Placental adaptation to maternal malnutrition

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Abstract

Maternal malnutrition gives rise to both short- and long-term consequences for the survival and health of the offspring. As the intermediary between mother and fetus, the placenta has the potential to interpret environmental signals, such as nutrient availability, and adapt to support fetal growth and development. While this potential is present, it is clear that at times placental adaptation fails to occur resulting in poor pregnancy outcomes. This review will focus on placental responses to maternal undernutrition related to changes in placental vascularization and hemodynamics and placental nutrient transport systems across species. While much of the available literature describes placental responses that result in poor fetal outcomes, novel models have been developed to utilize the inherent variation in fetal weight when dams are nutrient restricted to identify placental adaptations that result in normal-weight offspring. Detailed analyses of the spectrum of placental responses to maternal malnutrition point to alternations in placental histoarchitectural and vascular development, amino acid and lipid transport mechanisms, and modulation of immune-related factors. Dietary supplementation with selected nutrients, such as arginine, has the potential to improve placental growth and function through a variety of mechanisms including stimulating cell proliferation, protein synthesis, angiogenesis, vasodilation, and gene regulation. Improved understanding of placental responses to environmental cues is necessary to develop diagnostic and intervention strategies to improve pregnancy outcomes.

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Introduction

A tremendous body of literature now exists to describe mechanisms whereby the fetus adapts to the intrauterine environment to alter critical physiological set points in preparation for life in the extra-uterine environment (El Hajj *et al.* 2014). Despite these recent advances, our understanding of the mechanisms employed by the placenta to adapt to maternal and/or fetal cues to optimize its function for the overall well-being of mother and fetus is limited. Despite a biologically diverse array of placental types and shapes, the role of the placenta among eutherian mammals is similar. The placenta mediates the exchange of gases, hormones, nutrients, and waste products between the maternal and fetal circulations (Dunlap *et al.* 2015). Importantly, placental growth precedes fetal growth, and due to its intermediary role, growth of the placenta is highly correlated with the eventual growth of the fetus (Burton & Jauniaux 2018). While gross measures of placental growth, such as weight, are predictive of fetal growth, placental transport capacity is a complex biological process. Uteroplacental blood flow, surface area for exchange, transporter availability and activity, and substrate availability are all factors in determining placental transport capacity (Reynolds *et al.* 2005b,

Burton & Jauniaux 2018). The consequences of placental impairment or insufficiency are significant. Suboptimal placental function is associated with low birth weight, perinatal morbidity and mortality, and a lifelong predisposition to the development of a myriad of metabolic, cardiovascular, and other disorders (El Hajj *et al.* 2014, Cirulli *et al.* 2020). The purpose of this review is to summarize what is currently known about placental responses to maternal malnutrition, with a particular emphasis on the identification of potentially novel targets or strategies to promote placental growth and function essential for normal development of the fetus and well-being of the offspring.

Malnutrition: a global challenge faced by humans and livestock species alike

The World Health Organization's definition of malnutrition includes undernutrition (e.g. inadequate intake of amino acids or protein), insufficient vitamin or mineral intake, overweight, and obesity (WHO 2018). According to the 2018 Global Nutrition Report, there are currently 462 million underweight adults, while 1.9 billion adults are either overweight or obese. These nutritional imbalances contribute to the incidence of

low birth weight, which encompasses roughly one in every ten newborns. The incidence of malnutrition in humans, particularly undernutrition, is a common problem shared among agricultural production systems of mammalian livestock species, such as cattle, swine, sheep, and goats. Importantly, increased losses associated with undernutrition in livestock production systems also contribute to food insecurity for humans in many parts of the world. While overnutrition is a prominent component of the nutritional spectrum, to provide sufficient depth, this review will focus on placental responses to maternal undernutrition with the belief that described studies and approaches likely have relevance across the entirety of the nutritional spectrum.

Nutritional influences on placental vascularization and hemodynamics

Placentae can be classified both by shape and the number of layers separating the maternal and fetal circulations (Table 1). These structural differences play a key role in the efficiency of nutrient transport and the requisite maternal cardiac output necessary to facilitate adequate exchange between mother and fetus.

