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Hannah Roberts
Schulich School of Medicine & Dentistry

Stephane L. Bourque
University of Alberta

Stephen J. Renaud
Schulich School of Medicine & Dentistry, srenaud4@uwo.ca

REPRODUCTION
REVIEW

Maternal iron homeostasis: effect on placental development and function

Hannah Roberts[1], Stephane L Bourque[2] and Stephen J Renaud[1,3]

[1]*Department of Anatomy and Cell Biology, Schulich School of Medicine and Dentistry, University of Western Ontario, London, Ontario, Canada,* [2]*Departments of Anesthesiology & Pain Medicine, Pharmacology, and Pediatrics, University of Alberta, Edmonton, Alberta, Canada and* [3]*Children's Health Research Institute, Lawson Health Research Institute, London, Ontario, Canada*

Correspondence should be addressed to S L Bourque or S J Renaud; Email: sbourque@ualberta.ca or srenaud4@uwo.ca

Abstract

Iron is an essential mineral that participates in oxygen transport, DNA synthesis and repair, and as a cofactor for various cellular processes. Iron deficiency is the most common nutritional deficiency worldwide. Due to blood volume expansion and demands from the fetal–placental unit, pregnant women are one of the populations most at risk of developing iron deficiency. Iron deficiency during pregnancy poses major health concerns for offspring, including intrauterine growth restriction and long-term health complications. Although the underlying mechanisms remain unclear, maternal iron deficiency may indirectly impair fetal growth through changes in the structure and function of the placenta. Since the placenta forms the interface between mother and baby, understanding how the placenta changes in iron deficiency may yield new diagnostic indices of fetal stress in affected pregnancies, thereby leading to earlier interventions and improved fetal outcomes. In this review, we compile current data on the changes in placental development and function that occur under conditions of maternal iron deficiency, and discuss challenges and perspectives on managing the high incidence of iron deficiency in pregnant women.

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Introduction

Iron is an essential mineral that participates in many cellular processes, including oxygen transport, DNA synthesis and repair, and as an essential cofactor for various enzymes. Iron deficiency (ID) is the most common nutrient deficiency worldwide (McLean *et al.* 2009). It occurs when iron demands chronically exceed intake, leading to progressive depletion of tissue iron stores, and eventually, functional iron. Since iron is required for hemoglobin (Hb) synthesis, ID can result in anemia – a condition in which the quantity of circulating Hb, and thus oxygen-carrying capacity, falls below clinical thresholds and begins to impact cellular function (Maria de Regil 2010). One of the populations most at risk for ID anemia is pregnant women, due to the increased iron requirements of pregnancy. Iron is essential to support placental and fetal growth and maintain increased maternal red blood cell mass. An estimated 38% of women develop anemia during pregnancy, including 22% in high-income countries, with most cases attributed to ID (Stevens *et al.* 2013, Lopez *et al.* 2016).

ID during pregnancy can detrimentally affect fetal health. ID increases the risk of fetal death, preterm birth, and intrauterine growth restriction, and is associated with altered growth trajectories and long-term cognitive, cardiovascular, metabolic, and immune system complications in affected offspring (Krantman *et al.* 1982, Doom & Georgieff 2014, Alwan *et al.* 2015, Grandone *et al.* 2015). Despite repletion of iron stores in children through iron supplementation, health complications persist in children whose mothers exhibited ID during pregnancy, underscoring the importance of sufficient iron being supplied *in utero* for long-term health (Pasricha *et al.* 2013). Mechanisms through which maternal ID impairs fetal growth and results in long-term sequelae remain to be elucidated; however, the placenta may play a key role.

The placenta forms the interface between mother and fetus and supports fetal growth and development by facilitating respiratory gas, nutrient, and waste transport between maternal and fetal circulations, producing hormones, and providing immunological support. As an adaptive organ, the placenta can respond to environmental signals through alterations in structure and function, which in turn influence blood flow, nutrient transport, and hormone metabolism to optimize the intrauterine environment. While these adaptations may convey short-term benefits, in some circumstances, they may impair long-term function and have profound impacts on a fetus' ability to cope in the intrauterine environment. Placental

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dysfunction may, therefore, connect adverse maternal nutrition and offspring health, and potentially be used as an 'early warning system' for fetal complications. In this review, we will briefly introduce placental development and discuss iron requirements during pregnancy. We will then summarize current knowledge detailing how ID affects placental development and function, and discuss new challenges and opportunities to address the impact of ID on placental development and pregnancy outcome.

The placenta

The human placenta is discoid-shaped, hemochorial (maternal blood directly contacts fetal chorion), and is composed of tissues derived from both mother and fetus. The maternal aspect of the placenta is the decidua basalis, which contains stromal cells, glands, leukocytes, and blood vessels (e.g. spiral arteries). The fetal aspect of the placenta is arranged in chorionic villi, which are highly branched structures consisting of an outer layer of syncytialized trophoblast and mononuclear cytotrophoblasts surrounding an inner core containing fibroblasts, macrophages, and blood vessels (Fig. 1). The syncytialized trophoblast layer is the major source of hormones necessary for pregnancy establishment and maintenance. At the tips of large anchoring villi, cytotrophoblasts differentiate into extravillous trophoblasts. Extravillous trophoblasts proliferate into stratified cell columns and then invade into the uterine

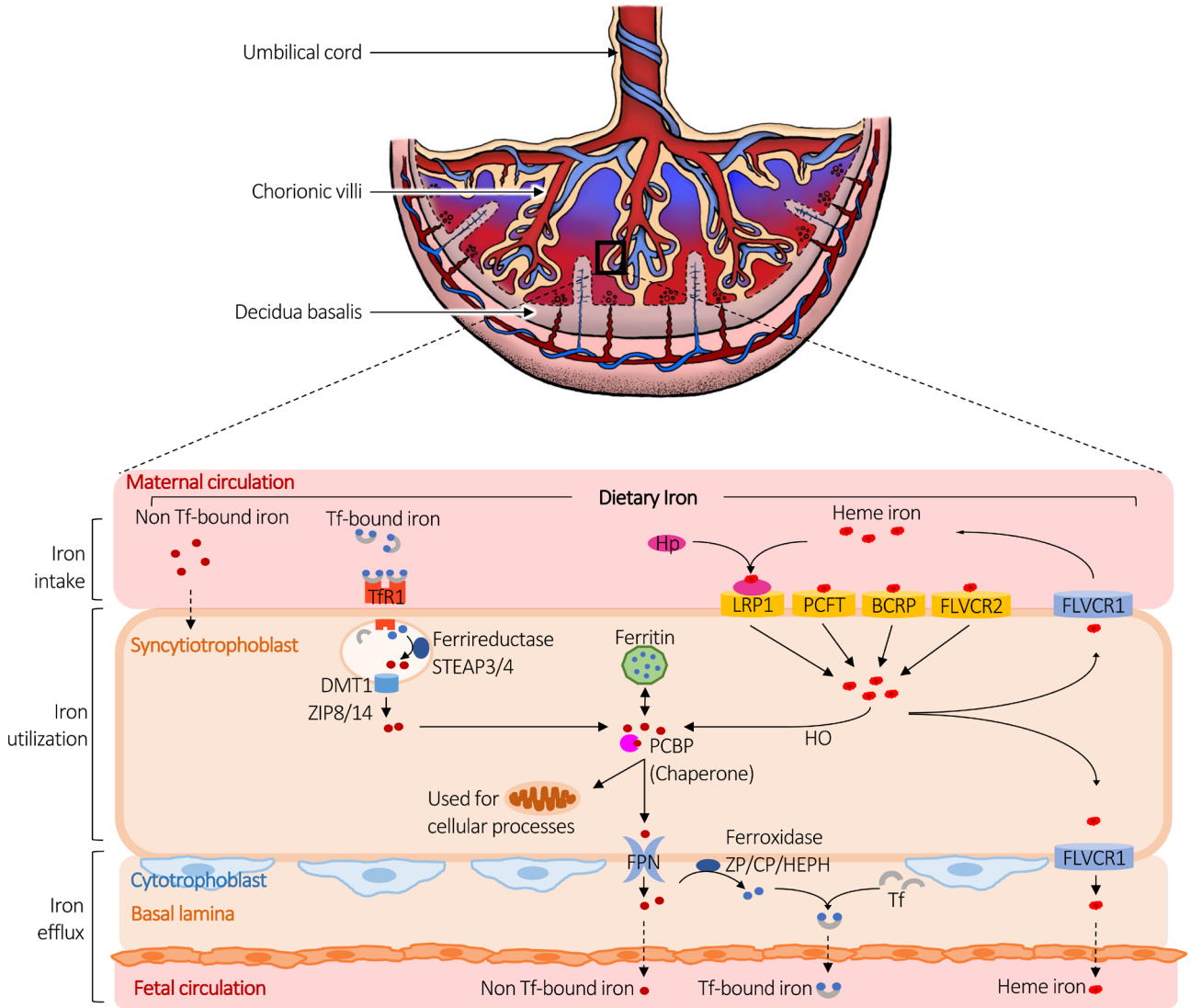

Figure 1 Schematic illustration of placental iron transport. Top: illustration of a human placenta. Bottom: diagram showing iron transport from the maternal circulation to the fetal circulation. BCRP, breast cancer resistance protein; CP, ceruloplasmin; DMT, divalent metal transporter-1; FLVCR1/2, feline leukemia subgroup C receptor 1/2; FPN, ferroportin; HO, heme oxygenases; Hp, hemopexin; LRP1, low-density lipoprotein receptor-related protein-1; PCBP, poly(rC)-binding protein; PCFT, proton-coupled folate transporter; STEAP, six-transmembrane epithelial antigen of prostate; Tf, transferrin; TfR1, transferrin receptor 1; ZIP, ZRT/IRT-like protein; ZP, zyklopen.

wall to modify spiral arteries and promote consistent, low-velocity maternal blood flow toward the space surrounding chorionic villi. Blood vessels in the villous core bring deoxygenated, nutrient-poor blood from the fetus. Oxygen and nutrients dissolved in maternal blood pass across the trophoblast layer into blood vessels within the villous core, where they are carried to the fetus. Carbon dioxide and wastes pass from fetal blood into the intervillous space to be carried away via endometrial veins. Substances can move across the placenta by simple diffusion (e.g. oxygen), facilitated diffusion (e.g. glucose), or active transport (e.g. iron). Placental nutrient transfer capacity can be influenced by a wide variety of factors, including villous surface area and thickness, the abundance and activity of transporters, concentration gradients between maternal and fetal blood, placental metabolism, and uteroplacental blood flow, all of which are sensitive to environmental stimuli (Tarrade *et al.* 2015).

