Placental mitochondria and reactive oxygen species in the physiology and pathophysiology of pregnancy

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Summary

Mitochondria are central to cell function. The placenta forms the interface between maternal and fetal systems, and placental mitochondria have critical roles in maintaining pregnancy. The placenta is unusual in having two adjacent cell layers (cytotrophoblasts and the syncytiotrophoblast) with vastly different mitochondria that have distinct functions in health and disease. Mitochondria both produce the majority of reactive oxygen species (ROS), and are sensitive to ROS. ROS are important in allowing cells to sense their environment through mitochondrial-centred signalling, and this signalling also helps cells/tissues adapt to changing environments. However, excessive ROS are damaging, and increased ROS levels are associated with pregnancy complications, including the important disorders preeclampsia and gestational diabetes mellitus. Here we review the function of placental mitochondria in healthy pregnancy, and also in pregnancy complications. Placental mitochondria are critical to cell function, and mitochondrial damage is a feature of pregnancy complications. However, the responsiveness of mitochondria to ROS signalling may be central to placental adaptations that mitigate damage, and placental mitochondria are an attractive target for the development of therapeutics to improve pregnancy outcomes.

Keywords: mitochondria, reactive oxygen species, oxidative stress, placenta, trophoblast

1. Introduction

Mitochondria are central to regulating cellular metabolism, redox state, and determining cell fate[1]. Pregnancy is a time of large physiological changes, many of which are driven by the placenta, and placental mitochondria are critical to a healthy pregnancy, and also in pregnancy complications.

The placenta forms the interface between maternal and fetal physiological systems, performing functions including metabolic and hormonal regulation as well as transport of oxygen from maternal to fetal circulations. The placenta is capable of responding to the signals from both mother and fetus, optimising fetal growth by modifying the maternal supply of nutrients and oxygen[2,3]. As with most tissues, the primary use of oxygen in the placenta is to release energy through oxidative phosphorylation. This process also generates reactive oxygen species (ROS). ROS production is a normal part of cell biology, with intracellular levels balanced by antioxidants[4], and ROS important for intracellular signalling and tissue adaptions[4] (Figure 1). However, excessive ROS production leads to oxidative stress and the oxidation of macromolecules that has been linked to pregnancy pathologies[2-5]; additionally mitochondrial dysfunction is associated with an array of disorders including gestational complications[5,6].

Placental function is critical to successful fetal development, but is also important in ongoing health[7], with poor pregnancy conditions linked to later life diseases in mother and offspring[7,8]. Here we consider two of the most prevalent and dangerous pregnancy complications, gestational diabetes mellitus (GDM) and preeclampsia, and review evidence that placental mitochondria are important in a healthy pregnancy, and implicated in the pathogenesis of these conditions.

2. Placental mitochondria in cytotrophoblasts and the syncytiotrophoblast

The human placenta is composed of several cell types. We focus on the villous trophoblast cell lineage, which consist predominantly of two cell types; cytotrophoblasts and the syncytiotrophoblast, as these cells are central to important placental functions and have distinct mitochondrial populations with varying susceptibility to dysfunction. Cytotrophoblasts have typical organelle organisation, whereas the syncytiotrophoblast is a poorly organised extended cytoplasm with many thousands of nuclei and specific organelle morphology/function[9]. Cytotrophoblast mitochondria are relatively larger (0.2–0.8 μm) and have a stereotypical shape with lamellar cristae, whereas syncytiotrophoblast mitochondria are small (≤0.1 μm), more spherical, have tubular cristae and a less dense matrix (Figure 2)[10-12]. The small size of syncytiotrophoblast mitochondria has been suggested to aid in their steroidogenic function, as the most common sterol carrier protein family member responsible for mitochondrial cholesterol transport in other cells — steroidogenic acute regulatory protein — is not expressed in the placenta, and cholesterol may be transported more efficiently across membranes of smaller mitochondria[11,13]. Further, the atypical morphology of syncytiotrophoblast mitochondria has been attributed to reduced F_1F_0 ATP synthase supercomplex dimerization, the structural component of ATP synthase, which alters inner membrane curvature and affects formation of mitochondria cristae[14-17]. ATP synthase is also critical in maintaining membrane potential (ΔΨm), and uses the proto-motive force for ATP biosynthesis. Therefore, lower dimeric ATP synthase levels may explain decreased ATP production in the syncytiotrophoblast compared to cytotrophoblasts[14]. In the syncytiotrophoblast, most ΔΨm regulates ATP-diphosphohydrolase (an enzyme involved in cholesterol transport), and the majority of ATP produced is utilized for cholesterol transport and steroidogenesis[18].

As the syncytiotrophoblast is formed via the fusion of cytotrophoblasts, these mitochondrial differences represent an important shift in cellular function. Cytotrophoblast differentiation into the syncytiotrophoblast leads to decreased mitochondrial respiration and altered metabolism, including an increase in lactate production[19]. The syncytiotrophoblast forms the outer layer of the placenta in direct contact with maternal systems, and appears to be the placental region most responsive to changes in oxygen levels; this responsiveness is likely partly due to the unique mitochondria of the syncytiotrophoblast, and may help the placental/fetal unit adapt to maternal conditions[20]. Further, relative to cytotrophoblasts, the syncytiotrophoblast has lower antioxidant function[20,21], which would otherwise dampen ROS levels that may be critical to signalling within this cell layer. Additionally, mitochondrial response to oxygen levels has a role in the endovascular phenotype of extravillous cytotrophoblasts[22] (critical for early placental uterine attachment and ongoing placental function), and in murine trophoblast differentiation[23], demonstrating the importance of mitochondrial oxygen responses to a range of placental behaviours.