Blood flow through the uterus and placenta facilitates the crucial transfer of nutrients, wastes, and gases between the maternal and fetal compartments. Uteroplacental blood flow exponentially increases as pregnancy progresses in humans and livestock, thus correlating with placental function and fetal growth (Reynolds et al. 2006). The absolute rate at which uterine blood flow increases during the second half of pregnancy is different between species, at least 4.5-fold in cattle, 3-fold in sheep, and 2.5-fold in humans and is associated with increases in umbilical flow as well as decreases in umbilical resistance (Reynolds et al. 2006, Dunlap et al. 2015).

The effect of maternal undernutrition on uteroplacental blood flows is surprisingly modest and varies widely among studies, likely due to differences in severity and duration of restriction (Reynolds et al. 2005a). Uterine blood flow was reduced by 17% when adult sheep

were severely undernourished (60–70% reduction in the diet compared to controls) during mid-gestation, while there was a 20–33% reduction in uterine blood flow if the nutritional restriction was applied during late gestation (Chandler et al. 1985, Newnham et al. 1991, Reynolds et al. 2006). Similarly, a 40% reduction in dietary intake beginning in early gestation results in a reduction in umbilical blood flow and increased arterial indices of resistance (Lemley et al. 2012, Lekatz et al. 2015). Investigators, however, have not observed differences in placental capillary densities in response to the maternal nutrient restriction (Lekatz et al. 2010, Eifert et al. 2015). Consistent with previously described differences in vascular changes between sheep and cattle, a similar 40% reduction in maternal intake in beef cattle did not affect uterine blood flows even after 110 days of nutrient restriction (Camacho et al. 2014). However, when nutritionally restricted cows were realimented to the control diet, uterine blood flow to the gravid horn increased. A subsequent study found that an earlier realimentation to a control diet following early gestational nutrient restriction increased uterine blood flow above that of both control-fed cows as well as cows experiencing an extended period of nutrient restriction prior to late gestation realimentation (Camacho et al. 2018).

Much of the previously described results were based on Doppler ultrasound, and while this technology has vastly improved in recent decades, its utilization is only suitable for the determination of blood flow measurements in major vessels (Roberts et al. 2018). Indeed, the umbilical artery pulsatility index (PI) is frequently used as an indicator of the hemodynamic properties of the placental villi, such that an increase in PI reflects an increase in microvascular resistance (Acharya et al. 2004). In practice, however, an increase in PI only becomes detectable when ~60% of terminal vascular branches are disrupted (Thompson and Trudinger 1990), leading to significant concerns regarding the sensitivity of available technologies to detect less severe incidences of placental dysfunction. Roberts et al. (2016) recently established methodologies to utilize contrast-enhanced ultrasound to assess microvascular perfusion in the intervillous space

Table 1 Characteristics of the placenta across species.

Species	Placental type	Placental shape	Placental layers	Length of gestation (days)	Timing of peak placental mass (proportion of pregnancy)
Pig	Epitheliochorial	Diffuse	6	114	Day 100 (0.87) ¹
Sheep	Synepitheliochorial	Cotyledonary	5–6	147	Day 90 (0.61) ²
Cow	Synepitheliochorial	Cotyledonary	5–6	283	Day 283 (1.00) ³
Human	Hemochorial	Mono-discoid	3	268	Day 268 (1.00) ⁴
Baboon	Hemochorial	Mono-discoid	3	184	NA
Rhesus monkey	Hemochorial	Bi-discoid	3	164	Day 164 (1.00) ⁵
Mouse	Hemochorial	Mono-discoid	3	20	Day 20 (1.00) ⁶
Rat	Hemochorial	Mono-discoid	3	22	Day 19 (0.86) ⁷

¹Michael et al. (1983); ²Alexander (1964); ³Laven & Peters (2001); ⁴Molteni et al (1978); ⁵van Wageningen & Catchpole (1965); ⁶Kulandavelu et al. (2006); ⁷Furukawa et al. (2011).

of human and non-human primate placentae. These methods were then employed to assess the effect of a 50% protein restriction, for the entirety of pregnancy, in the rhesus macaque. Ultrasound assessment of placental hemodynamics found that umbilical artery, but not uterine artery PI, was elevated in protein-restricted primates in late gestation. The increase in umbilical artery PI was associated with a decrease in placental perfusion (Roberts *et al.* 2018). It should be noted that the 50% protein restriction for the entirety of gestation is a substantial nutritional insult, as evidenced by a 50% rate of pregnancy loss in restricted monkeys (Roberts *et al.* 2018). Additional studies are needed to ascertain the sensitivity of the contrast-enhanced ultrasonography in less severe cases of maternal malnutrition that may lead to poor pregnancy outcomes.