A discoid-shaped, hemochorial placenta also features in many rodent species, including those commonly used in laboratory settings: mice and rats (referred to as 'laboratory rodents' unless specified otherwise) (Soares *et al.* 2012). Although there are inherent differences in placentation between humans and laboratory rodents (e.g. length of gestation, number of conceptuses, and organization of trophoblast subtypes within the placenta), there are also anatomical and functional similarities (Soncin *et al.* 2018). For instance, placentas of laboratory rodents are composed of both maternal and fetal compartments. The maternal compartment includes the decidua basalis and mesometrial triangle, and contains spiral arteries and a similar composition of cells to that found in humans. In laboratory rodents, the site at which nutrients are exchanged between maternal and fetal blood, referred to as the labyrinth zone because of its maze-like resemblance in cross-section, is compositionally similar to the chorionic villi in humans. Nutrients dissolved in maternal blood pass across three trophoblast layers (including two syncytialized layers) to access the fetal circulation. The junctional zone is a transitional region sandwiched between the labyrinth zone and decidua basalis. It is composed of stratified layers of trophoblasts, a subset of which invade into the maternal compartment and, particularly in rats, contribute to spiral artery transformation. Thus, the junctional zone is functionally analogous to extravillous trophoblasts in human placenta. The junctional zone also has an important endocrine function, which is a major difference between placentas of humans and laboratory rodents (Malassiné *et al.* 2003). Nevertheless, given the anatomical and functional similarities between human and laboratory rodent placentas and the capacity for experimental manipulation in the latter (Renaud *et al.* 2011), many studies have used mice and rats to deduce the impact of iron homeostasis on pregnancy outcome. Consequently, although this review will emphasize the impact of iron homeostasis on human placental development, we will also discuss findings using mice, rats, and other species where appropriate.

Iron requirements and regulation in pregnancy

The maternal requirement for iron changes considerably during pregnancy to facilitate maternal blood volume expansion, placental development, and fetal growth (reviewed in Fisher & Nemeth 2017). Most of this demand occurs in the third trimester, reaching an average daily iron requirement of approximately 4.4 mg/day (Milman 2006*a*). To accommodate the increased iron requirements, women entering pregnancy should have approximately 500 mg of stored iron (Milman *et al.* 1999), yet only 20–35% of women of reproductive age are estimated to meet this threshold (Milman 2006*b*, Means 2020). It is, therefore, apparent why many women experience ID during pregnancy.

Several mechanisms enhance iron availability in the maternal circulation throughout pregnancy, including increased intestinal absorption of iron and mobilization of maternal iron reserves. Dietary iron exists in two forms: nonheme (predominantly from plant sources) and heme (from animal sources). As pregnancy progresses, maternal absorption of nonheme iron increases and heme absorption likely follows a similar trend (Barrett *et al.* 1994, Young *et al.* 2012). Maternal iron stores become mobilized during pregnancy from depots such as liver and spleen, as shown by studies using pregnant rodents (Hubbard *et al.* 2013, Gao *et al.* 2015). Additionally, iron-binding capacity of transferrin (Tf), which transports nonheme iron in blood, increases throughout gestation (Choi *et al.* 2000). Together, these processes facilitate maternal hematologic adaptation, and increase iron availability for the placenta and fetus.

The regulation of iron availability during pregnancy is dependent on hepcidin, a hormone produced mainly by hepatocytes that controls levels of circulating iron (Nemeth & Ganz 2006). Multiple factors influence hepcidin production, including circulating and stored iron, erythropoietic activity, and inflammation (Sangkhae & Nemeth 2017). Hepcidin binds to the iron export protein, ferroportin (FPN), and promotes its degradation, thereby triggering iron sequestration and decreased efflux into plasma from key sites, including intestine and liver (Fisher & Nemeth 2017). In second and third trimesters, maternal hepcidin concentrations are decreased, presumably to increase iron availability and uptake by the placenta, which constitutes an important period of iron accretion for the fetus (van Santen *et al.* 2013, Bah *et al.* 2017). In a study of 19 pregnant women who ingested stable iron isotopes, net dietary nonheme and heme iron transferred to the fetus was inversely correlated with maternal serum hepcidin

(Young *et al.* 2012). Not surprisingly, maternal hepcidin is lowest in pregnant women with ID (Rehu *et al.* 2010).

Placental iron transport and regulation

Fetal growth and development are dependent on active iron transport across the placenta, therefore this organ plays a crucial role in iron flux throughout pregnancy. Placental iron transport is controlled mainly by the syncytiotrophoblast. A detailed description of placental iron transport mechanisms has recently been reviewed (Sangkhae & Nemeth 2019).

Placental nonheme iron transport

Nonheme iron is bound to Tf in maternal circulation. The iron-bound Tf binds to the Tf receptor (TfR) and undergoes unidirectional transport. Both TfR1 and TfR2 are expressed on the apical surface of the syncytiotrophoblast; however, the role of TfR2 remains undefined (McArdle *et al.* 2011). The iron-Tf-TfR1 complex is internalized via clathrin-mediated endocytosis. Acidification of the vesicle causes iron to dissociate from Tf as Fe^{3+} and becomes reduced by ferrireductases to Fe^{2+}. Potential ferrireductases include six-transmembrane epithelial antigen of prostate-3 (STEAP3) and STEAP4, which are highly expressed in the placenta (Ohgami *et al.* 2006). TfR and Tf return to the apical surface of syncytiotrophoblast, where Tf is recycled back into the maternal circulation. Fe^{2+} is transported from endosomes into the cytoplasm, possibly via divalent metal transporter-1 (DMT1), which has been implicated in maternal-fetal iron transfer (Chong *et al.* 2005). Interestingly, studies in mice suggest the likelihood of an alternative DMT1-independent iron-uptake pathway in the placenta, which may involve ZRT/IRT-like protein-14 (ZIP14), and ZIP8 (Gunshin *et al.* 2005).

Once in the cytosol, iron may be chaperoned by poly(rC)-binding protein (PCBP) to either be used for cellular processes, stored in ferritin, or transported to the basolateral surface of syncytiotrophoblast, where it is exported into the villous core via FPN. As ferritin levels are low in syncytiotrophoblast, most iron entering the placenta is likely used for cellular processes or transported to the fetus (Bastin *et al.* 2006). Once exported from the syncytiotrophoblast via FPN, iron may be delivered to fetal Tf via oxidation by placental ferroxidases which may include ceruloplasmin, hephaestin, and zyklopen (Danzeisen *et al.* 2000, Chen *et al.* 2010). Alternatively, non-Tf-bound iron is present in the fetal circulation, indicating that it may be transported directly across fetal endothelial cells, although this mechanism is unknown (Evans *et al.* 2011).

Placental heme iron transport

Placental expression of putative heme influx transporters includes proton-coupled folate transporter (PCFT)/heme carrier protein-1 (HCP1), breast cancer resistance protein (BCRP), and feline leukemia subgroup C receptor-2 (FLVCR2) (Maliepaard *et al.* 2001, Qiu *et al.* 2006, Duffy *et al.* 2010). Low-density lipoprotein receptor-related protein-1 (LRP1) is highly expressed in placenta and may also facilitate heme uptake by recognizing heme complexed to a high-affinity heme-binding plasma protein, hemopexin (Hvidberg *et al.* 2005). Once imported, heme iron can be processed by heme oxygenases, releasing iron into the cytosol, or may be directly exported by FLVCR1, which may serve to prevent heme toxicity (Jaacks *et al.* 2011, Levytska *et al.* 2013). The functional significance of heme iron transport across the placenta is largely unknown. However, following maternal ingestion of ^{57}Fe-heme and ^{58}Fe-nonheme iron, a greater proportion of ^{57}Fe was detected in neonates, suggesting that heme-based iron is more efficiently transferred compared to nonheme iron (Young *et al.* 2012). Although there are multiple factors that may account for increased enrichment of heme versus nonheme iron in neonatal blood, it is nevertheless apparent that heme is an important source of iron for the fetus.

Regulation of placental iron transport

Several factors influence transport of nutrients (including iron) across the placenta, including uteroplacental and umbilical hemodynamics, placental surface area, metabolism, and expression/activity of transporters. In response to changes in cytosolic iron status, iron regulatory proteins bind to untranslated regions (UTRs) of transcripts encoding transporters and storage proteins (e.g. TfR1, DMT1, ferritin, and FPN), as reviewed in (Lipiński *et al.* 2013). In general, binding of iron regulatory proteins to the 3′-UTR increases mRNA stability (e.g. for TfR1 and DMT1), whereas binding to the 5′-UTR prevents translation (e.g. for ferritin and FPN). Thus, when cytosolic iron levels are plentiful, iron storage and export are stimulated and import is inhibited.

Maternal and fetal hepcidin levels influence placental iron trafficking. Hepcidin may interact with FPN to facilitate its internalization from the basolateral membrane of syncytiotrophoblast, and also downregulates TfR1 expression in mouse placenta (Martin *et al.* 2004, Nemeth *et al.* 2004). Notably, in mouse embryos that overexpress hepcidin, either as a consequence of transgenic hepcidin overexpression or mutations in the hepcidin regulator matriptase-2, placental FPN is downregulated resulting in severe fetal ID (Nicolas *et al.* 2002, Willemetz *et al.* 2014). Additionally, mouse embryos lacking hereditary hemochromatosis protein (HFE), a protein that promotes hepcidin production, exhibit increased placental expression of TfR1, DMT1, and FPN (Balesaria *et al.* 2012). HFE is also expressed on the apical surface of

syncytiotrophoblast. In addition to stimulating hepcidin production, it may negatively regulate iron uptake by blocking TfR1 binding to Tf, as it does in hepatocytes (Parkkila *et al.* 1997, Gruper *et al.* 2005). In rats, fetal hepcidin levels tightly correlate with maternal liver iron levels, more so even than the association between maternal hepcidin and maternal iron status (Gambling *et al.* 2009). This may help to ensure fetal iron demands are met during pregnancy. Fetal hepcidin is also upregulated during maternal inflammation, possibly as a protective mechanism to limit iron availability to pathogenic bacteria (Fisher *et al.* 2020). Notably, hepcidin is also expressed by the placenta at low levels, but its role is unclear (Evans *et al.* 2011). No correlation exists between placental hepcidin expression and either maternal or neonatal iron status, nor with placental iron transporter expression (Rehu *et al.* 2010, Best *et al.* 2016). Similarly, placental hepcidin in pregnant rats is not associated with iron content of maternal diet, or with maternal or fetal liver iron content; in mice, placental hepcidin does not correlate with fetal iron endowment in normal pregnancy or in iron-deficient states (Gambling *et al.* 2009, Sangkhae *et al.* 2020*a*,*b*).