The syncytiotrophoblast also appears to be sensitive to damage from oxygen, which may be a consequence of high oxygen sensitivity through mechanisms such as low antioxidant function[20,21]. The production of steroids by the syncytiotrophoblast may also be linked to syncytiotrophoblast susceptibility to ROS-mediated damage, through increased production of superoxide (O_2-*) and hydrogen peroxide (H_2O_2). Syncytiotrophoblast mitochondria are central to placental steroidogenesis, in particular progesterone production[13]. Increased expression of cytochrome P450scc (an enzyme that converts cholesterol to pregnenolone) in syncytiotrophoblast compared to cytotrophoblast

mitochondria is associated with changes in mitochondrial morphology[11,24], and also increased production of ROS[25].

3. Placental mitochondrial adaptations

3.1 Placental mitochondrial dynamics and apoptosis in preeclampsia

Mitochondria are constantly changing in a dynamic cycle[26]; fission segregates damaged mitochondria for disposal by autophagy, whereas fusion allows dysfunctional/damaged mitochondria to be rescued by amalgamation with more functional parts of the mitochondrial network[27] (Figure 3). In the placenta, mitochondrial fission/fusion appears to be impaired in cases of preeclampsia, but this relationship is complex and not well understood. Changes in molecular mechanisms of placental mitochondrial fission/fusion may relate to preeclampsia severity and gestational length. In severe preeclampsia, increases in pro-fusion regulator Optic atrophy protein 1 (OPA-1) and decreases in pro-fission regulator Dynamin-related protein 1 (DRP1) have been reported[28,29], but no apparent changes were found in other fission/fusion regulators including Mitochondrial fission factor[29] and Mitofusins (MFN1 and MFN2)[28]. In contrast, other research has found decreases in MFN1 and MFN2 associated with preeclampsia, indicating a pro fission status[30,31]. Placental mitochondrial dynamics is also affected in GDM, with hypergylcemia inducing mitochondrial fragmentation through a proposed mechanism involving DRP1[32,33], and this may be a reason GDM is a risk factor for preeclampsia. Damaged mitochondria are removed by autophagy (Figure 3), and in both severe preeclampsia and GDM increased placental autophagy markers have been observed[34]. Placental autophagy markers are altered in association with excessive ROS generation,

decreased antioxidant capacity and enhanced mitochondrial turnover[34], directly linking changes in mitochondrial dynamics to complications of pregnancy.

Our laboratory and others have shown that in more severe forms of preeclampsia, markers of mitochondrial fission are increased, whereas in less severe disease fusion markers predominate[28,35]. Therefore, in less severe preeclampsia, salvage mechanisms may be operating to rescue damaged placental mitochondria and protect trophoblasts from apoptosis, thereby preventing excessive generation of placental cell debris that enters the maternal circulation and is a hallmark of preeclampsia. Whether this mitochondrial salvage is a positive adaptation allowing pregnancies to reach greater gestational age, and what drives fission versus fusion in normal versus diseased placentae, remains uncertain.

Mitochondria regulate apoptosis through intrinsic activation with cytochrome C release[36-38]. Increases in apoptotic markers are associated with the development of preeclampsia and GDM [39], however, we have also shown that anti-apoptotic B-cell lymphoma 2 (BCL2) is increased in placentae from preeclamptic pregnancies that reach term delivery, and that this protective effect is not seen in preeclamptic pregnancies that are delivered preterm [35]. Further, decreased pro-apoptotic FAS receptor, FAS ligand and Caspase-3, in addition to increased BCL2 have been observed in GDM placentae [40]. Therefore, differences in apoptotic signalling in GDM and preeclampsia may relate to disease severity, with a mitochondrial-linked suppression of apoptosis in less severe cases of both.

3.3 Placental mitochondrial content

Mitochondrial content can alter in response to a variety of stimuli, allowing tissue adaption via changes in metabolism. Exercise promotes mitochondrial biogenesis in skeletal muscle that increases aerobic capacity[41,42], whereas various pathologies either increase or decrease placental mitochondrial content[5]. Placentae of rats exposed to maternal undernutrition increase content and alter expression of biogenesis/bioenergetic pathways[43], suggesting alterations in mitochondrial content may help increase bioenergetic efficiency under adverse conditions. Placental mitochondrial content is also altered in overweight/obese women and those with hypercholesterolemia[44,45], therefore these changes in mitochondrial content may represent a similar placental response in humans.

Changes in placental mitochondrial content may be a consequence of pathology-induced damage, or an active cellular adaptation to better align organ function with the altered external environment. There appears to be no consistent pattern of either increase or decrease in placental mitochondrial content associated with specific pregnancy pathologies[5], suggesting that both damage and adaptive changes may be occurring. It is also likely that the distinct mitochondrial subpopulations in cytotrophoblasts and the syncytiotrophoblast respond differently to stimuli, and that these subpopulations will need to be investigated separately to understand placental responses.

Oxygen pressure and possible signalling through ROS likely influence placental mitochondrial content[46,47]. Oxygen-related mechanisms operate in other tissues; in cardiac and neurological pathologies, hypoxia and mtDNA damage can trigger increased mitochondrial content[48-50]. Placental mitochondrial content/respiration have been linked to oxygen fluctuations over gestation[51], and mitochondrial responses are correlated with changes in placental antioxidant status[52], demonstrating the ability of placental

mitochondria to adapt to oxygen stimuli. Additionally, insulin resistance may drive mitochondrial content changes in multiple tissues[53], and could be a mechanism that affects placental mitochondrial biogenesis regulation in GDM[54].