Influence of maternal nutrition on nutrient transporter expression and activity

While uteroplacental blood flows are critical for the movement of factors involved in fetal growth, placental transport capacity for many substances is mediated at the cellular level via complex transporter gene families known as the solute carrier (SLC) family of molecules (Broer 2008, Satterfield *et al.* 2010b). Indeed, the presence and activity of specific transporters is likely the rate-limiting factor in the transit of glucose and amino acids in uteroplacental cells. In mice, a modest 20% reduction in the diet compared to controls, beginning at day 3 of gestation, resulted in decreased placental, but not fetal weight at day 16, although there was a decrease in both fetal and placental weights at day 19 (Coan *et al.* 2010). This corresponded to reduced placental mRNA expression of glucose transporter *SLC2A1* at day 16, but at day 19 of gestation, both *SLC2A1* and sodium-dependent amino acid transporter *SLC38A2* had increased gene expression (Coan *et al.* 2010). Amino acid transport systems are down-regulated in the placenta in early gestation, prior to the start of fetal growth restriction, in rats exposed to a maternal dietary protein restriction for the duration of pregnancy (Malandro *et al.* 1996, Jansson *et al.* 2006). The reduced dietary protein intake was associated with lower fetal and maternal serum concentrations of amino acids as well as reduced fetal and placental weights at day 20 (Malandro *et al.* 1996). The activity of cationic amino acid transporters was reduced, as was the activity of anionic sodium-dependent transporters in placentae of rats on a low protein diet, but the activity of anionic sodium-independent transporters was unaffected (Malandro *et al.* 1996).

In a second study, rats on a low protein diet, at day 21 of pregnancy had down-regulated placental protein and mRNA levels of transporters in the sodium-dependent

system A (*SLC38A2* and *SLC38A4*), which have an affinity for neutral amino acids, but neutral amino acid transporters in the sodium-dependent system ASC were not affected (Jansson *et al.* 2006). The system A transporter family has been hypothesized to be essential for fetal growth in rats (Cramer *et al.* 2002). Indeed, administration of 2-(methylamino)isobutyric acid between days 7 and 20 of gestation, to inhibit the system A transporter family, resulted in decreased fetal weight via a mechanism that has not been fully elucidated (Cramer *et al.* 2002).

Additional evidence regarding the influence of dietary intake on the expression of placental amino acid transporters has been found in studies with the baboon. Maternal total caloric nutrient restriction from days 30 to 165 of gestation reduced fetal circulation concentrations of select amino acids and was associated with alterations in the nutrient-sensing, mammalian target of rapamycin (MTOR) signaling pathway (Kavitha *et al.* 2014). A subsequent study detailed reductions in the placental microvillous system A plasma membrane vesicle activity, culminating in reduced placental transport of leucine (Pantham *et al.* 2015). Similar to previous work in the rat, downregulation of placental amino acid transport precedes the development of fetal growth restriction in the nutrient-restricted non-human primate (Pantham *et al.* 2016).

While several studies have identified alterations in placental transport of amino acids in response to maternal nutrient restriction in sheep (Kwon *et al.* 2004, Satterfield *et al.* 2010a), the influence of nutrient restriction on the expression of placental amino acid transporter genes is not well known. Despite the limited number of transporters that have been investigated, expression of *SLC38A2* mRNA was reduced in placentomes from nutrient-restricted sheep at mid-gestation, similar to that found for protein-restricted rats (Dunlap *et al.* 2015). Data regarding the effect of maternal malnutrition on the placental expression of amino acid and glucose transporter genes are similarly lacking in the cow. Available studies with cattle investigated the effect of maternal nutrient restriction only to day 50 of pregnancy and found no difference in expression of glucose, cationic, neutral, or acidic amino acid transporters in the endometrium after implantation (Crouse *et al.* 2017, Greseth *et al.* 2017). This was despite modest differences in concentrations of amino acids in the amniotic and allantoic fluid between control-fed and nutrient-restricted cattle (Crouse *et al.* 2019). It should be noted that analysis of expression of amino acid transporters in the placentomes was not undertaken. Given that the placentome is the primary site of hematotropic support during pregnancy, the differences in amounts of amino acids in allantoic and amniotic fluids may reflect changes in the developing placentomes.

