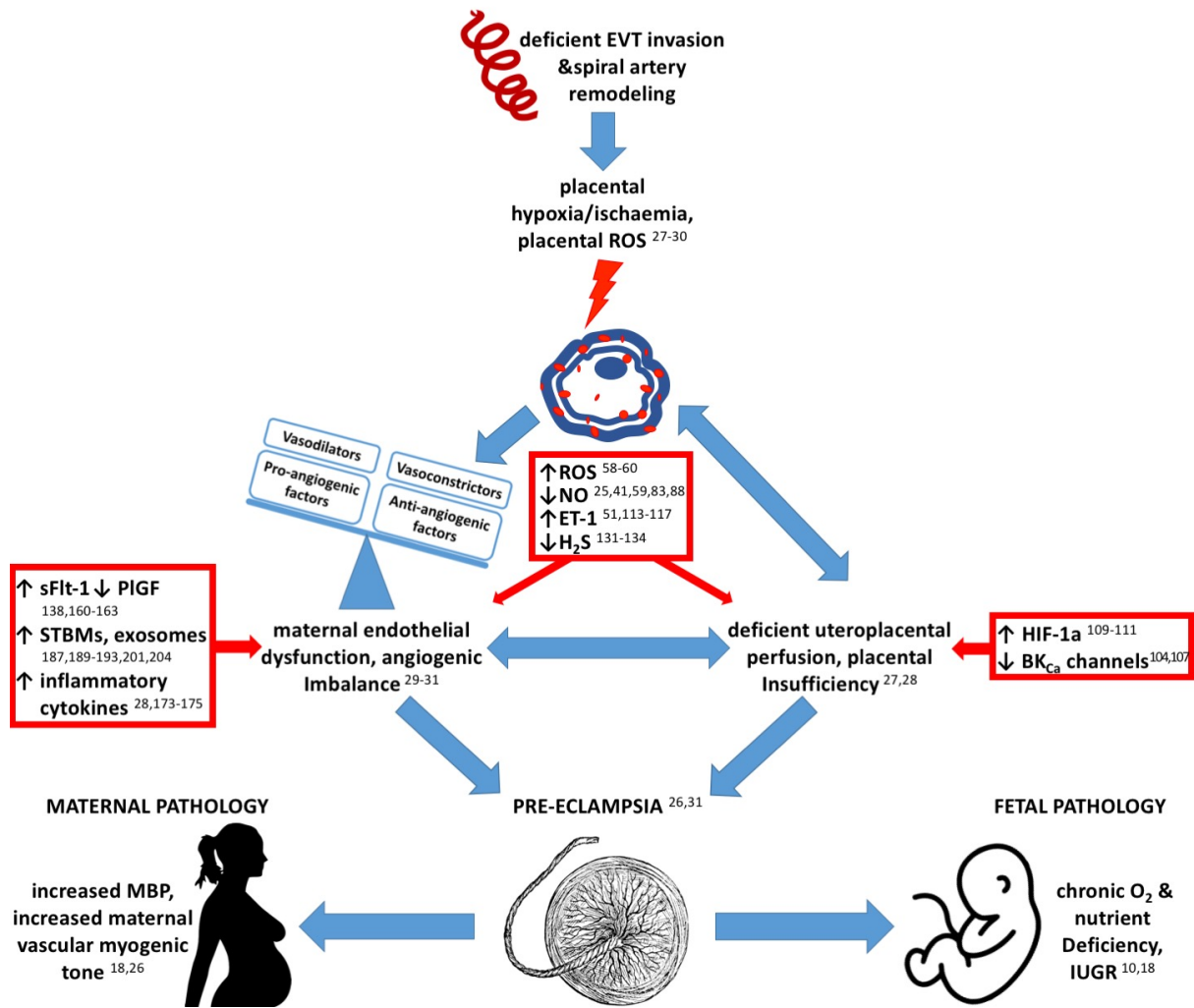


Fig. 1. The pathogenesis of pre-eclampsia.

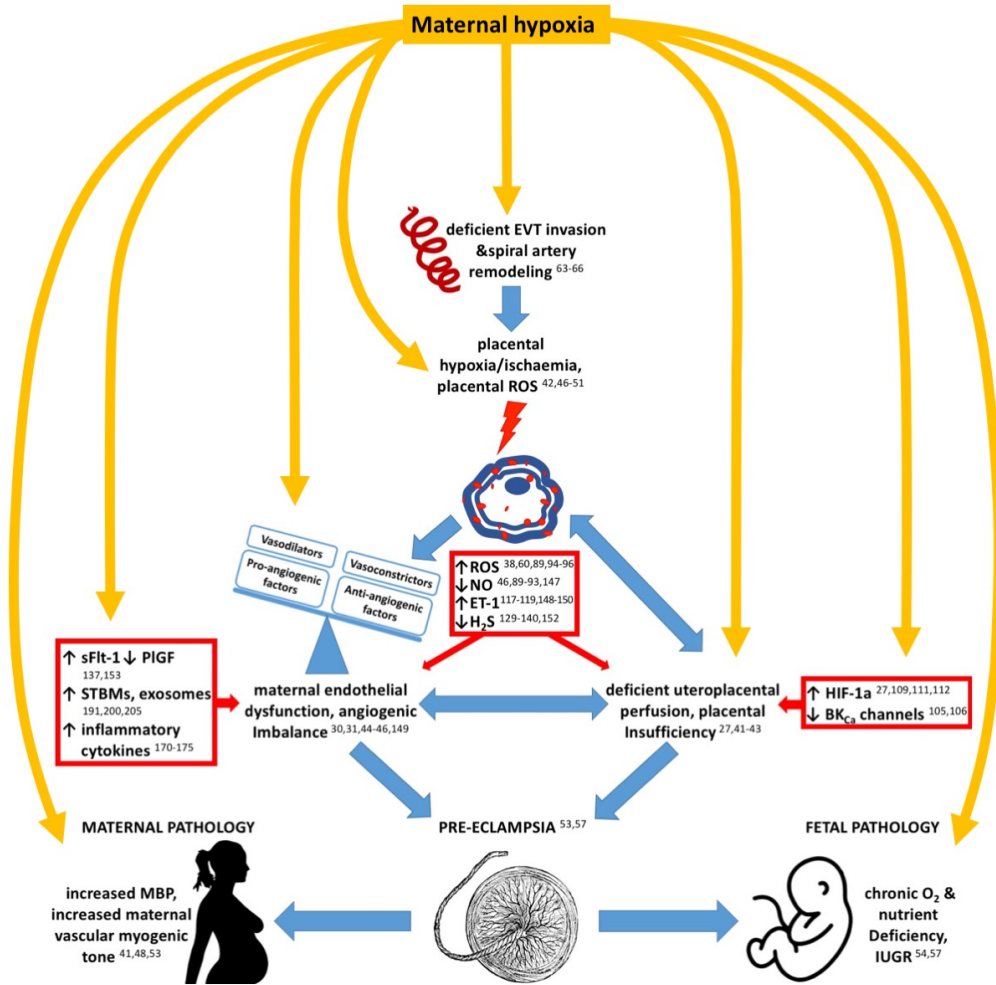


Fig. 2. Maternal hypoxia modulates placental, fetal and maternal pathways to mimic the pathogenesis of pre-eclampsia.

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