4. Endoplasmic reticulum and mitochondria in the placenta

Mitochondria and endoplasmic reticulum (ER) form close functional units, and the role of placental mitochondria and ER has been extensively reviewed[55]. Contact between mitochondria and ER occur through mitochondrial associated ER membranes (MAM)[56] that enable $Ca^{2+}$and phospholipid trafficking, and act as mitochondrial fission/fusion sites[56-58]. Ca^{2+} release must be regulated to prevent mitochondrial dysfunction and apoptotic activation, and ER control over mitochondrial Ca^{2+} release/up-take regulates mitochondrial bioenergetics[59,60]. Additionally, MAM help coordinate mitochondrial fission[58], may have roles in transitioning mitochondria from cytotrophoblasts into the syncytiotrophoblast through the generation of smaller mitochondria.

In pregnancy pathologies, placental mitochondrial dynamics and content can be variable[55]. This variability in MAM-based responses may indicate that stimuli are at a level to induce positive adaptations, or are high enough to cause damage. Low levels or early phases of ER stress are associated with increased mitochondrial metabolism, mediated by organelle linkage through MAM formation and Ca^{2+} transfer[61]. However, high levels of ER stress/dysfunction can lead to high levels of Ca^{2+} release, enhancing leakage of electrons from complex I and III of the electron transfer chain (ETS). Electron leakage from the ETS may be facilitated through Ca^{2+} activating isocitrate dehydrogenase and α-ketoglutarate, and in turn stimulation NADPH[62]. This turnover has been suggested to contribute to ROS

generation[63] and mitochondrial damage, with excessive ROS shown to drive mitochondrial swelling through mitochondrial permeability transition pore[64].

Placental ER stress occurs in pregnancies affected by GDM and preeclampsia[65,66]. Preeclamptic placentae have decreases in intracellular Ca^{2+} and decreased expression of electron transport system (ETS) enzymes[35,67,68], which are associated with ER dysfunction. Further, ER stress markers GRP78, phospho-PERK, X-box binding protein1 (XBP1) and eukaryotic initiation factor 2 alpha (eiF2α) increase in response to inducible nitric oxide synthase (iNOS) in preeclampsia[69]. Similarly, low grade ER stress has been observed in GDM, with increased XBP1 and phosphorylated-eiF2α[70]. Additionally, MFN2 acts as a tethering antagonist preventing over accumulation of ER-mitochondrial association[57], therefore, ER dysregulation may affect mitochondrial biogenesis and content in gestational disorders through changes to mitochondrial fission/fusion.

5. Oxygen and reactive oxygen species in cellular signalling

ROS are important to physiological homeostasis through mitochondrial-centred cellular signalling that allows response to external and internal stimuli. At every stage of implantation and the establishment of the placenta, ROS are critical cell function mediators. ROS may be important in preimplantation embryonic development through a shift in metabolic substrate preference with blastulation, and effects on redox-sensitive transcription factors such as Hypoxia inducible factors and Nuclear factor kappaB that have a range of downstream actions[71]. In rabbit blastocysts, ROS are produced throughout implantation[72], and inhibiting murine embryo ROS *in vivo* altered production of the important second messenger cyclic guanosine monophosphate[73], suggesting ROS function in ongoing embryonic cellular messaging. In early pregnancy, ROS can trigger activation of

vascular endothelial growth factor (VEGF) and glucose transporters that promote angiogenesis, and therefore the oxygen delivery critical to placental mitochondrial function continuing development[74].

Differing oxygen levels across pregnancy also shape placental function. Maternal blood flow to the human placenta is not fully established until ~12 gestational weeks, and before this the fetal-placental unit exists in relatively low oxygen (~2%)[52]. Extravillous cytotrophoblasts adapt to this environment — proliferating more rapidly and being resistant to apoptosis in low oxygen[75] — and therefore functioning in placental implantation, as the most distal trophoblasts are exposed to higher oxygen concentrations that promote an invasive phenotype and lead to the remodelling of maternal spiral arteries that is critical to pregnancy success[76]. The redox-sensitive Hypoxia inducible factor 1α (HIF-1α) is also important for placentation, influencing trophoblast and uterine cell behaviour based on oxygen concentration[77]. Additionally, inhibiting HIF-1α in human first trimester placental explants causes inhibition of $TGF\beta_3$ and arrest cell proliferation, suggesting that trophoblast differentiation may be partially mediated by TGFβ3 through HIF-1α transcription factors[78].

ROS are also important in later pregnancy. Towards the end of the first trimester, maternal intraplacental circulation is established[79]. The sudden increase in oxygen in and around an ETS saturated with electrons may cause reverse electron flow[80], leading to increased ROS generation[52]. The syncytiotrophoblast is central to placental/fetal endocrine function and nutrient transport, and renewal of this terminally differentiated cell has also been linked to oxygen concentration, with secretion of human chorionic gonadotrophin modulated by ROS-responsive potassium channels[81]. The final phase in placental vascular development occurs in the third trimester[82], and ROS are also important at this point in

gestation, with ROS-responsive transcription factors regulating angiogenesis and tissue remodelling[83].

5.1 Reactive oxygen species in gestational pathologies

ROS are important in cellular signalling during placental-fetal development, but excessive levels can cause tissue damage, and high levels of ROS are found in several pregnancy complications, including preeclampsia and GDM. Oxidative stress caused by excessive ROS generation and/or diminished antioxidant function is associated with hypoxia, inflammation and immune responses, all characteristics of gestational disorders[84]. ROS are central to preeclampsia aetiology, with inadequate placental perfusion due to poor trophoblast invasion of maternal spiral arteries[85] leading to ischemic-reperfusion injury resulting in ROS generation and oxidative stress[86]. This increased ROS production is associated with anti-angiogenic pathways central to preeclampsia pathogenesis[86], as well as placental damage and release of factors including soluble fms-like tyrosine kinase (sFlt-1) into the maternal periphery that are thought to lead to the classic maternal symptoms of endothelial dysfunction and hypertension[3,5]. GDM pathophysiology has also been linked to ROS and placental mitochondrial dysfunction, with insulin resistance potentially a response to varying hormones levels — e.g. progesterone and human placental lactogen (hPL) — synthesised by placental mitochondria[87]. Oxidative stress is observed in GDM[88,89] and excessive ROS is also associated with hypertension, insulin resistance and hyperglycemia, all characteristics of GDM[5,32]. Indeed, placental mitochondrial damage and ROS production may be one reason GDM predisposes women to develop preeclampsia.