Exploring population variance as a means to understand placental responses to maternal malnutrition

Heterogeneity of a population is a persistent challenge in the conduct of research on factors affecting outcomes of pregnancy. As heterogeneity increases, sample sizes must be increased to provide sufficient statistical power to identify significant differences. Compared to humans and large animal models, laboratory rodent models exhibit less genetic variation that contributes, in part, to their adoption as a valuable research tool despite significant differences in fetal physiology. In the course of our research with the pregnant sheep model, we consistently observed a subset of pregnant sheep that, despite long-term maternal nutrient restriction at 50% of NRC requirements, supported rates of fetal growth similar to those for well-fed control sheep (Edwards *et al.* 2020b, Sandoval *et al.* 2020). We have initiated a line of investigation focusing on each end of the spectrum of responses to maternal nutrient restriction as a means to identify critical factors regulating placental growth and function.

Results of our recent research identified a quantifiable reduction in caruncular crypt density coupled with limited contact and juxtaposition of the maternal caruncle and fetal cotyledonary tissues in nutrient-restricted (NR) sheep having growth-restricted fetuses compared to NR sheep having fetuses of normal weight (Edwards *et al.* 2020b). At the molecular level, we observed reductions in the expression of the amino acid transporters *SLC7A6*, *SLC7A7*, and *SLC38A2* in the placentomes of NR sheep having growth-restricted fetuses compared to NR sheep having fetuses of normal weight. Importantly, these changes in placental histoarchitecture and amino acid transporter expression were associated with reduced amino acid availability in the fetal circulation, even when there were no differences in amino acid concentrations in maternal circulation. One additional observation of interest in this study was that the expression of the amino acid transporter *SLC7A2* in the placenta was reduced in all NR animals irrespective of fetal growth rate. While clearly, these data suggest that *SLC7A2* expression is highly sensitive to maternal nutritional intake, its expression does not appear to correlate with actual rates of fetal growth.

In the same cohort of animals, an assessment of gene expression for known angiogenic factors at day 125 of gestation found that *VEGFA* mRNA expression in the placenta was upregulated in NR sheep having normal-weight fetuses compared to NR sheep having growth-restricted fetuses as well as well-fed controls (Fig. 1). Reynolds *et al.* (2005a) observed no difference in *VEGF* mRNA expression in placentomes from NR vs well-fed sheep at either day 90 or day 130 of gestation but did detect decreases in the VEGF receptors (*FLT1* and *KDR*) in NR sheep on day 130. The apparent

Figure 1 Expression of mRNAs for angiogenic factors in placentomes at day 125 of gestation was determined by qPCR as previously described (Edwards *et al.* 2020b). The following primers were used: *VEGFA*, F-GCCCACTGAGGAGTTCAACATC R-GCTGGCTTTGGT GAGGTTTG; *VEGFB*, F-TCCAAGGCTGCCATCCA R-GACCCCTCT TGGTCTCCTCAT; *VEGFC*, F-CCAGGCTGCAACAAGACTTG R-CATCTGCAGACGTGGTTATTCC; *FLT1* F-TGGATTCA GGTGAGCTTGA R-TCACCGTGCAAGACAGCTTC; *KDR*, F-GCTTGGCCCCGGGATATTA R-GGCGAGCATCTCTTTTCTG; *FLT4* F-GAGCATCGCGCCTGAT R-CACGTTGAGGTGGTTACCAATG. Singleton fetuses from the ewes fed 50% NRC diets from days 35 to 125 of pregnancy were categorized based on fetal weight at day 125 into NR non-IUGR (top quartile of fetuses) and NR IUGR (bottom quartile of fetuses), while fetuses from ewes fed 100% NRC diets were controls. Expression of *VEGFA* mRNA was greater ($P < 0.05$) in placentomes from NR non-IUGR fetuses than either control or NR IUGR fetuses. *VEGFB* mRNA expression was lower in NR IUGR compared to control placentomes ($P < 0.05$). Expression of *FLT1* mRNA in placentomes from control ewes tended to be lower ($P < 0.10$) than for NR ewes. Expression of *KDR* mRNA tended ($P < 0.10$) to be lower in control placentomes vs NR non-IUGR placentomes.