Maternal ID: effect on the placenta

Transplacental iron and nutrient transport

In maternal ID, placental iron transfer is a balancing act to ensure that sufficient iron is available to support its own functions and delivered to the fetus despite low levels in maternal blood. Table 1 summarizes alterations in placental expression of proteins involved in iron transport and metabolism in relation to iron status. The placental protein that is most frequently reported to exhibit altered levels relative to iron status is TfR1. Increased TfR1 expression is frequently reported in placentas from ID pregnancies, and is recapitulated in human trophoblast cells and cell-lines cultured in the presence of the iron chelator, desferoxamine (Kroos *et al.* 1996, Georgieff *et al.* 1999, Gambling *et al.* 2001, Li *et al.* 2008, 2012, Young *et al.* 2010, Garcia-Valdes *et al.* 2015, Best *et al.* 2016, Sangkhae *et al.* 2020*a*). Likewise, increased placental TfR1 expression is evident in pregnant rodents fed ID diets (Gambling *et al.* 2009, Balesaria *et al.* 2012, Cornock *et al.* 2013), whereas rodents fed an iron-replete diet or given parenteral iron supplementation exhibit decreased placental TfR1 expression (Martin *et al.* 2004, Balesaria *et al.* 2012). Thus, dynamic expression of TfR1 by the placenta is potentially a key adaptation to guarantee appropriate uptake of iron from maternal blood.

Expression of other proteins involved in iron transport or metabolism has also been measured in maternal ID, although patterns are less clear. Placental expression of DMT1 is reported to be increased in some studies using rodent models of maternal ID, which may facilitate intracellular iron trafficking (Gambling *et al.* 2001, Cornock *et al.* 2013); although in other studies, no change in DMT1 was detected (Sangkhae *et al.* 2020*a*). In placentas collected from mothers with mild-to-moderate anemia, no change in FPN expression is evident (Li *et al.* 2008, Best *et al.* 2016, Sangkhae *et al.* 2020*a*). However, in placentas collected from mice exposed to severe iron deficiency, or primary human trophoblasts treated with chelating agents, FPN expression is paradoxically reduced, suggesting that the placenta may sequester iron to preserve its own metabolic function rather than ensure fetal sustenance (Sangkhae *et al.* 2020*a*).

Maternal ID may also affect the expression of putative heme transporters. In pregnant adolescents, maternal anemia is associated with decreased expression of the placental heme export protein FLVCR1 (Jaacks *et al.* 2011). Fetal iron status more closely reflects the expression of the placental heme transporters FLVCR1 and PCFT than with nonheme transporters (Best *et al.* 2016), suggesting that heme transporters may respond to fetal iron demand rather than maternal iron availability. Given that the placenta efficiently uptakes heme iron, a better understanding of placental heme transport during normal and ID pregnancies is warranted.

In addition to increased expression of placental iron transporters, maternal adaptations also occur that facilitate iron availability for the placenta (Cao & Fleming 2016). In rat models of ID, anemic dams exhibit increased intestinal absorption of iron, reduced liver iron content, and decreased maternal hepcidin expression compared to controls (Gambling *et al.* 2004, 2009, Cornock *et al.* 2013). Despite various adaptive changes in maternal metabolism and placental transport to optimize iron transfer to the fetus, babies born from severely anemic women have lower cord Hb levels compared to controls (Kumar *et al.* 2008, El-Farrash *et al.* 2012). Thus, under conditions of moderate iron deficiency, placental iron transport may be accelerated in favor of fetal demands. However, there appears to be a threshold of maternal iron status when a sufficient supply of iron cannot be delivered to the fetus, at which point the placenta establishes a new hierarchy in which iron is prioritized for its own function rather than delivered to the fetus.

Appropriate fetal growth and development also depend on the availability of nutrients, including glucose, amino acids, and fatty acids. Dysfunction in the transplacental transport of these nutrients may contribute to intrauterine growth restriction and other complications observed in maternal ID. In rats, maternal ID causes reduced fetal growth, correlating with decreased fetal plasma levels of triacylglycerol, cholesterol, and several amino acids, including taurine, phenylalanine, and tyrosine. Maternal plasma levels of these nutrients are unaffected, suggesting impaired placental transport (Lewis *et al.* 2001*a*). However, no studies to date have directly assessed placental expression or activity of nutrient transporters in the context of maternal ID.

Table 1 Expression of factors implicated in placental iron trafficking during ID.

Name	Gene	Changes in mRNA and protein expression			
		Human Studies	References	Animal studies	References
Iron influx					
TfR1	*TFRC*	↑ expression in human placentas ↑ expression in BeWo cells and primary trophoblasts exposed to DFO	Kroos *et al.* (1996), Georgieff *et al.* (1999), Gambling *et al.* (2001), Li *et al.* (2008, 2012), Young *et al.* (2010), Garcia-Valdes *et al.* (2015), Best *et al.* (2016), Sangkhae *et al.* (2020*a*)	↑ expression in rat placentas	Gambling *et al.* (2009), Balesaria *et al.* (2012), Cornock *et al.* (2013)
HFE	*HFE*			↔ mRNA expression in rat and mouse placentas	Gambling *et al.* (2009), Sangkhae *et al.* (2020*a*)
BCRP	*BCRP*	↔ protein expression in human placentas	Jaacks *et al.* (2011)		
Intracellular iron trafficking and storage					
DMT1	*SLC11A2*	↑ expression in human placentas ↑ expression in BeWo cells exposed to DFO	Gambling *et al.* (2001), Venkata Surekha *et al.* (2020)	↔ mRNA expression in mouse placentas ↑ mRNA expression in rat placentas	Cornock *et al.* (2013), Sangkhae *et al.* (2020*a*)
Ferritin	*FTH1* (heavy chain), *FTL* (light chain)	↓ mRNA expression in human placentas	Li *et al.* (2008)	↔ in expression in rat placentas	Gambling *et al.* (2009)
Iron efflux					
FPN	*SLC40A1*	↔ or ↑ protein expression in human placentas ↑ expression in BeWo cells exposed to DFO ↓ protein expression in trophoblast cells exposed to DFO	Li *et al.* (2008, 2012), Best *et al.* (2016), Sangkhae *et al.* (2020*a*), Venkata Surekha *et al.* (2020)	↔ in mRNA expression in rat and mouse placentas ↓ protein expression in mouse placentas	Gambling *et al.* (2001), Cornock *et al.* (2013), Sangkhae *et al.* (2020*a*)
Ceruloplasmin	*CP*	↔ in mRNA expression in human placentas ↔ in mRNA expression in BeWo cells exposed to DFO ↑ protein expression in BeWo cells exposed to DFO	Danzeisen *et al.* (2000), Li *et al.* (2008), Best *et al.* (2016)	↔ in mRNA expression in rat placentas	Fleming and Gitlin (1990)
Hephaestin	*HEPH*	↑ expression in BeWo cells exposed to DFO	Li *et al.* (2012)		
Zyklopen	*ZP*	↑ expression in human placentas	Venkata Surekha *et al.* (2020)	↔ in mRNA expression in mouse placentas	Sangkhae *et al.* (2020*a*)
FLVCR1	*FLVCR1*	↓ protein expression in human placentas	Jaacks *et al.* (2011)	↔ in mRNA expression in mouse placentas	Sangkhae *et al.* (2020*a*)

↑ = Increased; ↔ = No change; ↓ = Decreased; BCRP, breast cancer resistance protein; DMT1, divalent metal transporter 1; FLVCR1, feline leukemia virus subgroup C receptor 1; FPN, ferroportin; HFE, human hemochromatosis protein; TfR1, transferrin receptor 1.

Placental hypoxia, oxidative stress, and inflammation

Hypoxia is a condition in which oxygen availability is reduced in cells or tissues. Since ID restricts Hb synthesis and oxygen transport, hypoxia is a potential consequence. Cells adapt to hypoxic conditions in part through stabilization of hypoxia-inducible factor alpha (HIFA) subunits, which interact with HIFA subunits to alter transcription of numerous genes that alter cell growth, metabolism, and survival. Stabilization of HIFA subunits is facilitated by reduced activity of prolyl hydroxylases, which are enzymes that hydroxylate HIFA when oxygen is replete, leading to its ubiquitination and degradation. Expression of HIF1A in the placenta is high in early pregnancy due to the low oxygen environment in which the placenta develops, and in normal circumstances expression declines by the end of the first trimester as oxygen tension increases (Ietta *et al.* 2006). However, in ID pregnancies, term placentae exhibit increased expression of HIF1A (Michalitsi *et al.* 2015). Likewise, in rat models of ID anemia, no change in mRNA expression of *Hif1a* in placenta is evident, but higher HIF1A protein expression is detected (Lewis *et al.* 2001*b*, Toblli *et al.* 2012). Whether the increased HIF1A expression is attributed to hypoxia *per se* is unclear, because iron is a critical cofactor for prolyl hydroxylase activity (Bishop & Ratcliffe 2015), and therefore HIF1A expression may be increased irrespective of oxygen

levels (Woo *et al.* 2006). Indeed, using pimonidazole, which forms stable adducts with thiol groups of proteins in hypoxic cells, Woodman *et al*. found no evidence of placental hypoxia with maternal ID in pregnant rats, despite robust signals in various fetal organs (Woodman *et al.* 2017). Notwithstanding, the increased placental HIF1A may contribute to dysregulated expression of genes, such as those encoding inflammatory cytokines, which affect placental function and contribute to pregnancy complications (Gambling *et al.* 2002).