ROS are generated by several mechanisms, and increased ROS lead to numerous downstream effects. In the preeclamptic placenta, increased NADPH oxidase isoform NOX1 is overexpressed in the syncytiotrophoblast, possibly due to increased O_2^{-*} and related cytokine production, which may then contribute to maternal endothelial dysfunction[90,91]. Additionally, NOX may be activated by ROS in a positive feedback loop, inducing further oxidative stress and generating systemic maternal inflammation[92,93], possibly directly linking placental ROS and maternal effects. Excessive ROS production also causes mitochondrial transition pore opening and the intrinsic activation of apoptosis, and is another mechanism of leading increased trophoblast shedding into the maternal circulation and therefore induction of maternal symtoms[94]. Further, xanthine oxidase (XO) and XO-induced damage also occurs in umbilical cord blood from GDM pregnancies[89], further associating similar mitochondrial mechanisms of ROS overproduction in the pathogenesis of both preeclampsia and GDM. Hyperglycemia is associated with increased NOX, and hyperglycemia-induced ROS production has been attributed to mitochondrial morphological changes across multiple tissues including the placenta[32,95], suggesting that hypergylcemic-related mitochondrial damage may lead to ROS through an imbalance of metabolic function and mitochondrial bioenergetics.

Both preeclampsia and GDM range widely in severity. As the placenta and ROS are central to the pathogenesis of both disorders, the ability of the placenta to respond to ROS may be important in determining disease severity. ROS production is countered by antioxidants (Figure 1). In preeclamptic placentae there is decreased function of antioxidants Superoxide dismutase (SOD), Thioredoxin reductase (ThRed) and Glutathione peroxidase (GPx)[96,97]. The severity of preeclampsia may be linked to antioxidant responses, with placentae from preeclampsia pregnancies that reached term exhibiting increased ROS,

but also potentially responding via compensatory mechanisms including increased GPx activity[35,98]. Indeed, correct ROS signalling and antioxidant responses may be critical in the development of preeclampsia, with animal work showing increased activity of the antioxidant response element Nrf2 linked to repressed angiogenesis[99]. Further, modulation of trophoblast antioxidant function can affect mitochondrial performance and ultimately determine cell fate[46,100]. ROS overproduction in GDM may be a result of impaired glycaemic control, rather than due to deficiencies in antioxidants[101]. However, antioxidant function is still important in GDM; increased placental protein carbonyls (formed via protein oxidation) are associated with increased placental SOD and catalase activity. This may represent an adaptive response of antioxidant upregulation to counter damage[102], although mitochondria may respond differently in different severities of the disorder, with less severe forms of GDM exhibit decreased maternal circulating SOD, catalase and GPx function[103].

Indeed, in animal models of pregnancies experiencing hypoxia, therapy in the form of the mitochondrial targeted MitoQ (a ubiquinone derivative) limits activation of mitochondrial and ER stress pathways in placentae and protects fetal brain development[104]. Together, these results demonstrate the importance of supporting placental mitochondrial function (e.g. through endogenous antioxidants) under stressful conditions, and also the utility of targeting mitochondria with exogenous therapeutics to improve pregnancy outcomes.

6. Conclusions

Mitochondria are critical to placental function, and therefore in supporting the fetus. ROS are fundamental messaging molecules that allow cells to sense their environment and to respond through mitochondrial-centred signalling cascades. Mitochondrial response to ROS is adaptive, and

can be critical to allowing tissues to cope with adverse environments; this can be observed in alterations such as increased antioxidant function in some preeclamptic placentae. Conversely, excessive ROS can lead to tissue damage, and ROS-induced mitochondrial adaptations are central to two important pregnancy complications; preeclampsia and GDM.

The effects of ROS in the placenta are complex and require further investigation, especially around the different placental mitochondrial subpopulations. The critical functions of mitochondria mean they are sensitive to damage, but the ability of mitochondria to sense and respond to stimuli also makes them attractive targets for future therapies that may improve pregnancy outcomes.