compensatory upregulation of *VEGFA* mRNA in a subset of NR sheep described in the former study highlights the value of evaluating population variance for the discovery of novel factors that may promote placental angiogenesis to increase placental nutrient exchange. Of note, Reynolds *et al.* (2005a) found that *VEGF* mRNA expression within the placenta was most highly correlated with capillary area density, capillary number density, and capillary surface density of the fetal cotyledon, but not caruncle. Taken together, this suggests that the upregulation of *VEGFA* in placentomes from NR sheep having normal-weight fetuses is a mechanism whereby conceptuses increase placental nutrient exchange.

An exploratory analysis of placental gene expression within this experimental paradigm identified 103 differentially expressed genes in placentomes from NR ewes having either normal or growth-restricted fetuses on day 125 of gestation (Edwards *et al.* 2020a). Pathway enrichment analyses, not surprisingly, identified nutrient

and amino acid transport as two of the top ten most highly enriched gene clusters among genes that were upregulated in NR ewes having growth-restricted fetuses. Interestingly, five of the remaining eight most highly enriched clusters were associated with immune system activation. Similar findings were observed in the nutritionally restricted pregnant mouse model where genes associated with immunologically mediated processes such as vascular inflammation, ER stress, and autophagy were differentially expressed in the placenta in response to the maternal nutrient restriction (Chu *et al.* 2016). Collectively, results suggest that mechanisms to temper inappropriate immunologically related responses to nutritional stress may be beneficial in preventing fetal growth restriction.

One of the challenges hindering our understanding of placental adaptation to a given insult is that the placenta develops prior to the fetus, therefore, it is difficult to conclusively illustrate that an early gestation placental phenotype gives rise to a late gestation fetal phenotype. Ultrasound-based approaches fail to identify the earliest changes in placental development. Placental biopsies provide minimal tissue for study and may not be a representative sample of both fetal and maternal tissues. In an attempt to overcome these challenges, we developed a surgical method whereby a single placentome is removed from pregnant sheep during early gestation without compromising the pregnancy (Lambo *et al.* 2020). This allows the pregnancy to progress to a later point in gestation for retrospective analyses of how the developing placenta responds to an insult (i.e. nutrient restriction) and then a second assessment of the maximally functioning placentome in late gestation, along with a fetal phenotype, in the same sheep.

Utilizing this surgical approach, we recently found that concentrations of triglycerides and bile acids in allantoic fluid of NR ewes on day 70 of gestation are negatively correlated with fetal weight in late gestation (Steinhauser *et al.* 2021). The accumulation of triglycerides and bile acids in the allantoic fluid of growth-restricted pregnancies likely highlights a reduced ability of the allantoic epithelium to transport these molecules out of the allantoic fluid reservoir for utilization by the fetus and removal of waste products, respectively. Indeed, growth-restricted fetuses in our study had lower concentrations of triglycerides and elevated bile acids in the fetal circulation. We further found that protein abundance of the fatty acid transporter protein SLC27A6 (also known as FATP6) in the mid-gestation placentome was decreased in NR ewes having growth-restricted fetuses compared to those having normal-weight fetuses. Interestingly, while the expression of other members of the fatty acid transporter family are modest in mid-gestation and increase to term, expression of SLC27A6 is abundantly expressed in mid-gestation, during placental development, and declines

dramatically to term. Thus, the potential importance of SLC27A6 as an early mediator of placental growth would likely have been overlooked if only placental samples from late gestation were evaluated. In the baboon, a 30% maternal nutrient restriction resulted in increased expression of both SLC27A2 and SLC27A6 in late gestation (Chassen *et al.* 2020). Investigators hypothesized that this upregulation was a compensatory mechanism by which the nutritionally challenged fetus attempts to increase its nutritional supply.