Oxidative stress is characterized by amplified production of reactive oxygen species beyond the capacity of antioxidant defense mechanisms, and is a normal occurrence throughout pregnancy (Mannaerts *et al.* 2018). As gestation advances, antioxidant defenses are enhanced to prevent oxidative damage to the placenta and fetus (Furukawa *et al.* 2016). In pregnant rat models of ID anemia, increased oxidative stress, lipid peroxidation, mitochondrial damage, and inflammatory cytokine production (e.g. tumor necrosis factor (TNF) and interleukin-6) is evident (Gambling *et al.* 2002, Walter *et al.* 2002, Toblli *et al.* 2012). Like the case with prolyl hydroxylases described above, iron is an essential cofactor for several antioxidant enzymes, including catalase. ID may thus impair the function of antioxidant defenses, exacerbating generation of reactive oxygen species and contributing to placental damage and dysfunction.

Placental structure

Since the placenta forms the interface between mother and fetus, its structure is a key determinant for oxygen and nutrient delivery to the fetus. The efficiency of nutrient and oxygen transfer to the fetus can be affected by changes in the total surface area available for exchange, thickness of the placental exchange surface, proportion of specialized placental regions, and vascularization. Therefore, the impact of maternal ID on placental structure is an important consideration.

When compared to fetal/neonatal weight, placental weight can be used as a proxy for placental efficiency, and an indication of how placental development and function has adapted to sustain fetal requirements (Fowden *et al.* 2009). Studies in both humans (Table 2) and animals (Table 3) show that maternal ID has an effect on placental weight and placental:fetal birth weight ratios. For example, several studies suggest that placental weight and placental:fetal weight ratios increase with maternal anemia (Beischer *et al.* 1970, Godfrey *et al.* 1991, Lao & Wong 1997, Lao & Tam 2000, Huang *et al.* 2001, Baptiste-Roberts *et al.* 2008, Lelic *et al.* 2014, Larsen *et al.* 2016). Among these studies, two were conducted using large cohorts of 8648 (Godfrey *et al.* 1991), and 57,062 (Larsen *et al.* 2016) human pregnancies, in which a negative correlation between placental weight and maternal Hb concentration was reported. It is possible that decreased iron and/or Hb evokes compensatory placental hypertrophy that increases surface area and enhances oxygen and nutrient exchange. Conversely, some studies have found no correlation between low Hb and placental weight (Reshetnikova *et al.* 1995) or decreased placental weights in anemic mothers (Rusia *et al.* 1988, Mongia *et al.* 2011, Kiran *et al.* 2015). Multiple factors may account for these discrepancies, including severity of anemia, timing of anemia onset, number of samples analyzed, ethnic populations, and possibility of maternal malnutrition or other nutritional deficits. In some cases, gestational age was not controlled, which confounds the interpretation of placental size and its relationship to fetal weight.

Rodents fed iron-deficient diets have been used to study the impact of ID anemia on pregnancy outcome. In these models, diets lacking iron are invariably associated with reduced fetal size (Lewis *et al.* 2001*a*, Gambling *et al.* 2002, Toblli *et al.* 2012, Woodman *et al.* 2017, 2018). However, conflicting effects of maternal anemia on placental weight have been reported, with many studies showing an increase in placental weight (Lewis *et al.* 2001*a*,*b*, Gambling *et al.* 2009, Woodman *et al.* 2017, 2018), and others reporting a decrease in placental weight (Toblli *et al.* 2012). As in the clinical studies, the differing effects may relate to the duration and severity of anemia. Low maternal iron and/or Hb may result in a compensatory hypertrophic response by the placenta to offset reduced oxygen or iron supply to the fetus. However, there may be a critical threshold of maternal iron and Hb, below which placental oxidative stress, inflammation, and damage occur, hindering the capacity of the placenta to adapt.

Maternal ID is also associated with altered placental composition and morphology. In placentas exposed to ID, increased placental vascularization, capillary density, and dilation of villous sinusoids is observed (Reshetnikova *et al.* 1995, Burton *et al.* 1996, Kadyrov *et al.* 1998, Mongia *et al.* 2011, Lelic *et al.* 2014, Lelić *et al.* 2014). Such changes are a principal adaptation of the placenta to hypoxia or result from differences in hemodynamic forces during fetal development (Stanek 2013). Indeed, fetal cardiovascular adaptations have been associated with maternal anemia in sheep, including increased heart weight and cardiac output, which may explain altered placental capillary vascularization (Davis & Hohimer 1991). Placentas exposed to maternal ID have smaller placental villous trees and thinner chorionic villous membranes, the latter possibly an adaptation to maintain the diffusion capacity of the exchange surface (Reshetnikova *et al.* 1995). Similar results are observed in rat models of maternal ID, in which the labyrinth zone (the region of rodent placentas where nutrients and gases are exchanged between maternal and fetal blood) is reduced in thickness (Awad *et al.* 2017).

Table 2 Placental and fetal outcomes from maternal ID anemia in human pregnancy.

Placental morphology	Fetal features	Maternal Hb (g/L)	*n*	References
↑ placental weight, placental volume and/or placental/fetal weight ratio				
↑ placental weight ↑ chorionic plate area	Not reported	Control: >100 Anemic: <100	23,420	Baptiste-Roberts *et al.* (2008)
↑ placental weight ↑ placental/fetal weight ratio	Not reported	Control: >100 Anemic: <80	4974	Beischer *et al.* (1970)
↑ placental/fetal weight ratio	↓ fetal weight	Control: >110 Anemic: <110	8648	Godfrey *et al.* (1991)
↑ placental weight ↑ surface area of capillaries in maternal villi	Not reported	122	PS: 1650; PM: 17	Hindmarsh *et al.* (2000)
↑ placental volume ↑ area of intervillous space ↔ membrane thickness ↔ placental Hb or iron	↑ fetal weight	Control: 127 Moderate: 106 Severe: 86	26	Huang *et al.* (2001)
↑ placental weight ↑ chorionic plate area ↑ placental/fetal weight ratio	↔in fetal weight	Control: >100 Anemic: <100	511	Lao and Wong (1997)
↔ placental weight ↑ placental/fetal weight ratio	↓ fetal weight ↓ gestational age	Control: >100 Anemic: <100	437	Lao and Tam (2000)
↑ placental weight and placental/fetal weight ratio with ↓ Hb concentration	↓ fetal weight in low and high Hb concentrations	High: 135 Intermediate: 90–135 Low: <90	57,062	Larsen *et al.* (2016)
↑ placental/fetal weight ratio	↓ fetal weight	Control: >110 Anemic: <110	2507	Williams *et al.* (1997)
↔ or ↓ placental weight, placental volume or placental/fetal weight ratio				
↓ placental weight with increasing severity of ID ↑ placental infarcts ↑ number of syncytial knots	Not reported	Control: >110 Mild: 100–109 Moderate: 70–99 Severe: <70	75	Kiran *et al.* (2015)
↔ placental weight and volume ↑ volume of villous blood vessels	↓ fetal weight ↓ gestational age	Control: >105 Anemic: <105	100	Lelic *et al.* (2014)
↓ placental weight ↑ fibrosis and syncytial knots ↑ cytotrophoblast proliferation ↑ capillaries per villus	↓ fetal weight Premature birth	Control: >110 Anemic: <110	120	Mongia *et al.* (2011)
↔ placental weight ↓ villous surface area ↑ volume of fetal capillaries ↓ placental membrane thickness	↔ in fetal weight	Control: 119 Anemic: 90	20	Reshetnikova *et al.* (1995)
↓ placental weight and volume ↔ placental surface area ↓ number of cotyledons	↓ fetal weight	Control: >110 Mild: 86–109 Moderate: 61–85 Severe: <60	85	Singla *et al.* (1978)

↑, increased; ↔, no change; ↓, decreased; Hb, hemoglobin; PM, placental morphology; PS, placental size.

Table 3 Placental and fetal outcomes in rodent models of maternal ID.

Animals	Placental morphology	Fetal features	ID model diets	References
Studies reporting increased placental weight, placental volume, or placental/fetal weight ratio				
Rowett Hooded Lister Rats	↔placental weight ↓ placental iron ↑ placental/fetal weight	↓ fetal bodyweight Asymmetric growth ↓ fetal Hb	Dams fed diet starting 4w before pregnancy Control: 50 mg iron/kg Mild: 37.5 mg iron/kg Moderate: 12.5 mg iron/kg Severe: 7.5 mg iron/kg	Gambling *et al.* (2002)
Wistar Rats	↑ placental weight ↔labyrinth zone area ↑ placental *Hif1a* mRNA	↓ fetal bodyweight ↓ fetal Hb ↓ hematocrit ↓ red blood cell volume	Control: Dams fed 150 mg iron/kg diet starting 2w before pregnancy Moderate: Dams fed 3 mg iron/kg diet starting 1w before pregnancy Severe: Dams fed 3 mg iron/kg diet starting 2w before pregnancy	Lewis *et al.* (2001*a*)
Mice (C57BL/6J)	↔placental weight ↑ placental/fetal weight ratio ↔ placental heme iron ↓ placental non-heme iron	↓ fetal Hb ↓ hematocrit	Control: Dams fed 185 ppm iron diet starting 2w before pregnancy ID: Mice fed 4 ppm iron diet starting 2w before pregnancy	Sangkhae *et al.* (2020*a*)
Sprague Dawley Rats	↑ placental weight ↑ placental/fetal weight	↓ fetal bodyweight ↓ fetal Hb	Control: Dams fed 37 mg iron/kg diet starting 2w before pregnancy Moderate: Dams fed 10 mg iron/kg starting 2w before pregnancy Severe: Dams fed 3 mg iron/kg starting 2w before pregnancy	Woodman *et al.* (2018)
Sprague Dawley Rats	↑ placental weight ↑ placental/fetal weight ↔ placental hypoxia ↑ umbilical artery resistance ↑ pulsatility index (in severe ID)	↓ fetal bodyweight ↓ fetal Hb	Control: Dams fed 35 mg iron/kg diet throughout pregnancy until GD 21.5 Moderate: 12w-old dams fed 3 mg iron/kg diet starting 2w before pregnancy Severe: 6w-old dams fed 3 mg iron/kg starting 2w before pregnancy	Woodman *et al.* (2017)
Studies reporting decreased placental weight, placental volume, or placental/fetal weight ratio				
Sprague Dawley Rats	↓ placental weight ↓ placental/fetal weight	↓ fetal Hb	Control: Dams fed standard chow starting 3-4w before pregnancy ID: Dams fed <6 ppm iron diet starting 3-4w before pregnancy	Crowe *et al.* (1995)
Sprague Dawley Rats	↓ placental weight ↑ protein expression of HIF1A, TNF and IL6	↓ fetal bodyweight	Control: Rats fed 60 ppm iron diet 8w before mating ID: Rats fed 10-20 ppm iron diet 8w before pregnancy	Toblli *et al.* (2012)

↑, increased; ↔, no change; ↓, decreased; ID, iron deficiency; Hb, hemoglobin; w, weeks.