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References

1. Scheffler IE. *Mitochondria.* Vol Second edition: Hoboken, N.J. : John Wiley & Sons; 2007.
2. Sferruzzi-Perri AN, Camm EJ. The Programming Power of the Placenta. *Frontiers in physiology.* 2016;7:33.
3. Chamley LW, Holland OJ, Chen Q, Viall CA, Stone PR, Abumaree M. Review: where is the maternofetal interface? *Placenta.* 2014;35 Suppl:S74-80.
4. Jiang F, Zhang Y, Dusting GJ. NADPH oxidase-mediated redox signaling: roles in cellular stress response, stress tolerance, and tissue repair. *Pharmacological reviews.* 2011;63(1):218-242.
5. Holland O, Dekker Nitert M, Gallo LA, Vejzovic M, Fisher JJ, Perkins AV. Review: Placental mitochondrial function and structure in gestational disorders. *Placenta.* 2017.
6. Murphy MP, Hartley RC. Mitochondria as a therapeutic target for common pathologies. *Nature reviews Drug discovery.* 2018.
7. Cuffe JS, Turton EL, Akison LK, Bielefeldt-Ohmann H, Moritz KM. Prenatal corticosterone exposure programs sex-specific adrenal adaptations in mouse offspring. *The Journal of endocrinology.* 2017;232(1):37-48.
8. Cheong JN, Wlodek ME, Moritz KM, Cuffe JS. Programming of maternal and offspring disease: impact of growth restriction, fetal sex and transmission across generations. *The Journal of physiology.* 2016;594(17):4727-4740.
9. Fogarty NM, Burton G, Ferguson-Smith A. Different epigenetic states define syncytiotrophoblast and cytotrophoblast nuclei in the trophoblast of the human placenta. *Placenta.* 2015;36(8):796-802.
10. Jones CJ, Fox H. Ultrastructure of the normal human placenta. *Electron microscopy reviews.* 1991;4(1):129-178.
11. Martinez F, Kiriakidou M, Strauss JF, 3rd. Structural and functional changes in mitochondria associated with trophoblast differentiation: methods to isolate enriched preparations of syncytiotrophoblast mitochondria. *Endocrinology.* 1997;138(5):2172-2183.
12. Fisher J, McKeating D, Pennell E, Cuffe J, Holland O, Perkins A. Mitochondrial isolation, cryopreservation and preliminary biochemical characterisation from placental cytotrophoblast and syncytiotrophoblast. *Placenta.* 2019;82:1-4.
13. Martinez F, Olvera-Sanchez S, Esparza-Perusquia M, Gomez-Chang E, Flores-Herrera O. Multiple functions of syncytiotrophoblast mitochondria. *Steroids.* 2015;103:11-22.
14. De los Rios Castillo D, Zarco-Zavala M, Olvera-Sanchez S, et al. Atypical cristae morphology of human syncytiotrophoblast mitochondria: role for complex V. *The Journal of biological chemistry.* 2011;286(27):23911-23919.
15. Strauss M, Hofhaus G, Schroder RR, Kuhlbrandt W. Dimer ribbons of ATP synthase shape the inner mitochondrial membrane. *The EMBO journal.* 2008;27(7):1154-1160.
16. Couoh-Cardel SJ, Uribe-Carvajal S, Wilkens S, Garcia-Trejo JJ. Structure of dimeric F1F0-ATP synthase. *The Journal of biological chemistry.* 2010;285(47):36447-36455.
17. Gavin PD, Prescott M, Luff SE, Devenish RJ. Cross-linking ATP synthase complexes in vivo eliminates mitochondrial cristae. *Journal of cell science.* 2004;117(Pt 11):2333-2343.
18. Flores-Herrera O, Olvera-Sánchez S, Esparza-Perusquía M, et al. Membrane potential regulates mitochondrial ATP-diphosphohydrolase activity but is not involved in progesterone biosynthesis in human syncytiotrophoblast cells. *Biochimica et Biophysica Acta (BBA) - Bioenergetics.* 2015;1847(2):143-152.
19. Poidatz D, Dos Santos E, Gronier H, et al. Trophoblast syncytialisation necessitates mitochondrial function through estrogen-related receptor-γ activation. *MHR: Basic science of reproductive medicine.* 2014;21(2):206-216.
20. Watson AL, Skepper JN, Jauniaux E, Burton GJ. Susceptibility of human placental syncytiotrophoblastic mitochondria to oxygen-mediated damage in relation to gestational age. *The Journal of Clinical Endocrinology & Metabolism.* 1998;83(5):1697-1705.

21. Schoots MH, Gordijn SJ, Scherjon SA, van Goor H, Hillebrands J-L. Oxidative stress in placental pathology. *Placenta.* 2018;69:153-161.
22. Genbacev O, Joslin R, Damsky CH, Polliotti BM, Fisher SJ. Hypoxia alters early gestation human cytotrophoblast differentiation/invasion in vitro and models the placental defects that occur in preeclampsia. *The Journal of clinical investigation.* 1996;97(2):540-550.
23. Xie Y, Zhou S, Jiang Z, et al. Hypoxic stress induces, but cannot sustain trophoblast stem cell differentiation to labyrinthine placenta due to mitochondrial insufficiency. *Stem cell research.* 2014;13(3):478-491.
24. Boguslawski W. Cytochrome P-450 and steroidogenic activities of the human placental mitochondria. *Journal of steroid biochemistry.* 1983;18(6):771-775.
25. Bustamante J, Ramírez-Vélez R, Czerniczyniec A, Cicerchia D, Aguilar de Plata AC, Lores-Arnaiz S. Oxygen metabolism in human placenta mitochondria. *Journal of Bioenergetics and Biomembranes.* 2014;46(6):459-469.
26. Archer SL. Mitochondrial dynamics—mitochondrial fission and fusion in human diseases. *New England Journal of Medicine.* 2013;369(23):2236-2251.
27. Youle RJ, van der Bliek AM. Mitochondrial Fission, Fusion, and Stress. *Science (New York, NY).* 2012;337(6098):1062-1065.
28. Vishnyakova PA, Volodina MA, Tarasova NV, et al. Mitochondrial role in adaptive response to stress conditions in preeclampsia. *Scientific Reports.* 2016;6:32410.
29. Ausman J, Abbade J, Ermini L, et al. Ceramide-induced BOK promotes mitochondrial fission in preeclampsia. *Cell death & disease.* 2018;9(3):298-298.
30. Zhou X, Han T-L, Chen H, Baker PN, Qi H, Zhang H. Impaired mitochondrial fusion, autophagy, biogenesis and dysregulated lipid metabolism is associated with preeclampsia. *Experimental Cell Research.* 2017;359(1):195-204.
31. Yu J, Guo X, Chen R, Feng L. Downregulation of mitofusin 2 in placenta is related to preeclampsia. *BioMed research international.* 2016;2016.
32. Yu T, Robotham JL, Yoon Y. Increased production of reactive oxygen species in hyperglycemic conditions requires dynamic change of mitochondrial morphology. *Proceedings of the National Academy of Sciences of the United States of America.* 2006;103(8):2653-2658.
33. Saben JL, Boudoures AL, Asghar Z, et al. Maternal metabolic syndrome programs mitochondrial dysfunction via germline changes across three generations. *Cell reports.* 2016;16(1):1-8.
34. Vishnyakova PA, Volodina MA, Tarasova NV, et al. Alterations in antioxidant system, mitochondrial biogenesis and autophagy in preeclamptic myometrium. *BBA clinical.* 2017;8:35-42.
35. Holland OJ, Cuffe JSM, Dekker Nitert M, et al. Placental mitochondrial adaptations in preeclampsia associated with progression to term delivery. *Cell Death & Disease.* 2018;9(12):1150.
36. Green DR, Galluzzi L, Kroemer G. Metabolic control of cell death. *Science.* 2014;345(6203):1250256.
37. Karbowski M, Lee Y-J, Gaume B, et al. Spatial and temporal association of Bax with mitochondrial fission sites, Drp1, and Mfn2 during apoptosis. *The Journal of cell biology.* 2002;159(6):931-938.
38. Kasahara A, Scorrano L. Mitochondria: from cell death executioners to regulators of cell differentiation. *Trends in cell biology.* 2014;24(12):761-770.
39. Shi Z, Long W, Zhao C, Guo X, Shen R, Ding H. Comparative proteomics analysis suggests that placental mitochondria are involved in the development of pre-eclampsia. *PLoS One.* 2013;8(5):e64351.