Observations of differential responses to maternal nutrient restriction have been observed in other species. Long *et al.* (2009) observed that only a subset of cows experienced IUGR at mid-gestation in response to maternal nutrient restriction and that IUGR was associated with a reduction in cotyledonary weights and surface area of placentomes compared to NR cows having calves with normal weights. These observations are further supported by research comparing the Baggs sheep vs University of Wyoming (UW) sheep. The Baggs sheep and University of Wyoming sheep arose from a similar genetic base; however, Baggs sheep were selected for survival in areas of limited nutrient availability, while UW sheep were raised in a more temperate environment. Interestingly, when nutritionally challenged at an identical level, UW sheep gave rise to IUGR offspring, while Baggs sheep did not (Jobgen *et al.* 2008). Amino acid analyses of blood revealed that Baggs sheep had greater placental transport activity compared to that for diet-matched UW sheep.

Even in relatively homogeneous populations of animals, placental adaptation to nutritional deficits has been observed. In WT mice with normal fetuses, the system A amino acid transporter system is adaptively upregulated in the lightest vs the heaviest placentae within a litter (McIntyre *et al.* 2019). While the lightest placentae were 23% lighter than the heaviest, there was a 154% increase in the glutamine transporter in placentae resulting in only a 5% difference in fetal weights at day 18.5. A subsequent study comparing the WT C57BL/6J and placenta-specific insulin-like growth factor 2 (*Igf2*) (P0) knockout mouse, a mutation known to induce fetal growth restriction, found that placentae from mutant mice initially upregulated system A transporter capacity, but by late gestation, this adaptation was insufficient to rescue fetal growth (McIntyre *et al.* 2019). Those results support previous findings for placentae from human babies across the normal spectrum of birth weights where system A transporter activity per microgram microvillous membrane protein was greatest in the lighter babies (Godfrey *et al.* 1998). Those researchers previously reported a decrease in system A transporter activity in placentae of growth-restricted babies and have suggested that they failed to adaptively increase system A transporter activity under conditions that result in fetal growth restriction (Sibley *et al.* 2010).

Nutritional strategies to improve placental growth and function

Amino acids are the central building blocks for protein synthesis required for normal fetal growth. However, their roles in promoting fetal growth and development go far beyond simply serving as a substrate for fetal protein synthesis. Arginine, a conditionally essential amino acid during pregnancy, not only serves as an important amino acid for protein synthesis but can also be catabolized into bioactive molecules of critical importance (Fig. 2). Indeed, arginine catabolism yields nitric oxide, ornithine, polyamines, creatine, proline, and glutamate (Wu *et al.* 2009). Nitric oxide serves as a key regulator of placental angiogenesis and vasodilation. Polyamines (polycationic molecules synthesized from the amino acid arginine (via two potential metabolic pathways)) regulate gene expression, signal transduction, ion channel function, DNA and protein synthesis, and angiogenesis, as well as proliferation, differentiation, and functions of cells (Lenis *et al.* 2017). To date, there have been no studies investigating the efficacy of polyamine supplementation as a means to stimulate placental function; however, a growing body of evidence supports the need for this research.

Inhibition of ornithine decarboxylase (ODC1), a key enzyme in polyamine synthesis, using alpha-difluormethylornithine during mid-pregnancy in mice results in embryonic growth arrest and impaired development of the yolk sac and placenta (Lopez-Garcia *et al.* 2008), while null mutation of *Odc1* results in early embryonic lethality (Pendevelle *et al.* 2001). In rats, inhibition of ODC1 activity results in intrauterine growth restriction as well as a reduction in placental weight and DNA content (Ishida *et al.* 2002). Further, pharmacological inhibition of ODC1 has been shown to decrease microvascular sprouting of human umbilical vein endothelial cells, *in vitro* (Kucharzewska *et al.* 2010). Sheep conceptuses fail to develop when the translation of both *ODC1* and *ADC* mRNAs were knocked down to

prevent the synthesis of polyamines (Lenis *et al.* 2018). Finally, increased amounts of polyamines and their amino acid precursors were found in the circulation of NR pregnant sheep that had normal-weight placentae and fetuses as compared to those that had small placentae and growth-restricted fetuses (Edwards *et al.* 2020b). These data highlight a positive association between increased polyamine availability and placental and fetal growth in the nutritionally challenged sheep.