Another feature of placentas exposed to maternal ID is an increased number of placental infarcts, syncytial knots, and fibrinoid necrosis, which could affect functional villous mass (Begum *et al.* 1970, Huang *et al.* 2001, Mongia *et al.* 2011, Nausheen Rumana 2012, Kiran *et al.* 2015). These features are typically associated with maternal vascular malperfusion resulting from insufficient or pulsatile uteroplacental blood flow and reduced oxygen supply, which may contribute to the increased incidence of adverse pregnancy outcomes in maternal ID. Women with maternal ID also exhibit increased cytotrophoblast proliferation, possibly to facilitate syncytiotrophoblast repair in response to ischemia (Kosanke *et al.* 1998, Biswas *et al.* 2014). Together, these studies demonstrate that maternal ID impacts several aspects of placental structure.

Challenges and opportunities

Although screening for ID is recommended for all pregnant women (Abdulrehman *et al.* 2019), a study commissioned by the United States Preventive Services Task Force reported that the evidence is inconclusive about the efficacy of supplementation for improving maternal and infant health outcomes despite improving maternal hematological indices (Cantor *et al.* 2015, Achebe & Gafter-Gvili 2017). Routine screening for ID and iron supplementation is only recommended in women of reproductive age if anemia is diagnosed. Consequently, many women enter pregnancy with marginal iron stores and do not receive treatment until they exhibit symptoms of severe anemia. Treatment recommendations for maternal ID anemia include increased dietary intake of iron-rich foods and oral iron supplementation, most commonly with ferrous sulfate. Although inexpensive and readily available in many developed countries, oral iron supplementation causes gastrointestinal discomfort in more than 70% of those to whom it is prescribed, including metallic taste, gastric irritation, and worsening constipation, resulting in poor adherence (Tolkien *et al.* 2015). Furthermore, serum hepcidin levels are increased for approximately 48 h after ingestion of iron supplements, restricting iron absorption from the intestine (Moretti *et al.* 2015). Prolonged-release preparations of oral iron supplements may offer solutions to these issues while conferring similar bioavailability (Santiago 2012). Other formulations are available that purport enhanced efficacy and tolerability (iron-polysaccharide complexes, heme iron polypeptide), though these claims have been questioned in light of several clinical trial outcomes (Santiago 2012, Moe *et al.* 2019). Although more expensive, intravenous iron has lower toxicity and is more effective than orally-administered iron (Auerbach *et al.* 2017); although it has not been rigorously tested in pregnant women and it is uncertain how well it is tolerated by the mother, placenta, and fetus.

Given the high incidence of maternal ID and its potential impacts on placental and fetal health, there is urgent need to better understand placental iron metabolism and develop strategies to prevent, detect, and treat maternal ID during pregnancy. Early detection

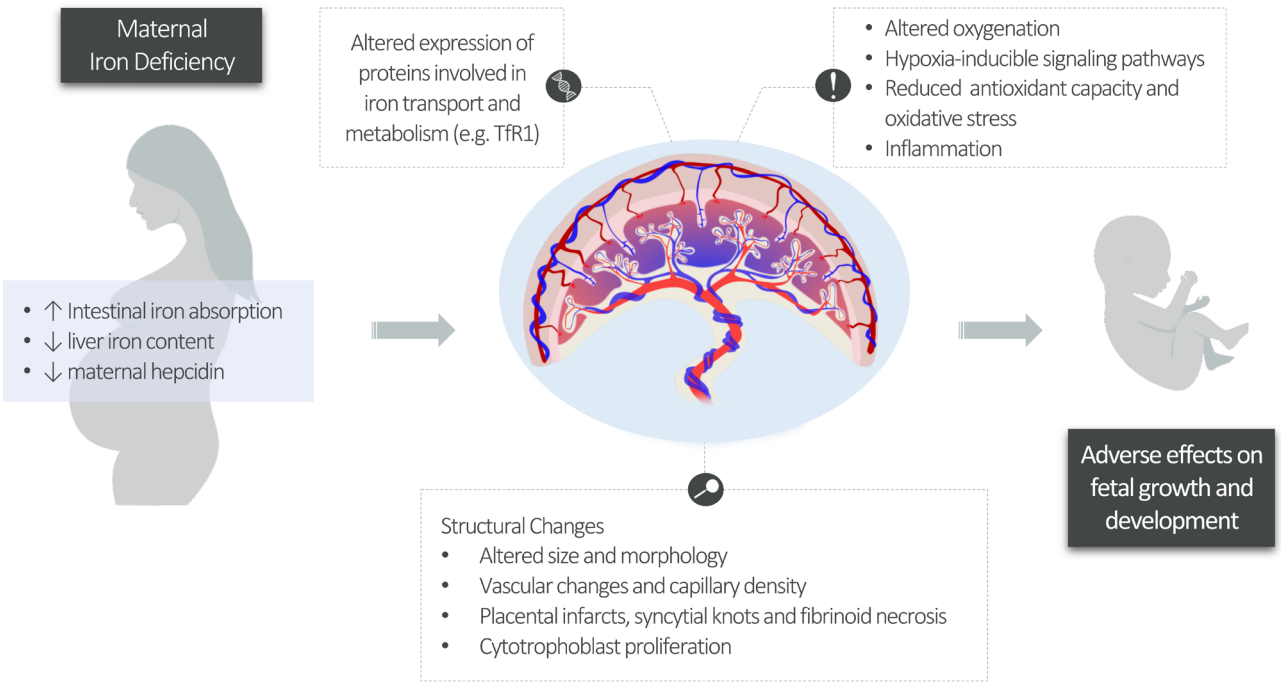

Figure 2 Schematic depicting the potential impact of maternal iron deficiency on the placenta.

of ID can be improved by implementing routine prenatal screening, irrespective of Hb level. Public health strategies to prevent ID include improvements in dietary diversity, food fortification with iron and other micronutrients, and distribution of iron-containing supplements; although indiscriminate iron supplementation may be problematic due to gastric irritation and potential toxicity described previously, and may be deleterious in regions where certain infectious diseases are prevalent. Other public health strategies include better control of infections like malaria that compromise red blood cell integrity, and access to education for reproductive health and family planning. Additionally, a better understanding of how ID affects placental and fetal development may lead to identification of biomarkers that better reflect fetal/placental distress in ID (e.g. ratio of placental FPN/TfR1 (Sangkhae *et al.* 2020*a*)), and provide opportunities to intervene *in utero*, thereby reducing the risk of long-term health complications. Accessible and cost-effective interventions will help to reduce the burden of maternal ID and long-term consequences on offspring health.

Conclusions

Iron is a crucial mineral for many cellular and physiological processes. ID during pregnancy remains a global problem with significant health implications for both mothers and offspring. Depending on the severity and duration, low levels of maternal iron and Hb may result in decreased oxygenation that progress to hypoxic and inflammatory conditions in the placenta. This may stimulate placental hypertrophy, increased vascularization, structural changes at the fetal-maternal exchange surface, and altered expression or activity of nutrient transporters (Fig. 2). Future studies are needed to clarify structural and functional modifications in the placenta during maternal ID, and determine whether these modifications are adaptive responses striving to maintain iron sustenance and support fetal growth and development, or pathological changes contributing to maternal and fetal adversity. Since the placenta forms the interface between mother and baby, understanding how the placenta changes in ID may yield new diagnostic indices of fetal stress in affected pregnancies, thereby leading to earlier interventions and improved fetal outcomes.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

H R and S J R wrote the initial draft of the manuscript. S L B and S J R critically revised the manuscript. All authors read, revised, and approved the final manuscript.

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References

Abdulrehman J, Lausman A, Tang GH, Nisenbaum R, Petrucci J, Pavenski K, Hicks LK & Sholzberg M 2019 Development and implementation of a quality improvement toolkit, iron deficiency in pregnancy with maternal iron optimization (IRON MOM): a before-and-after study. *PLoS Medicine* **16** e1002867. (https://doi.org/10.1371/journal.pmed.1002867)

Achebe MM & Gafter-Gvili A 2017 How I treat anemia in pregnancy: iron, cobalamin, and folate. *Blood* **129** 940–949. (https://doi.org/10.1182/blood-2016-08-672246)

Alwan NA, Cade JE, McArdle HJ, Greenwood DC, Hayes HE & Simpson NAB 2015 Maternal iron status in early pregnancy and birth outcomes: insights from the Baby's vascular health and iron in pregnancy study. *British Journal of Nutrition* **113** 1985–1992. (https://doi.org/10.1017/S0007114515001166)

Auerbach M, James SE, Nicoletti M, Lenowitz S, London N, Bahrain HF, Derman R & Smith S 2017 Results of the first american prospective study of intravenous iron in oral iron-intolerant iron-deficient gravidas. *American Journal of Medicine* **130** 1402–1407. (https://doi.org/10.1016/j.amjmed.2017.06.025)

Awad O, Malek A & Ogeng'o J 2017 Differential effects of chronic iron deficiency anaemia on junctional and labyrinthine zones of placenta in Sprague dawely rat. *Anatomy Journal of Africa* **6** 840–846.