40. Magee TR, Ross MG, Wedekind L, Desai M, Kjos S, Belkacemi L. Gestational diabetes mellitus alters apoptotic and inflammatory gene expression of trophobasts from human term placenta. *Journal of diabetes and its complications.* 2014;28(4):448-459.
41. Holloszy JO. Biochemical adaptations in muscle. Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. *The Journal of biological chemistry.* 1967;242(9):2278-2282.
42. Broskey NT, Greggio C, Boss A, et al. Skeletal muscle mitochondria in the elderly: effects of physical fitness and exercise training. *The Journal of clinical endocrinology and metabolism.* 2014;99(5):1852-1861.
43. Mayeur S, Lancel S, Theys N, et al. Maternal calorie restriction modulates placental mitochondrial biogenesis and bioenergetic efficiency: putative involvement in fetoplacental growth defects in rats. *American journal of physiology Endocrinology and metabolism.* 2013;304(1):E14-22.
44. Mele J, Muralimanoharan S, Maloyan A, Myatt L. Impaired mitochondrial function in human placenta with increased maternal adiposity. *American journal of physiology Endocrinology and metabolism.* 2014;307(5):E419-E425.
45. Le ZY, Dong S, Zhang R, et al. Placental mitochondrial biogenesis and function was slightly changed by gestational hypercholesterolemia in full-term pregnant women. *Journal of developmental origins of health and disease.* 2018;9(4):395-400.
46. Khera A, Dong LF, Holland O, et al. Selenium supplementation induces mitochondrial biogenesis in trophoblasts. *Placenta.* 2015;36(8):863-869.
47. Radenkovic F, Holland O, Vanderlelie JJ, Perkins AV. Selective inhibition of endogenous antioxidants with Auranofin causes mitochondrial oxidative stress which can be countered by selenium supplementation. *Biochemical pharmacology.* 2017;146:42-52.
48. Lee HC, Yin PH, Lu CY, Chi CW, Wei YH. Increase of mitochondria and mitochondrial DNA in response to oxidative stress in human cells. *Biochemical Journal.* 2000;348(Pt 2):425-432.
49. Lee HM, Greeley GH, Englander EW. SUSTAINED HYPOXIA MODULATES MITOCHONDRIAL DNA CONTENT IN THE NEONATAL RAT BRAIN. *Free radical biology & medicine.* 2008;44(5):807-814.
50. Costa LE, Boveris A, Koch OR, Taquini AC. Liver and heart mitochondria in rats submitted to chronic hypobaric hypoxia. *The American journal of physiology.* 1988;255(1 Pt 1):C123-129.
51. Holland OJ, Hickey AJ, Alvsaker A, et al. Changes in mitochondrial respiration in the human placenta over gestation. *Placenta.* 2017;57:102-112.
52. Burton GJ. Oxygen, the Janus gas; its effects on human placental development and function. *Journal of anatomy.* 2009;215(1):27-35.
53. Szendroedi J, Phielix E, Roden M. The role of mitochondria in insulin resistance and type 2 diabetes mellitus. *Nature Reviews Endocrinology.* 2012;8(2):92.
54. Muralimanoharan S, Maloyan A, Myatt L. Mitochondrial function and glucose metabolism in the placenta with gestational diabetes mellitus: role of miR-143. *Clinical Science.* 2016;130(11):931-941.
55. Burton GJ, Yung HW, Murray AJ. Mitochondrial - Endoplasmic reticulum interactions in the trophoblast: Stress and senescence. *Placenta.* 2017;52:146-155.
56. Marchi S, Patergnani S, Pinton P. The endoplasmic reticulum–mitochondria connection: One touch, multiple functions. *Biochimica et Biophysica Acta (BBA) - Bioenergetics.* 2014;1837(4):461-469.
57. Sugiura A, Nagashima S, Tokuyama T, et al. MITOL regulates endoplasmic reticulum-mitochondria contacts via Mitofusin2. *Molecular cell.* 2013;51(1):20-34.
58. Korobova F, Ramabhadran V, Higgs HN. An actin-dependent step in mitochondrial fission mediated by the ER-associated formin INF2. *Science.* 2013;339(6118):464-467.