The efficacy for maternal arginine supplementation to prevent fetal growth restriction has been varied and likely depends on the timing, dose, chemical form, and duration of supplementation. Dietary supplementation with 1% arginine-HCl to gilts between days 30 and 114 of gestation increases litter birth weight of live-born piglets (Mateo *et al.* 2007). Similarly, dietary supplementation with 0.83% arginine to gilts between days 14 and 30 of gestation enhances placental angiogenesis (Wu *et al.* 2017), placental growth, and the number of live embryos (Li *et al.* 2014). In nutritionally restricted sheep, maternal arginine supplementation from day 60 of gestation to term increased the birth weights of lambs by 21% (Lassala *et al.* 2010). Dietary supplementation with either L-arginine or N-carbamylglutamate, a metabolically stable activator of intestinal synthesis of citrulline and arginine from glutamine, glutamate, and proline (Wu *et al.* 2004), to NR sheep from days 35 to 110 of gestation was successful in increasing fetal weights at necropsy compared to NR sheep receiving no supplement (Zhang *et al.* 2016). In contrast, supplementation from days 100 to 125 of gestation did not increase fetal weights at necropsy on day 125; however, brown adipose tissue and pancreatic mass were both increased in response to arginine supplementation (Satterfield *et al.* 2013). Thus, arginine supplementation may reduce cold-induced perinatal mortality of ruminant livestock species (McKnight *et al.* 2020) and may ameliorate some of the negative aspects of metabolic programming in a wide array of animal species following maternal malnutrition.

Figure 2 Roles of arginine and associated molecules in placental function. Arginine is a precursor to proline, agmatine, ornithine, citrulline, nitric oxide, creatine, glutamine, and urea but can also be synthesized from citrulline, therefore, the roles of citrulline are associated with downstream actions of arginine. Arginine is also a potent stimulator of protein synthesis. This figure is compiled from these sources (Wu *et al.* 2009, 2011, Dickinson *et al.* 2014, Laube & Bernstein 2017, Hsu & Tain 2019).

In human pregnancy, L-arginine supplementation has mostly been investigated as a means to combat gestational hypertension or improve placental function in pre-eclampsia. Results of those studies suggest that L-arginine supplementation improves maternal diastolic blood pressure and extends the length of gestation (Facchinetti *et al.* 2007, Neri *et al.* 2010). There was a varied response in birth outcome most typically associated with duration of supplementation or severity of fetal growth restriction at the initiation of supplementation. When hypertensive mothers were administered 20 g/day of L-arginine for 5 days after a preeclampsia diagnosis made between weeks 20 and 36 of gestation, there was no effect on birth weight (Facchinetti *et al.* 2007) nor was there an improved birth weight when L-arginine was administered at 12 g/day for only 5 days between weeks 28 and 36 (Staff *et al.* 2004). In contrast, the majority of studies reporting a significant improvement in birth outcome used a prolonged, low-dose strategy for arginine supplementation where L-arginine was administered at 3–5 g/day from approximately week 20 of pregnancy until delivery (Vadillo-Ortega *et al.* 2011, Camarena Pulido *et al.* 2016). As most of these studies have been performed in women considered high risk for, or diagnosed with, gestational hypertension, and since supplementation commenced at least halfway through pregnancy, the question still remains as to the benefits of L-arginine supplementation during placental development in the first half of pregnancy. Of specific interest would be whether early administration of L-arginine would occlude the onset of hypertension, or, at a minimum, continue to temper the severity as is observed with administration later in pregnancy. Additionally, the use of L-arginine or L-citrulline supplementation in low resource settings that likely experience significant maternal malnutrition has not been undertaken (Weckman *et al.* 2019).