Bah A, Pasricha SR, Jallow MW, Sise EA, Wegmuller R, Armitage AE, Drakesmith H, Moore SE & Prentice AM 2017 Serum hepcidin concentrations decline during pregnancy and may identify iron deficiency: analysis of a longitudinal pregnancy cohort in the Gambia. *Journal of Nutrition* **147** 1131–1137. (https://doi.org/10.3945/jn.116.245373)

Balesaria S, Hanif R, Salama MF, Raja K, Bayele HK, McArdle H & Srai SKS 2012 Fetal iron levels are regulated by maternal and fetal Hfe genotype and dietary iron. *Haematologica* **97** 661–669. (https://doi.org/10.3324/haematol.2011.055046)

Baptiste-Roberts K, Salafia CM, Nicholson WK, Duggan A, Wang NY & Brancati FL 2008 Maternal risk factors for abnormal placental growth: the national collaborative perinatal project. *BMC Pregnancy and Childbirth* **8** 44. (https://doi.org/10.1186/1471-2393-8-44)

Barrett JF, Whittaker PG, Williams JG & Lind T 1994 Absorption of non-haem iron from food during normal pregnancy. *BMJ* **309** 79–82. (https://doi.org/10.1136/bmj.309.6947.79)

Bastin J, Drakesmith H, Rees M, Sargent I & Townsend A 2006 Localisation of proteins of iron metabolism in the human placenta and liver. *British Journal of Haematology* **134** 532–543. (https://doi.org/10.1111/j.1365-2141.2006.06216.x)

Begum M, Ara S, Kishwara S, Nurunnabi ASM & Rayhan KA 1970 Microscopic changes of the placental components in maternal anaemia. *Bangladesh Journal of Anatomy* **8** 59–63. (https://doi.org/10.3329/bja.v8i2.7017)

Beischer NA, Sivasamboo R, Vohra S, Silpisornkosal S & Reid S 1970 Placental hypertrophy in severe pregnancy anaemia. *Journal of Obstetrics and Gynaecology of the British Commonwealth* **77** 398–409. (https://doi.org/10.1111/j.1471-0528.1970.tb03541.x)

Best CM, Pressman EK, Cao C, Cooper E, Guillet R, Yost OL, Galati J, Kent TR & O'Brien KO 2016 Maternal iron status during pregnancy compared with neonatal iron status better predicts placental iron transporter expression in humans. *FASEB Journal* **30** 3541–3550. (https://doi.org/10.1096/fj.201600069R)

Bishop T & Ratcliffe PJ 2015 HIF hydroxylase pathways in cardiovascular physiology and medicine. *Circulation Research* **117** 65–79. (https://doi.org/10.1161/CIRCRESAHA.117.305109)

Biswas S, Meyur R, Adhikari A, Bose K & Kundu P 2014 Placental changes associated with maternal anaemia. *European Journal of Anatomy* **18** 165–169.

Burton GJ, Reshetnikova OS, Milovanov AP & Teleshova OV 1996 Stereological evaluation of vascular adaptations in human placental villi to differing forms of hypoxic stress. *Placenta* **17** 49–55. (https://doi.org/10.1016/s0143-4004(05)80643-5)

Cantor AG, Bougatsos C, Dana T, Blazina I & McDonagh M 2015 Routine iron supplementation and screening for iron deficiency anemia in pregnancy: a systematic review for the U.S. Preventive Services Task Force. *Annals of Internal Medicine* **162** 566–576. (https://doi.org/10.7326/M14-2932)

Cao C & Fleming MD 2016 The placenta: the forgotten essential organ of iron transport. *Nutrition Reviews* **74** 421–431. (https://doi.org/10.1093/nutrit/nuw009)

Chen H, Attieh ZK, Syed BA, Kuo YM, Stevens V, Fuqua BK, Andersen HS, Naylor CE, Evans RW, Gambling L *et al.* 2010 Identification of Zyklopen, a new member of the vertebrate multicopper ferroxidase family, and characterization in rodents and human cells. *Journal of Nutrition* **140** 1728–1735. (https://doi.org/10.3945/jn.109.117531)

Choi JW, Im MW & Pai SH 2000 Serum transferrin receptor concentrations during normal pregnancy. *Clinical Chemistry* **46** 725–727. (https://doi.org/10.1093/clinchem/46.5.725)

Chong WS, Kwan PC, Chan LY, Chiu PY, Cheung TK & Lau TK 2005 Expression of divalent metal transporter 1 (DMT1) isoforms in first trimester human placenta and embryonic tissues. *Human Reproduction* **20** 3532–3538. (https://doi.org/10.1093/humrep/dei246)

Cornock R, Gambling L, Langley-Evans SC, McArdle HJ & McMullen S 2013 The effect of feeding a low iron diet prior to and during gestation on fetal and maternal iron homeostasis in two strains of rat. *Reproductive Biology and Endocrinology* **11** 32. (https://doi.org/10.1186/1477-7827-11-32)

Crowe C, Dandekar P, Fox M, Dhingra K, Bennet L & Hanson MA 1995 The effects of anaemia on heart, placenta and body weight, and blood pressure in fetal and neonatal rats. *Journal of Physiology* **488** 515–519. (https://doi.org/10.1113/jphysiol.1995.sp020986)

Danzeisen R, Ponnambalam S, Lea RG, Page K, Gambling L & McArdle HJ 2000 The effect of ceruloplasmin on iron release from placental (BeWo) cells; evidence for an endogenous Cu oxidase. *Placenta* **21** 805–812. (https://doi.org/10.1053/plac.2000.0582)

Davis LE & Hohimer AR 1991 Hemodynamics and organ blood flow in fetal sheep subjected to chronic anemia. *American Journal of Physiology* **261** R1542–R1548. (https://doi.org/10.1152/ajpregu.1991.261.6.R1542)

Doom JR & Georgieff MK 2014 Striking while the iron is hot: understanding the biological and neurodevelopmental effects of iron deficiency to optimize intervention in early childhood. *Current Pediatrics Reports* **2** 291–298. (https://doi.org/10.1007/s40124-014-0058-4)

Duffy SP, Shing J, Saraon P, Berger LC, Eiden MV, Wilde A & Tailor CS 2010 The Fowler syndrome-associated protein FLVCR2 is an importer of heme. *Molecular and Cellular Biology* **30** 5318–5324. (https://doi.org/10.1128/MCB.00690-10)

El-Farrash RA, Ismail EAR & Nada AS 2012 Cord blood iron profile and breast milk micronutrients in maternal iron deficiency anemia. *Pediatric Blood and Cancer* **58** 233–238. (https://doi.org/10.1002/pbc.23184)

Evans P, Cindrova-Davies T, Muttukrishna S, Burton GJ, Porter J & Jauniaux E 2011 Hepcidin and iron species distribution inside the first-trimester human gestational sac. *Molecular Human Reproduction* **17** 227–232. (https://doi.org/10.1093/molehr/gaq101)

Fisher AL & Nemeth E 2017 Iron homeostasis during pregnancy. *American Journal of Clinical Nutrition* **106** 1567S–1574S. (https://doi.org/10.3945/ajcn.117.155812)

Fisher AL, Sangkhae V, Presicce P, Chougnet CA, Jobe AH, Kallapur SG, Tabbah S, Buhimschi CS, Buhimschi IA, Ganz T *et al.* 2020 Fetal and amniotic fluid iron homeostasis in healthy and complicated murine, macaque, and human pregnancy. *JCI Insight* **5** e135321. (https://doi.org/10.1172/jci.insight.135321)

Fleming RE & Gitlin JD 1990 Primary structure of rat ceruloplasmin and analysis of tissue-specific gene expression during development. *Journal of Biological Chemistry* **265** 7701–7707.

Fowden AL, Sferruzzi-Perri AN, Coan PM, Constancia M & Burton GJ 2009 Placental efficiency and adaptation: endocrine regulation. *Journal of Physiology* **587** 3459–3472. (https://doi.org/10.1113/jphysiol.2009.173013)

Furukawa S, Nakajima A & Sameshima H 2016 The longitudinal change of extracellular antioxidant status during pregnancy using an electron spin resonance method. *Journal of Maternal-Fetal and Neonatal Medicine* **29** 2994–2999. (https://doi.org/10.3109/14767058.2015.1112370)

Gambling L, Danzeisen R, Gair S, Lea RG, Charania Z, Solanky N, Joory KD, Srai SK & McArdle HJ 2001 Effect of iron deficiency on placental transfer of iron and expression of iron transport proteins in vivo and in vitro. *Biochemical Journal* **356** 883–889. (https://doi.org/10.1042/0264-6021:3560883)

Gambling L, Charania Z, Hannah L, Antipatis C, Lea RG & McArdle HJ 2002 Effect of iron deficiency on placental cytokine expression and fetal growth in the pregnant rat. *Biology of Reproduction* **66** 516–523. (https://doi.org/10.1095/biolreprod66.2.516)

Gambling L, Andersen HS, Czopek A, Wojciak R, Krejpcio Z & McArdle HJ 2004 Effect of timing of iron supplementation on maternal and neonatal growth and iron status of iron-deficient pregnant rats. *Journal of Physiology* **561** 195–203. (https://doi.org/10.1113/jphysiol.2004.068825)

Gambling L, Czopek A, Andersen HS, Holtrop G, Srai SKS, Krejpcio Z & McArdle HJ 2009 Fetal iron status regulates maternal iron metabolism during pregnancy in the rat. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology* **296** R1063–R1070. (https://doi.org/10.1152/ajpregu.90793.2008)

Gao G, Liu SY, Wang HJ, Zhang TW, Yu P, Duan XL, Zhao SE & Chang YZ 2015 Effects of pregnancy and lactation on iron metabolism in rats. *BioMed Research International* **2015** 105325. (https://doi.org/10.1155/2015/105325)

Garcia-Valdes L, Campoy C, Hayes H, Florido J, Rusanova I, Miranda MT & McArdle HJ 2015 The impact of maternal obesity on iron status, placental transferrin receptor expression and hepcidin expression in human pregnancy. *International Journal of Obesity* **39** 571–578. (https://doi.org/10.1038/ijo.2015.3)

Georgieff MK, Berry SA, Wobken JD & Leibold EA 1999 Increased placental iron regulatory protein-1 expression in diabetic pregnancies complicated by fetal iron deficiency. *Placenta* **20** 87–93. (https://doi.org/10.1053/plac.1998.0339)

Godfrey KM, Redman CW, Barker DJ & Osmond C 1991 The effect of maternal anaemia and iron deficiency on the ratio of fetal weight to placental weight. *British Journal of Obstetrics and Gynaecology* **98** 886–891. (https://doi.org/10.1111/j.1471-0528.1991.tb13510.x)

Grandone A, Marzuillo P, Perrone L & Del Giudice EM 2015 Iron metabolism dysregulation and cognitive dysfunction in pediatric obesity: is there a connection? *Nutrients* **7** 9163–9170. (https://doi.org/10.3390/nu7115458)

Gruper Y, Bar J, Bacharach E & Ehrlich R 2005 Transferrin receptor co-localizes and interacts with the hemochromatosis factor (HFE) and the divalent metal transporter-1 (DMT1) in trophoblast cells. *Journal of Cellular Physiology* **204** 901–912. (https://doi.org/10.1002/jcp.20349)

Gunshin H, Fujiwara Y, Custodio AO, Direnzo C, Robine S & Andrews NC 2005 Slc11a2 is required for intestinal iron absorption and erythropoiesis but dispensable in placenta and liver. *Journal of Clinical Investigation* **115** 1258–1266. (https://doi.org/10.1172/JCI24356)

Hindmarsh PC, Geary MP, Rodeck CH, Jackson MR & Kingdom JC 2000 Effect of early maternal iron stores on placental weight and structure. *Lancet* **356** 719–723. (https://doi.org/10.1016/s0140-6736(00)02630-1)

Huang A, Zhang R & Yang Z 2001 Quantitative (stereological) study of placental structures in women with pregnancy iron-deficiency anemia. *European Journal of Obstetrics, Gynecology, and Reproductive Biology* **97** 59–64. (https://doi.org/10.1016/s0301-2115(00)00480-2)

Hubbard AC, Bandyopadhyay S, Wojczyk BS, Spitalnik SL, Hod EA & Prestia KA 2013 Effect of dietary iron on fetal growth in pregnant mice. *Comparative Medicine* **63** 127–135.