59. Decuypere JP, Monaco G, Bultynck G, Missiaen L, De Smedt H, Parys JB. The IP(3) receptor-mitochondria connection in apoptosis and autophagy. *Biochimica et biophysica acta.* 2011;1813(5):1003-1013.
60. Cardenas C, Miller RA, Smith I, et al. Essential regulation of cell bioenergetics by constitutive InsP3 receptor Ca2+ transfer to mitochondria. *Cell.* 2010;142(2):270-283.
61. Bravo R, Vicencio JM, Parra V, et al. Increased ER-mitochondrial coupling promotes mitochondrial respiration and bioenergetics during early phases of ER stress. *Journal of cell science.* 2011;124(Pt 13):2143-2152.
62. Bertero E, Maack C. Calcium Signaling and Reactive Oxygen Species in Mitochondria. *Circulation Research.* 2018;122(10):1460-1478.
63. Chaube R, Werstuck GH. Mitochondrial ROS versus ER ROS: which comes first in myocardial calcium dysregulation? *Frontiers in cardiovascular medicine.* 2016;3:36.
64. Lemasters JJ, Theruvath TP, Zhong Z, Nieminen AL. Mitochondrial calcium and the permeability transition in cell death. *Biochimica et biophysica acta.* 2009;1787(11):1395-1401.
65. Burton GJ, Yung HW, Cindrova-Davies T, Charnock-Jones DS. Placental endoplasmic reticulum stress and oxidative stress in the pathophysiology of unexplained intrauterine growth restriction and early onset preeclampsia. *Placenta.* 2009;30 Suppl A:S43-48.
66. Yung HW, Atkinson D, Campion-Smith T, Olovsson M, Charnock-Jones DS, Burton GJ. Differential activation of placental unfolded protein response pathways implies heterogeneity in causation of early- and late-onset pre-eclampsia. *The Journal of pathology.* 2014;234(2):262-276.
67. Vuorinen K, Remes A, Sormunen R, Tapanainen J, Hassinen IE. Placental Mitochondrial DNA and Respiratory Chain Enzymes in the Etiology of Preeclampsia. *Obstetrics & Gynecology.* 1998;91(6):950-955.
68. Hache S, Takser L, LeBellego F, et al. Alteration of calcium homeostasis in primary preeclamptic syncytiotrophoblasts: effect on calcium exchange in placenta. *Journal of cellular and molecular medicine.* 2011;15(3):654-667.
69. Du L, He F, Kuang L, Tang W, Li Y, Chen D. eNOS/iNOS and endoplasmic reticulum stress-induced apoptosis in the placentas of patients with preeclampsia. *Journal of human hypertension.* 2017;31(1):49-55.
70. Yung H-W, Alnæs-Katjavivi P, Jones CJP, et al. Placental endoplasmic reticulum stress in gestational diabetes: the potential for therapeutic intervention with chemical chaperones and antioxidants. *Diabetologia.* 2016;59(10):2240-2250.
71. Harvey AJ, Kind KL, Thompson JG. REDOX regulation of early embryo development. *Reproduction (Cambridge, England).* 2002;123(4):479-486.
72. Manes C, Lai N. Nonmitochondrial oxygen utilization by rabbit blastocysts and surface production of superoxide radicals. *Journal of reproduction and fertility.* 1995;104(1):69-75.
73. Chen HW, Jiang WS, Tzeng CR. Nitric oxide as a regulator in preimplantation embryo development and apoptosis. *Fertility and sterility.* 2001;75(6):1163-1171.
74. Al-Gubory KH, Fowler PA, Garrel C. The roles of cellular reactive oxygen species, oxidative stress and antioxidants in pregnancy outcomes. *The international journal of biochemistry & cell biology.* 2010;42(10):1634-1650.
75. Armant DR, Kilburn BA, Petkova A, et al. Human trophoblast survival at low oxygen concentrations requires metalloproteinase-mediated shedding of heparin-binding EGF-like growth factor. *Development (Cambridge, England).* 2006;133(4):751-759.
76. Seeho SKM, Park JH, Rowe J, Morris JM, Gallery EDM. Villous explant culture using early gestation tissue from ongoing pregnancies with known normal outcomes: the effect of oxygen on trophoblast outgrowth and migration. *Human Reproduction.* 2008;23(5):1170-1179.