While arginine supplementation has shown potential to increase fetal growth in humans, sheep, and rats, there are concerns about the efficacy of long-term supplementation due to potential effects on acid-base balance or chronic provision of chloride when administered as an HCl salt (Lassala *et al.* 2009). Further, cells and tissues which have high arginase activity contribute to the relatively short biological half-life of L-arginine in mammals, and intestinal arginase, specifically, inhibits the availability of orally provided supplemental L-arginine in humans (Castillo *et al.* 1993, Wu *et al.* 2007). L-citrulline is a neutral amino acid and is an effective precursor for arginine synthesis in humans, pigs, ruminants, and rodents (Gilbreath *et al.* 2020). In pregnant sheep, the half-life of L-citrulline is twice that of L-arginine and was more effective in sustaining high concentrations of arginine in both the maternal and fetal circulations, as well as ornithine in the fetal circulation (Lassala *et al.* 2009). Based on our recent discovery that extracellular citrulline is not

degraded by the ruminal microbes of adult ruminants (Gilbreath *et al.* 2020), dietary supplementation with unencapsulated citrulline may be an effective means to improve placental and fetal growth in cattle, sheep, and goats. In rats, both L-arginine and L-citrulline supplementation to pregnant rats fed a low protein diet increased fetal weight near term (Bourdon *et al.* 2016). However, L-citrulline supplementation increased the fetal/placental weight ratio (an indicator of placental efficiency) more than L-arginine supplementation. While L-arginine supplementation was associated with an increase in ornithine in maternal plasma, L-citrulline supplementation further increased concentrations of ornithine. This is consistent with a longer half-life of citrulline than arginine in gestating dams to sustain an elevated concentration of arginine for the production of ornithine by arginase (Wu *et al.* 2007). While L-arginine has been shown to have beneficial effects during pregnancy in multiple species, the supplemental use of L-citrulline moving forward certainly has merit for consideration in both humans and livestock species.

Leucine has also received consideration as a nutritional strategy to improve fetal growth as it has been shown to increase protein synthesis, resulting in increased skeletal muscle mass, as well as promote fetal pancreatic development and insulin secretion in fetal pigs and sheep (Wang *et al.* 2018, Boehmer *et al.* 2020). In the sheep study, leucine was infused into the fetal circulation in late gestation and potential impacts on placental function were not investigated (Boehmer *et al.* 2020). Leucine has been shown to activate the mechanistic target of rapamycin (mTOR) signaling pathway in trophectoderm cells *in vitro*, from both pigs and humans, and may play a role in the nutrient-sensing actions of the mTOR pathway and its effects on placental development (Roos *et al.* 2007, Kim *et al.* 2013).

Lipid-based supplements including omega-3 long-chain polyunsaturated fatty acids (PUFA) or mixed doses of essential fatty acids have demonstrated moderate improvements in pregnancy outcomes in women as well as the growth of the children in low nutrition environments (Adu-Afarwuah *et al.* 2015, 2016, Saccone *et al.* 2015, Middleton *et al.* 2018). In adequately fed sheep, supplementation of omega-3 PUFA during pregnancy had no effect on placental and fetal weights (Nickles *et al.* 2019, Rosa Velazquez *et al.* 2020). Further research is necessary to determine if omega-3 PUFA supplementation would enhance placental and fetal growth in a nutrient-restricted cohort.

Conclusions

The placenta serves as the critical intermediary between the maternal and fetal environments. Despite its transient role as the mediator of the exchange of nutrients, gases, and waste between mother and fetus, the capacity of the placenta to adapt to environmental stresses has

a lifelong impact on the health and well-being of the offspring. Maternal undernutrition is a common environmental stressor associated with increased risk for a myriad of postnatal health disorders such as cardiovascular disease and metabolic syndrome. A renewed focus on the scientific investigation of adaptive biological responses has identified compensatory changes in amino acid and lipid transport mechanisms, increased expression of angiogenic factors, and differential activation of immune-related factors as potential mediators of improved placental function, and fetal outcomes, in the undernourished dam. Further, new methodological approaches have the potential to identify early changes in placental development that are truly causative regulators of fetal growth. These observations and methodological advances are critical first steps toward the development of nutraceutical and therapeutic strategies, such as selected amino acid supplementation, to improve pregnancy outcomes in the face of limited nutrient availability.

Declaration of interest

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

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Author contribution statement

M C S was responsible for the original manuscript. A K E performed experiments and collected data. F W B, K A D, C B S, and G W provided crucial intellectual input into the design, writing, and editing of the manuscript.

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