Hvidberg V, Maniecki MB, Jacobsen C, Højrup P, Møller HJ & Moestrup SK 2005 Identification of the receptor scavenging hemopexin-heme complexes. *Blood* **106** 2572–2579. (https://doi.org/10.1182/blood-2005-03-1185)

Ietta F, Wu Y, Winter J, Xu J, Wang J, Post M & Caniggia I 2006 Dynamic HIF1A regulation during human placental development. *Biology of Reproduction* **75** 112–121. (https://doi.org/10.1095/biolreprod.106.051557)

Jaacks LM, Young MF, Essley BV, McNanley TJ, Cooper EM, Pressman EK, McIntyre AW, Orlando MS, Abkowitz JL, Guillet R *et al.* 2011 Placental expression of the heme transporter, feline leukemia virus subgroup C receptor, is related to maternal iron status in pregnant adolescents. *Journal of Nutrition* **141** 1267–1272. (https://doi.org/10.3945/jn.110.135798)

Kadyrov M, Kosanke G, Kingdom J & Kaufmann P 1998 Increased fetoplacental angiogenesis during first trimester in anaemic women. *Lancet* **352** 1747–1749. (https://doi.org/10.1016/s0140-6736(98)02069-8)

Kiran N, Zubair A, Malik TM, Ayyub M & Khan IM 2015 Placental morphology at different maternal hemoglobin levels: a histopathological study. *Pakistan Armed Forces Medical Journal* **65** 189–193.

Kosanke G, Kadyrov M, Korr H & Kaufmann P 1998 Maternal anemia results in increased proliferation in human placental villi. *Placenta* **19** 339–357. (https://doi.org/10.1016/S0143-4004(98)80024-6)

Krantman HJ, Young SR, Ank BJ, O'Donnell CM, Rachelesfsky GS & Stiehm ER 1982 Immune function in pure iron deficiency. *American Journal of Diseases of Children* **136** 840–844. (https://doi.org/10.1001/archpedi.1982.03970450082020)

Kroos MJ, Starreveld JS, Verrijt CE, van Eijk HG & van Dijk JP 1996 Regulation of transferrin receptor synthesis by human cytotrophoblast cells in culture. *European Journal of Obstetrics, Gynecology, and Reproductive Biology* **65** 231–234. (https://doi.org/10.1016/0301-2115(95)02368-2)

Kumar A, Rai AK, Basu S, Dash D & Singh JS 2008 Cord blood and breast milk iron status in maternal anemia. *Pediatrics* **121** e673–e677. (https://doi.org/10.1542/peds.2007-1986)

Lao TT & Tam KF 2000 Placental ratio and anemia in third-trimester pregnancy. *Journal of Reproductive Medicine* **45** 923–928.

Lao TT & Wong WM 1997 Placental ratio – its relationship with mild maternal anaemia. *Placenta* **18** 593–596. (https://doi.org/10.1016/0143-4004(77)90015-7)

Larsen S, Bjelland EK, Haavaldsen C & Eskild A 2016 Placental weight in pregnancies with high or low hemoglobin concentrations. *European Journal of Obstetrics, Gynecology, and Reproductive Biology* **206** 48–52. (https://doi.org/10.1016/j.ejogrb.2016.08.039)

Lelic M, Bogdanovic G, Ramic S & Brkicevic E 2014 Influence of maternal anemia during pregnancy on placenta and newborns. *Medical Archives* **68** 184–187. (https://doi.org/10.5455/medarh.2014.68.184-187)

Lelić M, Ramić S, Žigić Z, Bogdanović G & Marković S 2014 Stereological analysis of terminal villi of the placentas of pregnant woman with sideropenic anemia. *Bosnian Journal of Basic Medical Sciences* **14** 139–143. (https://doi.org/10.17305/bjbms.2014.3.44)

Levytska K, Kingdom J, Baczyk D & Drewlo S 2013 Heme oxygenase-1 in placental development and pathology. *Placenta* **34** 291–298. (https://doi.org/10.1016/j.placenta.2013.01.004)

Lewis RM, James LA, Zhang J, Byrne CD & Hales CN 2001*a* Effects of maternal iron restriction in the rat on hypoxia-induced gene expression and fetal metabolite levels. *British Journal of Nutrition* **85** 193–201. (https://doi.org/10.1079/bjn2000247)

Lewis RM, Doherty CB, James LA, Burton GJ & Hales CN 2001*b* Effects of maternal iron restriction on placental vascularization in the rat. *Placenta* **22** 534–539. (https://doi.org/10.1053/plac.2001.0679)

Li YQ, Yan H & Bai B 2008 Change in iron transporter expression in human term placenta with different maternal iron status. *European Journal of Obstetrics, Gynecology, and Reproductive Biology* **140** 48–54. (https://doi.org/10.1016/j.ejogrb.2008.02.012)

Li YQ, Bai B, Cao XX, Yan H & Zhuang GH 2012 Ferroportin 1 and hephaestin expression in BeWo cell line with different iron treatment. *Cell Biochemistry and Function* **30** 249–255. (https://doi.org/10.1002/cbf.1843)

Lipiński P, Styś A & Starzyński RR 2013 Molecular insights into the regulation of iron metabolism during the prenatal and early postnatal periods. *Cellular and Molecular Life Sciences* **70** 23–38. (https://doi.org/10.1007/s00018-012-1018-1)

Lopez A, Cacoub P, Macdougall IC & Peyrin-Biroulet L 2016 Iron deficiency anaemia. *Lancet* **387** 907–916. (https://doi.org/10.1016/S0140-6736(15)60865-0)

Malassiné A, Frendo JL & Evain-Brion D 2003 A comparison of placental development and endocrine functions between the human and mouse model. *Human Reproduction Update* **9** 531–539. (https://doi.org/10.1093/humupd/dmg043)

Maliepaard M, Scheffer GL, Faneyte IF, van Gastelen MA, Pijnenborg AC, Schinkel AH, van De Vijver MJ, Scheper RJ & Schellens JH 2001 Subcellular localization and distribution of the breast cancer resistance protein transporter in normal human tissues. *Cancer Research* **61** 3458–3464.

Mannaerts D, Faes E, Cos P, Briedé JJ, Gyselaers W, Cornette J, Gorbanev Y, Bogaerts A, Spaanderman M, Van Craenenbroeck E *et al.* 2018 Oxidative stress in healthy pregnancy and preeclampsia is linked to chronic inflammation, iron status and vascular function. *PLoS ONE* **13** e0202919. (https://doi.org/10.1371/journal.pone.0202919)

Maria de Regil L 2010 *Haemoglobin Concentrations for the Diagnosis of Anaemia and Assessment of Severity*. Geneva: World Health Organisation.

Martin ME, Nicolas G, Hetet G, Vaulont S, Grandchamp B & Beaumont C 2004 Transferrin receptor 1 mRNA is downregulated in placenta of hepcidin transgenic embryos. *FEBS Letters* **574** 187–191. (https://doi.org/10.1016/j.febslet.2004.08.010)

McArdle HJ, Lang C, Hayes H & Gambling L 2011 Role of the placenta in regulation of fetal iron status. *Nutrition Reviews* **69** (Supplement 1) S17–S22. (https://doi.org/10.1111/j.1753-4887.2011.00428.x)

McLean E, Cogswell M, Egli I, Wojdyla D & de Benoist B 2009 Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993–2005. *Public Health Nutrition* **12** 444–454.

Means RT 2020 Iron deficiency and iron deficiency anemia: implications and impact in pregnancy, fetal development, and early childhood parameters. *Nutrients* **12** 447. (https://doi.org/10.3390/nu12020447)

Michalitsi V, Dafopoulos K, Gourounti K, Messini C, Ioannou M, Christodoulaki C, Panagopoulos P & Messinis I 2015 Hypoxia-inducible factor-1α (HIF-1α) expression in placentae of women with iron deficiency anemia and β-thalassemia trait. *Journal of Maternal-Fetal and Neonatal Medicine* **28** 470–474. (https://doi.org/10.3109/14767058.2014.921672)

Milman N 2006*a* Iron and pregnancy – a delicate balance. *Annals of Hematology* **85** 559–565. (https://doi.org/10.1007/s00277-006-0108-2)

Milman N 2006*b* Iron prophylaxis in pregnancy – general or individual and in which dose? *Annals of Hematology* **85** 821–828. (https://doi.org/10.1007/s00277-006-0145-x)

Milman N, Bergholt T, Byg KE, Eriksen L & Graudal N 1999 Iron status and iron balance during pregnancy. A critical reappraisal of iron supplementation. *Acta Obstetricia et Gynecologica Scandinavica* **78** 749–757.

Moe S, Grill AK & Allan GM 2019 Newer iron supplements for anemia. *Canadian Family Physician Medecin de Famille Canadien* **65** 556.

Mongia SM, Jain SK & Yadav M 2011 Placenta: the wonder organ. *Journal of the Indian Academy of Forensic Sciences* **33** 140–143.