77. Kenchegowda D, Natale B, Lemus MA, Natale DR, Fisher SA. Inactivation of maternal Hif-1alpha at mid-pregnancy causes placental defects and deficits in oxygen delivery to the fetal organs under hypoxic stress. *Developmental biology.* 2017;422(2):171-185.
78. Caniggia I, Mostachfi H, Winter J, et al. Hypoxia-inducible factor-1 mediates the biological effects of oxygen on human trophoblast differentiation through TGFbeta(3). *J Clin Invest.* 2000;105(5):577-587.
79. Jauniaux E, Hempstock J, Greenwold N, Burton GJ. Trophoblastic oxidative stress in relation to temporal and regional differences in maternal placental blood flow in normal and abnormal early pregnancies. *The American journal of pathology.* 2003;162(1):115-125.
80. Hu H, Nan J, Sun Y, et al. Electron leak from NDUFA13 within mitochondrial complex I attenuates ischemia-reperfusion injury via dimerized STAT3. *Proceedings of the National Academy of Sciences.* 2017;114(45):11908-11913.
81. Diaz P, Sibley CP, Greenwood SL. Oxygen-Sensitive K+ Channels Modulate Human Chorionic Gonadotropin Secretion from Human Placental Trophoblast. *PLoS One.* 2016;11(2):e0149021.
82. Kingdom J, Huppertz B, Seaward G, Kaufmann P. Development of the placental villous tree and its consequences for fetal growth. *European Journal of Obstetrics & Gynecology and Reproductive Biology.* 2000;92(1):35-43.
83. Pereira RD, De Long NE, Wang RC, Yazdi FT, Holloway AC, Raha S. Angiogenesis in the placenta: the role of reactive oxygen species signaling. *Biomed Res Int.* 2015;2015:814543.
84. Gorlach A, Dimova EY, Petry A, et al. Reactive oxygen species, nutrition, hypoxia and diseases: Problems solved? *Redox biology.* 2015;6:372-385.
85. Guo X, Feng L, Jia J, Chen R, Yu J. Upregulation of VEGF by small activating RNA and its implications in preeclampsia. *Placenta.* 2016;46:38-44.
86. Sánchez-Aranguren LC, Prada CE, Riaño-Medina CE, Lopez M. Endothelial dysfunction and preeclampsia: role of oxidative stress. *Frontiers in physiology.* 2014;5:372.
87. Jameson JL, De Groot, L. J, Metzger B. E Endocrinology: Adult & pediatric. *Philadelphia, PA: Elsevier Saunders.* 2016;2015;7th;7th; ed.:Chapter 45, 788-804.
88. Sivitz WI, Yorek MA. Mitochondrial Dysfunction in Diabetes: From Molecular Mechanisms to Functional Significance and Therapeutic Opportunities. *Antioxidants & Redox Signaling.* 2010;12(4):537-577.
89. Biri A, Onan A, Devrim E, Babacan F, Kavutcu M, Durak I. Oxidant status in maternal and cord plasma and placental tissue in gestational diabetes. *Placenta.* 2006;27(2-3):327-332.
90. Cui XL, Brockman D, Campos B, Myatt L. Expression of NADPH oxidase isoform 1 (Nox1) in human placenta: involvement in preeclampsia. *Placenta.* 2006;27(4-5):422-431.
91. Matsubara K, Matsubara Y, Hyodo S, Katayama T, Ito M. Role of nitric oxide and reactive oxygen species in the pathogenesis of preeclampsia. *The journal of obstetrics and gynaecology research.* 2010;36(2):239-247.
92. Dikalov S. Crosstalk between mitochondria and NADPH oxidases. *Free radical biology & medicine.* 2011;51(7):1289-1301.
93. Dikalov S. Cross talk between mitochondria and NADPH oxidases. *Free radical biology & medicine.* 2011;51(7):1289-1301.
94. Pantham P, Viall CA, Chen Q, Kleffmann T, Print CG, Chamley LW. Antiphospholipid antibodies bind syncytiotrophoblast mitochondria and alter the proteome of extruded syncytial nuclear aggregates. *Placenta.* 2015;36(12):1463-1473.
95. Mandò C, Anelli GM, Novielli C, et al. Impact of Obesity and Hyperglycemia on Placental Mitochondria. *Oxidative medicine and cellular longevity.* 2018;2018.
96. Wang Y, Walsh SW. Increased Superoxide Generation is Associated with Decreased Superoxide Dismutase Activity and mRNA Expression in Placental Trophoblast Cells in Pre-eclampsia. *Placenta.*22(2):206-212.

97. Vanderlelie J, Venardos K, Clifton VL, Gude NM, Clarke FM, Perkins AV. Increased biological oxidation and reduced anti-oxidant enzyme activity in pre-eclamptic placentae. *Placenta.*26(1):53-58.
98. Cuffe JSM, Holland O, Salomon C, Rice GE, Perkins AV. Review: Placental derived biomarkers of pregnancy disorders. *Placenta.* 2017;54:104-110.
99. Nezu M, Souma T, Yu L, et al. Nrf2 inactivation enhances placental angiogenesis in a preeclampsia mouse model and improves maternal and fetal outcomes. *Science signaling.* 2017;10(479).
100. Khera A, Vanderlelie JJ, Holland O, Perkins AV. Overexpression of Endogenous Anti-Oxidants with Selenium Supplementation Protects Trophoblast Cells from Reactive Oxygen Species-Induced Apoptosis in a Bcl-2-Dependent Manner. *Biological trace element research.* 2017;177(2):394-403.
101. West IC. Radicals and oxidative stress in diabetes. *Diabetic Medicine.* 2000;17(3):171-180.
102. Coughlan MT, Vervaart PP, Permezel M, Georgiou HM, Rice GE. Altered placental oxidative stress status in gestational diabetes mellitus. *Placenta.* 2004;25(1):78-84.
103. López-Tinoco C, Roca M, García-Valero A, et al. Oxidative stress and antioxidant status in patients with late-onset gestational diabetes mellitus. *Acta Diabetologica.* 2013;50(2):201-208.
104. Nuzzo AM, Camm EJ, Sferruzzi-Perri AN, et al. Placental Adaptation to Early-Onset Hypoxic Pregnancy and Mitochondria-Targeted Antioxidant Therapy in Rat. *The American Journal of Pathology.* 2018.

Figure 1. Reactive oxygen species signalling and damage. Reactive oxygen species (ROS) are important in cellular signalling, and can lead to cellular adaptations to counter stressful environments. However, when ROS production is greater than antioxidant and other adaptive responses, ROS-mediated damage can led to cell death. Placental mitochondria are critical to cell function, and mitochondrial damage is a feature of pregnancy complications.

Figure 2. Placental mitochondria. Mitochondria of cytotrophoblasts have a classical morphology, whereas in the syncytiotrophoblast mitochondria are smaller with a less well defined cristae structure.

Figure 3. Mitochondrial dynamics. The mitochondrial fission/fusion cycle regulates mitochondria to prevent cell damage. Fission isolates depolarized segments of the mitochondrial reticular network, which can then disposed of via autophagy. More polarized mitochondrial segments re-join the network, and mitochondrial content can also be increased by biogenesis. Mitochondrial-endoplasmic reticulum interactions are important for assembly of fission and fusion apparatus.