Moretti D, Goede JS, Zeder C, Jiskra M, Chatzinakou V, Tjalsma H, Melse-Boonstra A, Brittenham G, Swinkels DW & Zimmermann MB 2015 Oral iron supplements increase hepcidin and decrease iron absorption from daily or twice-daily doses in iron-depleted young women. *Blood* **126** 1981–1989. (https://doi.org/10.1182/blood-2015-05-642223)

Nausheen Rumana ASAK 2012 A study of histo pathological changes of placenta in severe anaemia. *Journal of Evolution of Medical and Dental Sciences* **1** 616–623. (https://doi.org/10.14260/jemds/97)

Nemeth E & Ganz T 2006 Regulation of iron metabolism by hepcidin. *Annual Review of Nutrition* **26** 323–342. (https://doi.org/10.1146/annurev.nutr.26.061505.111303)

Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM, Ganz T & Kaplan J 2004 Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science* **306** 2090–2093. (https://doi.org/10.1126/science.1104742)

Nicolas G, Bennoun M, Porteu A, Mativet S, Beaumont C, Grandchamp B, Sirito M, Sawadogo M, Kahn A & Vaulont S 2002 Severe iron deficiency anemia in transgenic mice expressing liver hepcidin. *PNAS* **99** 4596–4601. (https://doi.org/10.1073/pnas.072632499)

Ohgami RS, Campagna DR, McDonald A & Fleming MD 2006 The steap proteins are metalloreductases. *Blood* **108** 1388–1394. (https://doi.org/10.1182/blood-2006-02-003681)

Parkkila S, Waheed A, Britton RS, Bacon BR, Zhou XY, Tomatsu S, Fleming RE & Sly WS 1997 Association of the transferrin receptor in human placenta with HFE, the protein defective in hereditary

hemochromatosis. *PNAS* **94** 13198–13202. (https://doi.org/10.1073/pnas.94.24.13198)

Pasricha SR, Hayes E, Kalumba K & Biggs BA 2013 Effect of daily iron supplementation on health in children aged 4–23 months: a systematic review and meta-analysis of randomised controlled trials. *Lancet: Global Health* **1** e77–e86. (https://doi.org/10.1016/S2214-109X(13)70046-9)

Qiu A, Jansen M, Sakaris A, Min SH, Chattopadhyay S, Tsai E, Sandoval C, Zhao R, Akabas MH & Goldman ID 2006 Identification of an intestinal folate transporter and the molecular basis for hereditary folate malabsorption. *Cell* **127** 917–928. (https://doi.org/10.1016/j.cell.2006.09.041)

Rehu M, Punnonen K, Ostland V, Heinonen S, Westerman M, Pulkki K & Sankilampi U 2010 Maternal serum hepcidin is low at term and independent of cord blood iron status. *European Journal of Haematology* **85** 345–352. (https://doi.org/10.1111/j.1600-0609.2010.01479.x)

Renaud SJ, Karim Rumi MA & Soares MJ 2011 Review: genetic manipulation of the rodent placenta. *Placenta* **32** (Supplement 2) S130–S135. (https://doi.org/10.1016/j.placenta.2010.12.017)

Reshetnikova OS, Burton GJ & Teleshova OV 1995 Placental histomorphometry and morphometric diffusing capacity of the villous membrane in pregnancies complicated by maternal iron-deficiency anemia. *American Journal of Obstetrics and Gynecology* **173** 724–727. (https://doi.org/10.1016/0002-9378(95)90330-5)

Rusia U, Bhatia A, Kapoor S, Madan N, Nair V & Sood SK 1988 Placental morphology and histochemistry in iron deficiency anemia. *Indian Journal of Medical Research* **87** 468–474.

Sangkhae V & Nemeth E 2017 Regulation of the iron homeostatic hormone hepcidin. *Advances in Nutrition* **8** 126–136. (https://doi.org/10.3945/an.116.013961)

Sangkhae V & Nemeth E 2019 Placental iron transport: the mechanism and regulatory circuits. *Free Radical Biology and Medicine* **133** 254–261. (https://doi.org/10.1016/j.freeradbiomed.2018.07.001)

Sangkhae V, Fisher AL, Wong S, Koenig MD, Tussing-Humphreys L, Chu A, Lelić M, Ganz T & Nemeth E 2020*a* Effects of maternal iron status on placental and fetal iron homeostasis. *Journal of Clinical Investigation* **130** 625–640. (https://doi.org/10.1172/JCI127341)

Sangkhae V, Fisher AL, Chua KJ, Ruchala P, Ganz T & Nemeth E 2020*b* Maternal hepcidin determines embryo iron homeostasis. *Blood In press*. (https://doi.org/10.1182/blood.2020005745)

Santiago P 2012 Ferrous versus ferric oral iron formulations for the treatment of iron deficiency: a clinical overview. *ScientificWorldJournal* **2012** 846824. (https://doi.org/10.1100/2012/846824)

Singla PN, Chand S, Khanna S & Agarwal KN 1978 Effect of maternal anaemia on the placenta and the newborn infant. *Acta Paediatrica Scandinavica* **67** 645–648. (https://doi.org/10.1111/j.1651-2227.1978.tb17816.x)

Soares MJ, Chakraborty D, Karim Rumi MA, Konno T & Renaud SJ 2012 Rat placentation: an experimental model for investigating the hemochorial maternal-fetal interface. *Placenta* **33** 233–243. (https://doi.org/10.1016/j.placenta.2011.11.026)

Soncin F, Khater M, To C, Pizzo D, Farah O, Wakeland A, Arul Nambi Rajan K, Nelson KK, Chang CW, Moretto-Zita M *et al.* 2018 Comparative analysis of mouse and human placentae across gestation reveals species-specific regulators of placental development. *Development* **145** dev156273. (https://doi.org/10.1242/dev.156273)

Stanek J 2013 Hypoxic patterns of placental injury: a review. *Archives of Pathology and Laboratory Medicine* **137** 706–720. (https://doi.org/10.5858/arpa.2011-0645-RA)

Stevens GA, Finucane MM, De-Regil LM, Paciorek CJ, Flaxman SR, Branca F, Peña-Rosas JP, Bhutta ZA, Ezzati M & **Nutrition Impact Model Study Group (Anaemia)** 2013 Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for 1995–2011: a systematic analysis of population-representative data. *Lancet: Global Health* **1** e16–e25. (https://doi.org/10.1016/S2214-109X(13)70001-9)

Tarrade A, Panchenko P, Junien C & Gabory A 2015 Placental contribution to nutritional programming of health and diseases: epigenetics and sexual dimorphism. *Journal of Experimental Biology* **218** 50–58. (https://doi.org/10.1242/jeb.110320)

Toblli JE, Cao G, Oliveri L & Angerosa M 2012 Effects of iron deficiency anemia and its treatment with iron polymaltose complex in pregnant rats, their fetuses and placentas: oxidative stress markers and pregnancy outcome. *Placenta* **33** 81–87. (https://doi.org/10.1016/j.placenta.2011.11.017)

Tolkien Z, Stecher L, Mander AP, Pereira DIA & Powell JJ 2015 Ferrous Sulfate supplementation causes significant gastrointestinal side-effects in adults: a systematic review and meta-analysis. *PLoS ONE* **10** e0117383. (https://doi.org/10.1371/journal.pone.0117383)

van Santen S, Kroot JJC, Zijderveld G, Wiegerinck ET, Spaanderman MEA & Swinkels DW 2013 The iron regulatory hormone hepcidin is decreased in pregnancy: a prospective longitudinal study. *Clinical Chemistry and Laboratory Medicine* **51** 1395–1401. (https://doi.org/10.1515/cclm-2012-0576)

Venkata Surekha M, Sujatha T, Gadhiraju S, Kotturu SK, Siva Prasad M, Sarada K, Bhaskar V & Uday Kumar P 2020 Effect of maternal iron deficiency anaemia on the expression of iron transport proteins in the third trimester placenta. *Fetal and Pediatric Pathology* 1–16. (https://doi.org/10.1080/15513815.2020.1725942)

Walter PB, Knutson MD, Paler-Martinez A, Lee S, Xu Y, Viteri FE & Ames BN 2002 Iron deficiency and iron excess damage mitochondria and mitochondrial DNA in rats. *PNAS* **99** 2264–2269. (https://doi.org/10.1073/pnas.261708798)

Willemetz A, Lenoir A, Deschemin JC, Lopez-Otin C, Ramsay AJ, Vaulont S & Nicolas G 2014 Matriptase-2 is essential for hepcidin repression during fetal life and postnatal development in mice to maintain iron homeostasis. *Blood* **124** 441–444. (https://doi.org/10.1182/blood-2014-01-551150)

Williams LA, Evans SF & Newnham JP 1997 Prospective cohort study of factors influencing the relative weights of the placenta and the newborn infant. *BMJ* **314** 1864–1868. (https://doi.org/10.1136/bmj.314.7098.1864)

Woo KJ, Lee TJ, Park JW & Kwon TK 2006 Desferrioxamine, an iron chelator, enhances HIF-1alpha accumulation via cyclooxygenase-2 signaling pathway. *Biochemical and Biophysical Research Communications* **343** 8–14. (https://doi.org/10.1016/j.bbrc.2006.02.116)

Woodman AG, Care AS, Mansour Y, Cherak SJ, Panahi S, Gragasin FS & Bourque SL 2017 Modest and severe maternal iron deficiency in pregnancy are associated with fetal anaemia and organ-specific hypoxia in rats. *Scientific Reports* **7** 46573. (https://doi.org/10.1038/srep46573)

Woodman AG, Mah R, Keddie D, Noble RMN, Panahi S, Gragasin FS, Lemieux H & Bourque SL 2018 Prenatal iron deficiency causes sex-dependent mitochondrial dysfunction and oxidative stress in fetal rat kidneys and liver. *FASEB Journal* **32** 3254–3263. (https://doi.org/10.1096/fj.201701080R)

Young MF, Pressman E, Foehr ML, McNanley T, Cooper E, Guillet R, Orlando M, McIntyre AW, Lafond J & O'Brien KO 2010 Impact of maternal and neonatal iron status on placental transferrin receptor expression in pregnant adolescents. *Placenta* **31** 1010–1014. (https://doi.org/10.1016/j.placenta.2010.08.009)

Young MF, Griffin I, Pressman E, McIntyre AW, Cooper E, McNanley T, Harris ZL, Westerman M & O'Brien KO 2012 Maternal hepcidin is associated with placental transfer of iron derived from dietary heme and nonheme sources. *Journal of Nutrition* **142** 33–39. (https://doi.org/10.3945/jn.111.145961)

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